An Electrophysiological Model of Spinal Transmission Deficits in Mouse Experimental Autoimmune Encephalomyelitis

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ABSTRACT

Chronic relapsing/remitting experimental autoimmune encephalomyelitis (EAE) can be induced in 8-week-old female SJL/J(H-2) mice via inoculation with the p139–151 peptide of myelin proteolipid protein (PLP), Mycobacterium tuberculosis (MT), complete Freund’s adjuvant (CFA), and Bordatella pertussis. EAE is a relevant preclinical model of MS that incorporates several aspects of the clinical disease. Chief among these are the inflammatory mediated neurological deficits. While the impact of localized spinal cord demyelination on neurotransmission has been modeled successfully, relatively little work has been done with spinal cord from animals with EAE. The goal of this study was to assess the utility of a grease-gap tissue bath methodology in the detection of transmission deficits in EAE spinal cord tissue. Spinal cords removed from EAE mice at different phases of the neurological deficit were assessed for their response to both lumbar and sacral application of one of several depolarizing agents (veratridine, potassium chloride [KCl], (−)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA]). The main finding of this study is that transmission deficits were detected in EAE mice at the onset of the neurological deficits. They were sustained for a period of approximately 2 to 3 weeks post disease onset followed by a gradual recovery of group function. The other finding is that there is a decrease in the latency to achieve AMPA-mediated depolarization in sacral spinal cord that is independent of the magnitude of the depolarization response. These results suggest that this methodology can be utilized to assess sensory and motor deficits in spinal cord from EAE animals.

Multiple sclerosis (MS) is a chronic, progressively debilitating disease presenting with neurological symptoms resulting from degenerative changes in the central nervous system (CNS). Axonal demyelination induced by autoimmune-triggered neuroinflammation has been reported to be at the core of the CNS lesions that characterize MS (Waxman, 1998, 2000). Recently, postmortem MS studies have correlated the degree of inflammation at the lesion site with the incidence of axonal transection (Trapp et al., 1998). Active demyelination has been correlated with a high incidence of axonal damage, compared with less damage in lesions with inactive centers, and still less damage in remyelinated plaques (Kornek et al., 2000). These findings suggest that neuroinflammatory events ultimately lead to the axonal degeneration that figures prominently in the progressive forms of MS (Trapp et al., 1998).

Although the precise physiological event by which the disease is triggered and progresses is unknown, both MS patients and preclinical EAE mice exhibit changes in CNS glutamate levels (Hardin-Pouzet et al., 1997; Stover et al., 1997). The preclinical findings suggest that glutamate excitotoxicity impacts upon the myelinating function of oligodendrocytes. Several investigators have reported that the AMPA/kainate antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) improves the neurological symptoms of EAE, increasing oligodendrocyte survival and decreasing axonal damage without affecting CNS neuroinflammation (Pitt et al., 2000; Smith, 2000; Matute, 2001). Both oligodendrocytes and spinal motor neurons exhibit AMPA-mediated excitotoxicity (Ikonomidou and Tsuji, 1996; Yoshioka et al., 1996; McDonald et al., 1998). Therefore rigorous examination of AMPA-mediated transmission in the mouse EAE model of demyelination may aid us in understanding the contribution of the glutamate pathway to the physiological progression of demyelinating disease.

