Conduction Abnormalities are Restricted to the Central Nervous System in Experimental Autoimmune Encephalomyelitis Induced by Inoculation with Proteolipid Protein but not with Myelin Basic Protein

Jonathan B. Chalk, Pamela A. McCombe and Michael P. Pender

Abstract

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) and can be induced by inoculation of animals with homogenized CNS tissue or highly purified myelin proteins such as myelin basic protein (MBP) or proteolipid protein (PLP). It is widely studied as a possible animal model of multiple sclerosis. We performed the present neurophysiological study to define the location of nerve conduction abnormalities in EAE induced by immunization with PLP (PLP-EAE) and in EAE induced by immunization with MBP (MBP-EAE) in the Lewis rat. In rats with tail weakness due to acute PLP-EAE, conduction was normal in the spinal nerve roots and peripheral nerves but there was evidence of conduction block in a high proportion of the fibres in the dorsal columns of the lumbosacral spinal cord. In contrast, in acute MBP-EAE, there was conduction block in a high proportion of fibres in the sacral dorsal and ventral roots of the peripheral nervous system (PNS) and in the dorsal columns of the lumbosacral spinal cord. The distribution of nerve conduction abnormalities is consistent with previous histological studies showing that inflammation and primary demyelination are restricted to the CNS in PLP-EAE, but are present in the CNS and in the spinal roots of the PNS in MBP-EAE. The restriction of functional abnormalities to the CNS in PLP-EAE but not in MBP-EAE may have implications for the human inflammatory demyelinating diseases, including multiple sclerosis.

Introduction

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) induced by the inoculation of susceptible animals, such as Lewis rats, with homogenized CNS tissue or highly purified myelin proteins, namely myelin basic protein (MBP) or proteolipid protein (PLP), together with complete Freund's adjuvant (CFA). It can also be induced by the passive transfer of MBP-specific or PLP-specific T cells. Experimental autoimmune encephalomyelitis is widely studied as a possible animal model of multiple sclerosis.

Neurophysiological techniques can be used to assess function in entire CNS and peripheral nervous system (PNS) pathways. In locating the site of functional abnormalities in EAE, such studies are complementary to histological examination, which generally assesses only small samples of functional pathways. In electrophysiological studies of whole spinal cord-induced acute EAE in the rabbit and Lewis rat, conduction abnormalities consistent with acute demyelination are present in the proximal PNS as well as in the CNS (Pender and Sears, 1982, 1984, 1985, 1986; Pender, 1986*a, b,* 1988*a,* 1989). Similar findings are present in MBP-induced acute EAE (MBP-EAE) (Pender, 1986*b,* 1988*b,* 1989), passively transferred MBP-EAE (Heininger *et al.,* 1989) and in whole spinal cord-induced chronic relapsing EAE in the Lewis rat (Stanley and Pender, 1991).

We performed the present study to assess the location of nerve conduction abnormalities in acute PLP-EAE and to compare the findings with those in acute MBP-EAE. Because tail weakness is the

main neurological sign of PLP-EAE in the Lewis rat and is also invariably present in MBP-EAE, the electrophysiological studies were carried out on the sacral segments of the CNS and PNS. We found that conduction abnormalities were confined to the CNS in PLP-EAE, but were present in both the CNS and proximal PNS in MBP-EAE.

Material and methods

Animals
Specific pathogen-free male Lewis rats (JC strain) were obtained from the University of Queensland Pinjarra Hills breeding colony and were kept five to a cage with an unrestricted supply of rat cubes and water. The experiments, performed in accordance with National Health and Medical Research Council of Australia guidelines for animal experimentation, were approved by the local ethics committee.

Preparation of proteins

Proteolipid protein
Proteolipid protein was prepared as previously described (Chalk *et al.,* 1994) using the methods of Folch *et al.* (1957) and Bizzozero *et al.* (1982). Briefly, 30 g of fresh bovine spinal cord, homogenized in a Waring blender with 570 ml of chloroform/methanol (2:1 vol/vol), was filtered and washed three times with water/methanol (1:1 vol/vol). Proteolipid protein in the concentrated (X 10), washed bovine spinal cord chloroform:methanol extract was precipitated by the addition of cold (4°C) diethyl ether. The PLP precipitate was collected by centrifugation, the solutes were discarded, dry nitrogen gas was used to remove remaining traces of diethyl ether and the partially dried PLP-containing precipitate was redissolved in chloroform/methanol/acetic acid (200:100:1 vol/vol/vol). The remaining lipids were separated from the PLP apoprotein by gel permeation chromatography using chloroform/methanol/acetic acid (200:100:1 vol/vol/vol) as the solvent and a column of LH-60 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) measuring 40 cm X25 mm with a flow rate of 20 ml/h. The absorbance of the eluate was monitored at 278 nm. The second protein peak from the diethyl ether-extracted PLP-containing sample, separated from brain lipids on the column of LH-60, was collected and repoured (without further concentration) onto the column of LH-60. The subsequently collected PLP apoprotein was converted to a water-soluble form according to the method of Sherman and Folch-Pi (1970) by the slow dropwise addition of water while at the same time evaporating the chloroform and methanol with dry gaseous nitrogen. The resultant clear solution was then dialysed with dialysis tubing of molecular weight cut-off 5 kD (Union Carbide Corporation, Chicago, Illinois, USA). The protein concentration was assayed with a Pierce protein assay kit (Pierce, Rockford, Illinois, USA).