Undoubtedly demyelination is a prominent causative fac-

ABBREVIATIONS: MS, multiple sclerosis; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; AMPA, (−)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; PLP, myelin proteolipid protein; CAP, compound action potential; 4-AP, 4-aminopyridine; MT, Mycobacterium tuberculosis; CFA, complete Freund’s adjuvant; PI, post inoculation; ACSF, artificial cerebral spinal fluid; ANOVA, analysis of variance.
tor in the pathology of the axonal transmission deficits associated with EAE (Chalk et al., 1994, 1995). Both conduction block and “ectopic” firing patterns have been observed. Although demyelinated central axons have been shown to conduct over large areas (2500 μm) they exhibit a decrease in conduction velocity and an increase in the refractory period of transmission (Felts et al., 1997). Whereas seminal electrophysiological studies (Hodgkin and Katz, 1949; Hodgkin and Huxley, 1952) have clearly defined the role of sodium and potassium channels in the generation of the action potential, the impact that demyelination has on the relationship between these ion channels and conduction is still evolving. The picture that is emerging suggests that demyelination influences both the location of the ion channels on the axons as well as whether subtypes are expressed. Increases in the concentration of sodium channels along the demyelinated regions of the axon may account for the reversal of conduction block (Foster et al., 1980). Transient redistribution of peripheral nerve potassium channels (Kv1.1) from the juxtaparanodal to the nodal region following demyelination translates into increases in both the amplitude and duration of the compound action potential (CAP) elicited by the potassium channel blocker, 4-aminopyridine (Rasband et al., 1998) as well as the incidence of ectopic action potentials (Felts et al., 1995; Kapoor et al., 1997). The sodium channel blocker tetradotoxin has been shown to block both the ectopic action potentials induced in demyelinated axons by 4-AP as well as the oscillations in the membrane potential recorded from sites near the lesion (Kapoor et al., 1997) suggesting that both the sodium and potassium current contribute to the ectopic discharge. Expression of sodium channel sensory neuron-specific mRNA in cerebellar Purkinje cells of both EAE mice and human patients with MS but not normal patients (Black et al., 2000a) may be responsible for the ataxia observed in clinical MS (Waxman, 2000, 2001b). Therefore examining the impact of EAE on spinal cord depolarization mediated via either the sodium channel opener veratridine or elevated levels of potassium may help elucidate the physiopathology of demyelinating disease.

Although there is extensive preclinical data on the behavioral expression and histopathology of EAE, less attention has been focused on analyzing the impact to spinal neurotransmission. Isolating the spinal cord of both EAE and untreated animals allows one to investigate the pharmacology of its sensory and motor components post-EAE insult. Therefore a series of studies were designed to characterize spinal cord conduction in EAE and naive animals using a grease-gap bath methodology. The overall goal of the study was to determine whether this method could be used to detect changes in spinal cord neurotransmission mediated by EAE and whether there was a correlation between the neurological deficit phase of EAE and spinal cord transmission.

Materials and Methods

Preparation of EAE Mice. EAE was induced in 8-week-old female, SJL/J(H-2) mice (Jackson Laboratorie, Bar Harbor, ME) using a protocol developed from referenced publications (Tuohy et al., 1989; Kuchroo et al., 1991, 1994). Briefly, on post inoculation day 0 (PI) subjects received both a subcutaneous inoculation of a combination of PLP 139–151 (75 μg) (Bachem Biosciences, King of Prussia, PA) and MT (400 μg) (Difco, Detroit, MI) in CFA H37 Ra (1 mg/ml Mycobacterium tuberculosis H37 Ra) and an intravenous inoculation of Bordatella pertussis (35 ng; List Biological Laboratories, Inc., Campbell, CA). A second inoculation of B. pertussis (50 ng) was administered on PI day 3. Sham controls (all inoculation components except PLP 139–151) were utilized in the veratridine studies. Naive controls (no injection) were utilized in both the KCl and AMPA studies.

Beginning on 7 days PI subjects were scored daily for the onset and progression of the behavioral symptoms of motoric impairment associated with the induction of EAE in the scientific literature. The scoring scale was from 0 to 5: 0 = normal; 0.5 = partial limp tail; 1.0 = complete limp tail; 2.0 = impaired righting reflex; 2.5 = delayed righting reflex; 3.0 = partial hind limb paralysis; 3.5 = one leg completely paralyzed, one leg partially paralyzed; 4.0 = complete hind limb paralysis; 4.5 = legs are completely paralyzed and moribund; 5.0 = death due to EAE. The subjects were scored over approximately a 2- to 5-week time period during which their progression from the acute attack, to first remission, relapse and second

![Graph](http://example.com/graph1.png)

**Fig. 1.** Representative graph of the progression of the neurological symptoms (EAE) typically observed in SJL/J (H-2) mice inoculated with PLP 139–151 (average ± S.E.M.: vehicle EAE n = 17; vehicle sham n = 10). Typically the acute phase occurs at 2 to 3 weeks PI, followed by remission 1 week later.

**Fig. 2.** Spinal cord depolarization elicited by motor and sensory application of veratridine (100 μM) in two behavioral subsets of EAE SJL/PLP mice, chronically relapsing/remitting and remitting. Spinal cords were removed (PI 39–41) and assessed for motor and sensory transmission evoked by application of veratridine (10, 30, and 100 μM). Subjects were divided into two behavioral subsets based on their neurological deficit profile per established criterion (see Materials and Methods). Statistical analysis of the response to 100 μM veratridine using an unpaired t test with Welch’s correction revealed a significant difference between the behavioral subsets in terms of both motor (*, p < 0.05; t = 2.738, df = 10) and sensory (**, p < 0.005; t = 3.878, df = 11) transmission. Panel A: open column, motor relapsing/remitting (n = 6); shaded column, motor remission (n = 11); panel B: open column, sensory relapsing/remitting (n = 4); shaded column, sensory remission (n = 11).
remission was tracked. The acute attack was defined as the first clinical episode with a score greater than 1.0 for a period of 2 consecutive days. Remission was defined as a phase of clinical improvement following an attack where the clinical score was decreased by at least one point for a period of 2 consecutive days. Relapse was defined as the period following remission where the clinical score rose by at least one point for a period of 2 consecutive days. Spinal cord transmission was assessed at different phases of the illness and is presented in relationship to the number of weeks PI.

**Electrophysiology.** After neurological assessment the test subjects (2–5 weeks PI) were anesthetized with urethane (0.5 ml of a 1.12 M solution; Sigma-Aldrich, St. Louis, MO), decapitated, and their spinal columns were exposed. The spinal column and surrounding visceras was dissected so that the portion remaining was comprised of mainly the lumbar and sacral portions. A 26-gauge 3/8-A needle attached to a 5-ml syringe filled with the artificial cerebral spinal fluid (ACSF), and 0.10 M urethane was inserted into the sacral portion of the vertebral column and the spinal cord was pressure ejected into a bath of cold oxygenated ACSF containing urethane (0.10 M). The ACSF was composed of 118 mM NaCl, 25 mM NaHCO₃, 4.7 mM KCl, 1.2 mM KH₂PO₄, 2.0 mM MgSO₄, 11 mM glucose, 2.0 mM CaCl₂, and 0.0033% H₂O₂ (pH 7.3–7.4). The tissue was further dissected so that the remaining block consisted of all of the tissue below the lumbar enlargement and approximately 5 mm of tissue above the lumbar enlargement. The cords were then transferred to one of eight, two-compartment grease-gap baths filled with cold oxygenated ACSF/urethane solution. The baths were constructed so that there was a greased slot in the central barrier separating the two compartments (Hayes and Simmonds, 1978, 1985; Black et al., 2000b). The cords were orientated in the baths so that the barrier was inserted across the spinal cord at the level of the beginning of the lumbar enlargement. Once all eight spinal cords were loaded into the baths, the baths were perfused with ACSF at a rate of 2 ml/min.

The tissues were allowed to equilibrate in the baths for approximately 60 min before the initial drug application. The direct current (d.c.) potential between the two compartments was measured using silver/silver chloride electrodes. Three studies were conducted. In each case, increasing concentrations of the selected drugs were applied sequentially to the lumbar portion of the cord followed by the sacral portion of the cord. For the purposes of this study, we have defined motor transmission as potential differences resulting from drug application to the lumbar portion of the spinal cord (corticospinal pathway). Sensory transmission is defined as potential differences resulting from drug application to the sacral portion of the cord (mechanosensory pathway). In the first study AMPA (Sigma-Aldrich) was assessed at concentrations of 1.0, 10.0, and 30.0 µM. In the second study, potassium was assessed at concentrations of 0.30, 216 Hanak et al.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Drug Application</th>
<th>Region</th>
<th>Group</th>
<th>Weeks PI</th>
<th>Avg. Sec. (±S.E.M.)</th>
<th>n</th>
<th>EAE Deficit Score Avg. Score (±S.E.M.)</th>
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<tbody>
<tr>
<td>Lumbar</td>
<td>Naive</td>
<td>Age matched for 2–4 weeks PI</td>
<td>306 (±15)</td>
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<tr>
<td>Lumbar</td>
<td>EAE</td>
<td>2 weeks PI</td>
<td>255 (±10)</td>
<td>10</td>
<td>3.0 (±0.4)</td>
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<tr>
<td>Lumbar</td>
<td>EAE</td>
<td>3 weeks PI</td>
<td>290 (±20)</td>
<td>26</td>
<td>2.3 (±0.3)</td>
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<tr>
<td>Lumbar</td>
<td>EAE</td>
<td>4 weeks PI</td>
<td>289 (±21)</td>
<td>13</td>
<td>1.2 (±0.2)</td>
<td></td>
</tr>
<tr>
<td>Sacral</td>
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<td>Age matched for 2–4 weeks PI</td>
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<tr>
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<td>2 weeks PI</td>
<td>303 (±35)</td>
<td>10</td>
<td>2.9 (±0.4)</td>
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<tr>
<td>Sacral</td>
<td>EAE</td>
<td>3 weeks PI</td>
<td>268 (±13)*</td>
<td>26</td>
<td>2.4 (±0.3)</td>
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<tr>
<td>Sacral</td>
<td>EAE</td>
<td>4 weeks PI</td>
<td>285 (±22)</td>
<td>12</td>
<td>1.3 (±0.2)</td>
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</table>