The purity of the PLP preparation and the possibility of MBP contamination were assessed with 12.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE) (Laemmli, 1970). Electroblotting of PLP was performed using the method of Macklin *et al.* (1982). The primary antibody was a rabbit anti-bovine-MBP antibody (Advanced Immunochemical Services Incorporated, Longbeach, California, USA, Cat. No. 021222B). The secondary antibody was a peroxidase-conjugated swine anti-rabbit antibody (Dako A/G, Glostrup, Denmark, Cat. No. P217).

Myelin basic protein
Myelin basic protein was prepared from guinea-pig spinal cord (after removal of the spinal roots) by the method of Deibler *et al.* (1972). Its purity was assessed by SDS-PAGE (Laemmli, 1970).

Induction of EAE

Proteolipid protein-experimental autoimmune encephalomyelitis
The PLP inoculum was prepared by mixing CFA (H37Ra Difco Laboratories, Detroit, Michigan,

USA) containing additional killed and dried *Mycobacterium tuberculosis* (H37RA Difco) (8 mg/ml of CFA) with an equal volume of the PLP-containing solution. Under anaesthesia, rats 9-12 weeks old were inoculated intradermally in the medial footpad of the right hind foot with 1000 µg of PLP in a total volume of 130 µl.

Myelin basic protein-experimental autoimmune encephalomyelitis
Myelin basic protein in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant (Difco) (IFA) containing a suspension of 4 mg of killed and dried *M. butyricum* (Difco) per millilitre of IFA. Rats were inoculated with 100 µl of the MBP-containing emulsion into the medial footpad of each hind foot. The total dose of MBP was 50 µg per rat.

Clinical assessment
Rats were assessed for tail, hindlimb and forelimb weakness graded in each region on a scale of 0 (no weakness) to 4 (complete paralysis) from day 7 as previously described (Pender, 1986b). The total clinical score was obtained by adding these three scores (maximum score = 12).

Electrophysiological studies

Preparation of animals
Anaesthesia was induced by the intraperitoneal (i.p.) injection of ketamine (74 mg/kg) (Mavlab Pty. Ltd., Slacks Creek, Australia), xylazine (9 mg/kg) (Parnell Laboratories, Silverwater, Australia) and atropine (36 µg/kg) (Apex Laboratories, St Marys, Australia) and maintained with further i.p. injections of half of the initial dose when a withdrawal response to pain, elicited in the left hindlimb by pinching the hindlimb digits, was present. An adequate depth of anaesthesia was maintained without depressing the corneal reflex. The rats breathed spontaneously through a tracheal cannula (Intramedic polyethylene tubing No. 7445, Becton Dickinson, New Jersey, USA). The tail was wrapped in cotton wool to minimize heat loss from this area. To maintain the temperature of the animal at 37°C, a metal box, containing circulating water at 42°C (Thermomix 1441, B. Braun Melsungen AG, Germany), was placed under the animal during the laminectomy and during the electrophysiological studies. An i.p. injection of 8 ml of compound sodium lactate intravenous infusion BP (Hartmann's solution) (Baxter Healthcare Pty Ltd, Old Toongabbie, Australia) was given at the beginning of each experiment, and 1 ml of Haemaccel (Behringwerke AG, Marburg, Germany) was given i.p. after the laminectomy had been performed, prior to neurophysiological recordings.

Dorsal root entry zone (DREZ) recordings
A T12-L5 laminectomy was performed, blood loss being minimized with local pressure and gelatin sponge (Gelfoam®) (The Upjohn Company, Kalamazoo, Michigan, USA). The rat was mounted on a frame and a pool was formed with raised skin flaps and the dura opened. After rinsing of the tissues with warmed (37°C) compound sodium lactate solution, warmed (37°C) paraffin oil was added to the pool to prevent tissue drying. A thermostatically controlled infrared lamp was used to maintain the temperature of the paraffin pool at 37°C. The rectal temperature was usually 36-37°C under these conditions. A segment of the left ventral caudal trunk, just distal to its junction with the S2 ventral primary ramus, was exposed and stimulated in continuity with two 0.25 mm diameter platinum wire electrodes 3 mm apart. The cathode was proximal to the anode. Stimuli were 0.1 ms square-wave voltage pulses (Isolated stimulator DS2, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK) delivered at 0.33 Hz with a programmable triggering device (Digitimer 4030, Digitimer Ltd). The threshold for tail movement was usually 0.3-0.4 V. During the course of the experiment the threshold for tail twitch often rose, probably from ischaemia of the ventral caudal trunk. When the threshold for tail twitch increased beyond 0.6 V, or if the corneal reflex became absent, the experiment was ceased.