* p < 0.05.
KCI (10.0 mM) latency to peak in EAE and naive mice

Latency to KCl-mediated (10.0 mM) depolarization in EAE and naive mice. Spinal cords were removed from SJL/PLP-inoculated EAE and naive (age-matched) mice at either 2 or 3 weeks PI and placed in a grease-gap bath. The d.c. potential between the two compartments was assessed in response to local application of KCl (0.30, 1.0, 3.0, 10.0 mM). The data are represented as the mean ± S.E.M. of the group for the latency to reach the maximum level of depolarization from the point of application of the 10.0 mM concentration of KCl. Induction of EAE did not affect the latency to maximal depolarization.

<table>
<thead>
<tr>
<th>Drug Application</th>
<th>Region</th>
<th>Group</th>
<th>Weeks PI</th>
<th>Avg. Sec. (±S.E.M.)</th>
<th>n</th>
<th>EAE Deficit Score Avg. Score (±S.E.M.)</th>
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</thead>
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<tr>
<td>Lumbar</td>
<td>Naive</td>
<td>Age-matched for 2–3 weeks PI</td>
<td>289 (±11)</td>
<td>16</td>
<td>0</td>
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<tr>
<td>Lumbar</td>
<td>EAE</td>
<td>2 weeks PI</td>
<td>294 (±13)</td>
<td>9</td>
<td>3.2 (±0.4)</td>
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<tr>
<td>Lumbar</td>
<td>EAE</td>
<td>3 weeks PI</td>
<td>345 (±61)</td>
<td>11</td>
<td>2.6 (±0.4)</td>
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<tr>
<td>Sacral</td>
<td>Naive</td>
<td>Age-matched for 2–3 weeks PI</td>
<td>264 (±7)</td>
<td>26</td>
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<tr>
<td>Sacral</td>
<td>EAE</td>
<td>2 weeks PI</td>
<td>276 (±11)</td>
<td>10</td>
<td>3.3 (±0.4)</td>
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</tr>
<tr>
<td>Sacral</td>
<td>EAE</td>
<td>3 weeks PI</td>
<td>259 (±11)</td>
<td>10</td>
<td>2.4 (±0.4)</td>
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</table>

1.0, 3.0, and 10.0 mM. In the third study, veratridine (Sigma-Aldrich) was assessed at concentrations of 10.0, 30.0, and 100.0 µM.

The standard drug application time was 2 min in length. The signals were captured, amplified and filtered using specialty hardware and software [Spike2, Cambridge Electronics Design Limited, Axon Instruments Inc. (Union City, CA) CyberAmp 380, programmable signal conditioner; Axoclamp filter].

**Data Analysis.** A Spike2 subprogram (min/max for peaks) was used to analyze the data in terms of the peak potential change (mV) caused by each drug application. Statistical comparison of the concentration-depolarization response curves elicited by drug application to EAE subjects at either 2, 3, 4, or 5 weeks PI or to their age-matched controls was accomplished via a repeated measures ANOVA (Statistics 5.0, Statsoft, Inc., Tulsa, OK).

The latency to the peak level of depolarization (time required to reach peak depolarization from the point of drug application) achieved by the highest concentration of each drug tested was also calculated. The values calculated for EAE subjects at either 2, 3, 4, or 5 weeks PI were compared with their age-matched controls using an ANOVA (GraphPad Prism 2.0).

**Results**

**Neurological Deficit Assessment in SJL/PLP EAE Mice.** Inoculation with PLP results in a clearly defined acute attack in approximately 90% of the subjects. Although it appears that all of the subjects go into remission, there are essentially two subsets within that group: those in chronic remission (approximately 60% of the inoculated subjects) and those that relapse and experience subsequent remissions (approximately 30% of the inoculated subjects). That the group’s behavior is the most synchronized at the acute attack and the first remission, those time points are also the most prominent features of the neurological deficit graph (Fig. 1). Subsequent relapse and remission phases demonstrate the least separation due to the effect of averaging the two subgroups.

**Neurological Deficits and Electrophysiological Assessment.** In a satellite study we observed that the subjects in chronic remission that were assessed between 39 and 41 days PI had significantly better spinal cord conduction than those that relapsed and remitted (Fig. 2). Clearly that finding would impact the ability to correlate overall group spinal cord transmission with the neurological deficit phase after the acute attack given that you can’t predict a priori which subgroup a subject would ultimately belong to at first remission. In fact preliminary analysis of spinal transmission in relationship to the phase of the neurological deficit (acute attack, first remission, relapse, second remission) did not...
demonstrate a clear correlation between the cyclic oscillations in the neurological deficit and the ability to depolarize spinal cord tissue (data analysis not presented). Therefore, for the purpose of developing a viable model for pharmaceutical development, overall spinal cord transmission for each group was assessed in relationship to the number of weeks PI. The concentration/response curves for AMPA, KCl, and veratridine are presented graphically in Figs. 3, 4, and 5 respectively. The latency to achieve the peak level of depolarization elicited by the highest concentration of each drug tested is presented in Tables 1, 2, and 3, for AMPA (30.0 μM), KCl (10.0 mM), and veratridine (100.0 μM), respectively.

Both sham (all inoculation components except PLP) and naive controls (age-matched for EAE subjects at 2–5 weeks PI) were generated for the veratridine studies. Initial comparison of the sham and naive animals (age-matched for EAE subjects at 2–5 weeks PI) response to veratridine did not reveal any discernable differences between the treatment groups (Table 3).

Electrophysiological Assessment of AMPA-Mediated Depolarization in EAE and Naive Age-Matched Controls. AMPA-mediated motor transmission was suppressed at both 2 and 3 weeks PI. Recovery of function was observed by 4 weeks PI (Fig. 3A). While sensory transmission was decreased by approximately 25% from 2 to 4 weeks PI (30.0 μM concentration) the effect was not statistically significant (Fig. 3B). EAE subjects exhibited a decrease in the latency to achieve sensory depolarization at 3 weeks PI (30.0 μM AMPA) (Table 1). Induction of EAE did not affect the latency to motor depolarization.

Electrophysiological Assessment of KCl-Mediated Depolarization in EAE and Naive Age-Matched Controls. KCl-mediated motor transmission (10.0 mM) was decreased by between 10 and 40% at 2 to 3 weeks PI but the effect was not statistically significant (Fig. 4A). There was, however, a significant decrease in KCl-mediated sensory transmission in the EAE group at both 2 and 3 weeks PI (Fig. 4B). Induction of EAE did not affect the latency to depolarize at any PI interval (10.0 mM KCl; Table 2).

Electrophysiological Assessment of Veratridine-Mediated Depolarization in EAE and Sham Age-Matched Controls. Lumbar application of veratridine in the EAE group demonstrated that motor transmission was suppressed at both 2 and 3 weeks PI (Fig. 5A). Recovery was noted by 4 to 5 weeks PI. Induction of EAE resulted in suppression of sensory transmission at 2 through 5 weeks PI (Fig. 5B). There was significant recovery between 2 and 5 weeks PI since spinal transmission was significantly better at 5 weeks PI then 2 weeks PI. Induction of EAE did not alter the latency to depolarize at any PI interval (100.0 μM veratridine; Table 3).

Discussion

The primary finding from this study is that PLP-mediated EAE resulted in deficits in spinal cord transmission that were quantifiable using a grease-gap bath methodology. In general, spinal cord tissues extracted from subjects expressing EAE at 2 to 4 weeks after inoculation were less responsive to local application of the depolarizing agents AMPA, KCl, and veratridine. Sensory, more so then motor transmission deficits appear to be a common, consistent feature of EAE animals.