Volume conductor recordings were made over the left S2 DREZ with a 0.5 mm diameter silver ball electrode as the active electrode (Fig. 1). The reference electrode was a platinum wire placed on the right paravertebral tissue at the same level as the active electrode. A ground electrode was placed, via

a steel supporting pin, on the right ilium. The recording electrodes were shielded leads connected to field effect transistor source-followers, and thence to a preamplifier bandwidth 5.3-10 kHz) and thence to an oscilloscope (Tektronix 5D10 Waveform Digitizer, Tektronix Inc., Beaverton, Oregon, USA) . For all recordings, negativity at the active recording electrode resulted in an upward deflection on the oscilloscope. Recordings were photographed with an oscilloscope camera. Conduction velocities were calculated after (allowing for the utilization time (0.1 ms) (Blair and Erlanger, 1936). Conduction distance was measured as the length of a piece of thread placed along the conduction pathway from the stimulation site on the ventral caudal trunk to the recording site on the S2 DREZ.
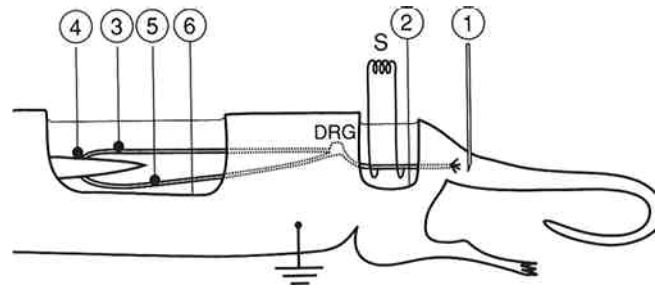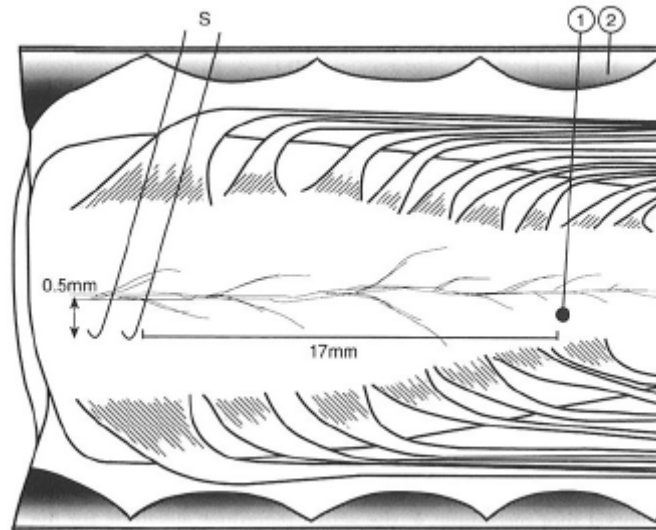

Fig. 1 Schematic diagram of the stimulating and recording arrangements for the S2 dorsal root, dorsal root entry zone and ventral root recordings and for the compound muscle action potential recording. DRG = dorsal root ganglion. S = stimulus. 1: the active recording electrode for the compound muscle action potential; 2: the reference recording electrode for the compound muscle action potential; 3: the active recording electrode for the S2 dorsal root recording; 4: the active recording electrode for the S2 dorsal root entry zone recording; 5: the active recording electrode for the S2 ventral root recording; 6: the reference electrode for the S2 dorsal root, dorsal root entry zone and ventral root recordings was placed on the right paravertebral tissue.

S2 dorsal root recordings
The left S2 dorsal root was identified and gently lifted with a Cawthorne's stapedectomy hook No. 3 (Downs Surgical Ltd, Mitcham, Surrey, UK) so that a small piece of plastic sheeting measuring 2.5 X6 mm could be placed underneath the root (Fig. 1). The 0.5 mm diameter silver ball electrode was placed on the segment of the S2 dorsal root overlying the plastic sheet (6-10 mm distal to the S2 DREZ), and the reference electrode was placed on the right paravertebral tissue at the same level as the active recording electrode. The ventral caudal trunk was stimulated as for the DREZ recordings.