Glutamate excitotoxicity has been hypothesized to be a central feature of the cycle of inflammation, demyelination, and axonal damage that occurs in both MS and EAE. CSF glutamate metabolite levels are altered in neuroinflammatory diseases, and chronic treatment with AMPA/kainate antagonists has been reported to improve the neurological outcome of EAE (Pitt et al., 2000; Smith et al., 2000). One phase of the current study was designed to assess whether this glutamatergic pathophysiology was reflected in our
Spinal cords were removed from SJL/PLP-inoculated EAE and sham (age-matched) mice at between 2 and 5 weeks PI and placed in a grease-gap bath. The d.c. potential between the two compartments was assessed in response to local application of veratridine (10.0, 30.0, and 100.0 μM). The data are represented as the mean ± S.E.M. of the group for the latency to reach the maximum level of depolarization from the point of application of the 100.0 μM concentration of veratridine. Induction of EAE did not affect the latency to maximal depolarization.

TABLE 3
Latency to veratridine-mediated (100.0 μM) depolarization in EAE and naive mice

<table>
<thead>
<tr>
<th>Drug Application</th>
<th>Region</th>
<th>Group</th>
<th>Weeks PI</th>
<th>Avg. Sec. (±S.E.M.)</th>
<th>n</th>
<th>EAE Deficit Score Avg. Score (±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Veratridine (100.0 μM) latency to peak in EAE and sham mice</td>
<td>Lumbar</td>
<td>Sham</td>
<td>2 weeks PI</td>
<td>501 (±31)</td>
<td>20</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3 weeks PI</td>
<td>519 (±23)</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 weeks PI</td>
<td>464 (±35)</td>
<td>18</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5 weeks PI</td>
<td>436 (±25)</td>
<td>19</td>
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</tr>
<tr>
<td></td>
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<td>EAE</td>
<td>2 weeks PI</td>
<td>411 (±30)</td>
<td>21</td>
<td>2.6 (±0.2)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3 weeks PI</td>
<td>570 (±53)</td>
<td>8</td>
<td>2.2 (±0.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 weeks PI</td>
<td>532 (±52)</td>
<td>16</td>
<td>2.4 (±0.3)</td>
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<td></td>
<td></td>
<td></td>
<td>5 weeks PI</td>
<td>526 (±42)</td>
<td>11</td>
<td>1.1 (±0.3)</td>
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<tr>
<td>B. Veratridine (100.0 μM) latency to peak in EAE and sham mice</td>
<td>Sacral</td>
<td>Sham</td>
<td>2 weeks PI</td>
<td>449 (±29)</td>
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<td></td>
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<td>3 weeks PI</td>
<td>453 (±18)</td>
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<td>4 weeks PI</td>
<td>478 (±28)</td>
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<td></td>
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<td>5 weeks PI</td>
<td>451 (±16)</td>
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<tr>
<td></td>
<td>Sacral</td>
<td>EAE</td>
<td>2 weeks PI</td>
<td>458 (±32)</td>
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<td>3 weeks PI</td>
<td>507 (±26)</td>
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<td>2.3 (±0.5)</td>
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<td>4 weeks PI</td>
<td>428 (±17)</td>
<td>17</td>
<td>2.4 (±0.2)</td>
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<td></td>
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<td>5 weeks PI</td>
<td>592 (±41)</td>
<td>13</td>
<td>1.2 (±0.3)</td>
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</table>

In fact AMPA-mediated transmission deficits were observed. When veratridine and KCl were used as general mediators of spinal cord depolarization they also revealed EAE-mediated transmission deficits.

The second major finding was that the cyclical pattern of neurological remissions and relapses was not mirrored in the spinal cord transmission studies. In general, transmission deficits appear to be sustained for a period of approximately 2–4 weeks PI. Signs of recovery as measured by spinal cord depolarization were observed at approximately 4 to 5 weeks after inoculation, corresponding with the period where there was a substantial decrease in the average neurological deficit score.

The failure to detect a cyclical pattern of spinal neurotransmission deficit and recovery for the group underscores the fact that there are multiple factors influencing the translation of physiological function into behavioral expression. One caveat to these findings is the inherent difficulty in categorizing the phase of EAE for each individual animal in order to assign them to test groups. Like others (Yu et al., 1996), we have observed a certain degree of intersubject variation in terms of the onset, duration, and relative magnitude of the acute attack, remission, and relapse phases. Clearly discernable transitions between the phases are less obvious after the first remission phase. This can be seen clearly in Fig. 1., which graphs the typical progression of neurological symptoms in a representative study. Only approximately 30% of the group expresses what is considered to be a behaviorally defined relapse. The majority of the group (approximately 60%) goes into chronic remission. Therefore the gradual attenuation of the group’s transmission deficits may well be a mathematical artifact of the heterogeneity of the group.

Similar distribution patterns have been reported by other groups (Yu et al., 1996). For their SJL/PLP EAE study they reported a 42% mortality rate. Of the 14 surviving animals with EAE approximately 36% went into chronic remission, whereas the remainder displayed either one relapse (36%) or two relapses (28%) in the 56 days following onset. The satellite study we reported on confirms that there is a physiological basis for the heterogeneity in the group’s behavioral phenotype. Both motor and sensory spinal transmission was significantly better in the subjects in chronic remission compared with those that relapsed and remitted subsequent to the acute attack.

Therefore, similar to MS, EAE is a phenotypically heterogeneous and evolving disease state. If you analyze the group data from the perspective of weeks after inoculation rather then by the neurological deficit phase, the picture that emerges suggests a gradual recovery of physiological function. It seems unlikely that the cycle of transmission deficit and recovery is age-dependent since developmental differences were not observed in the age-matched controls. Therefore, it’s possible that the time-dependent, gradual attenuation of the robust transmission deficits that we observed during early EAE is in reality a reflection of the fact that the groups physiological and behavioral state are most synchronized during the initial inflammatory phase of EAE that occurs during the acute attack.

In fact, qualitative histopathological analysis of EAE lumbar spinal cord tissue generated in a separate in-house study (n = 5 mice/PI interval [collected two times per week]; period = from PI 0 through PI 56) indicates that there is a large time lag between peak leukocyte infiltration and demyelination following inoculation with PLP (personal communication). Based on the percentage of subjects affected as well as their overall infiltrate score, leukocyte infiltration appears to peak at 14 to 21 days post inoculation. Demyelination was detected at approximately 56 days post inoculation. Samples were not assessed beyond PI 56 so the time course for further changes is unclear beyond that point. Therefore it appears that the transmission deficits that we observed for the whole group correspond with the time period when neuroinflammation was at its peak.

Overall these results suggest that inflammatory products produced during the initial phases of EAE are able to induce...
conduction block prior to the expression of neurodegenerative changes. Using a similar inoculation protocol Sobel et al. (1990) found that clinical disease onset occurred at between 10 and 15 days PI. Inflammatory infiltrates in CNS parenchyma occurred at between 7 and 10 days PI and reached a plateau by 14 days PI. The areas of parenchymal demyelination correlated with the neurological score at the time of death. Other investigators have demonstrated that during the acute attack neurological disability is tied to inflammatory changes whereas chronic disability is dependent upon axon loss (Wujek et al., 2002; Bjartmar et al., 2003).

The third major finding was that there was a decrease in the latency to AMPA-mediated sensory depolarization at 3 weeks post inoculation. It appears that the latency to depolarize and the magnitude of depolarization are independent measures since the magnitude of motor but not sensory depolarization was decreased in EAE.

It may be possible to interpret the magnitude of depolarization as a reflection of the overall integrity of the conduction pathway and the latency to depolarize as the qualitative sum of the physiological attributes of conduction. Demyelinated axons have been reported to exhibit changes in both the distribution and the expression pattern of both sodium and potassium ion channels (England et al., 1991, 1996; Rasband et al., 1998; Waxman, 2000a). These changes could impact conduction parameters (Waxman, 2000, 2001a,b). The fact that alterations in EAE spinal cord depolarization latencies were detected by AMPA alone may be a function of KCl and veratridine’s lack of selectivity for a specific transmitter pathway.

The overall aim of this study was to determine whether the neurological deficits observed in PLP-inoculated SJL mice would be paralleled by deficits in the motor and sensory transmission indices used in this study. In fact, both sensory and motor spinal cord transmission deficits were detected in EAE mice whether nonselective depolarizing agents such as potassium chloride and veratridine or a receptor selective agent such as AMPA was used. Although our data supports the hypothesized involvement of the glutamate system in the pathology of demyelinating diseases such as MS/EAE, the fact that EAE also affected robust changes in the response to the sodium channel opener veratridine indicates that other system pathologies are involved. Based on both our preliminary qualitative analysis and the literature findings, it appears that this methodology detects quantifiable changes in spinal cord transmission pathways during both the inflammatory period at the acute attack and the period of demyelination/axonal pathology occurring during chronic relapse/remission. Further studies will be needed to plot a quantitative time course for PLP-induced inflammation and axonal pathology and assess its impact on the expression of the neurological deficit and spinal cord transmission.

References