Dorsal column recordings
The active electrode was a 0.5 mm diameter silver ball electrode placed on the left dorsal column at the level of the S4 DREZ (Fig. 2). The reference electrode was a 0.2 mm diameter platinum wire electrode placed on the right paravertebral tissue at the same level as the active recording electrode. Two J-shaped 0.25 mm diameter stimulating platinum wire electrodes were placed 3 mm apart on the left dorsal column, with the cathode caudal, 0.5 mm from the midline and 17 mm rostral to the active recording electrode (Fig. 2). Stimulation was by 0.33 Hz square-wave voltage pulses of 20 μs duration. Preliminary studies revealed that cutting all dorsal roots caudal to, and including, the L4 dorsal root did not alter the duration or amplitude of the dorsal column compound action potential. Sectioning of the left dorsal column 4 mm caudal to the stimulating cathode resulted in an -50% reduction in the amplitude of the potential and further sectioning of the right dorsal column abolished the potential.

Fig. 2 Schematic diagram of the stimulating and recording arrangements for the dorsal column recordings. S = stimulus; 1: the active recording electrode; 2: the reference recording electrode which was placed on the right paravertebral tissue.

S2 ventral root recordings
The left ventral caudal trunk was stimulated as described above for the S2 DREZ recordings. The L5, L6, sacral and coccygeal dorsal roots were cut and folded back and the caudal spinal cord was rotated so that the left sacral and coccygeal ventral roots could be identified. The left and right S3, S4 and coccygeal ventral roots were cut and folded back. The left S2 ventral root was lifted gently with the Cawthorne's stapedectomy hook No. 3 and a small piece of plastic sheeting was placed beneath its proximal portion as described above for the left S2 dorsal root. A 0.5 mm diameter silver ball electrode was placed on the segment of the left S2 ventral root lying on the plastic sheeting (12-16 mm distal to the S2 ventral root exit zone) (Fig. 1). The reference electrode was placed on the right paravertebral tissue at the same level as the active recording electrode. Stimuli were 0.1 ms square-wave voltage pulses delivered at 0.33 Hz.

M wave recordings
The ventral caudal trunk was stimulated as described above for the S2 DREZ recordings, except that the polarity of the electrodes was reversed so that the cathode was closer than the anode to the recording site. Recordings were made of the compound muscle action potential from the left ventral muscles of the proximal tail, 1 cm distal to the base of the tail (Fig. 1). The active electrode was a 25 gauge needle inserted into the tail muscle, and the reference electrode was a similar needle inserted into the sacral vertebral column. Stimuli were 0.1 ms square-wave voltage pulses delivered at 0.33 Hz.

Histological studies
At the completion of the neurophysiological studies four rats (three with PLP-EAE and one with MBP-EAE) were perfused through the left ventricle of the heart with isotonic saline followed by modified Karnovsky's fixative. Specimens were taken from the lumbosacral spinal cord, and the tissue was processed for histological examination as described below.

Light microscopy
The tissue was processed according to the method of Nguyen and Pender (1989). The tissue samples were postfixed in osmium tetroxide (Dalton's solution), dehydrated in ascending alcohols and embedded in glycol methacrylate (Technovit 7100, Kulzer GmbH, Wehrheim, Germany). Sections (1.0 μm) were cut with a Ralph glass knife on a LKB retracting microtome and stained with cresyl fast violet.

Electron microscopy
Tissue samples were postfixed with osmium tetroxide, stained *en bloc* with uranyl acetate, dehydrated in a series of graded ethanol solutions followed by absolute acetone and embedded in epoxy resin. Ultrathin sections were stained with lead citrate and examined with a Jeol JEM-1200 EXII electron microscope.

Statistical analysis
To compare the recordings from normal rats with those from rats with PLP-EAE and MBP-EAE, one-way analysis of variance (ANOVA) was used when the data were normally distributed. Where a significant F result was obtained, further *post hoc* comparisons were performed between groups using a two-tailed *t* test. To correct the *P* value for multiple comparisons, the Bonferroni method was used. The statistical analysis program used was GraphPAD InStat (GraphPAD Software, San Diego California, USA).

Results

Normal controls
Electrophysiological studies were performed on 23 normal Lewis rats aged 9-13 weeks.

Table 1 Maximal compound muscle action potential evoked in the proximal ventral tail muscles by stimulation of the ventral caudal trunk

	Normal Controls		PLP-EAE		MBP-EAE		*F* value
	n Range	Mean ± SD	*n* Range	Mean ± SD	*n* Range	Mean ± SD	(ANOVA)
Peak-to-peak Amplitude (mV)	11 3.16-10.16	5.74±2.12	8 4.40-8.88	5.78±1.50 p_c = NS	10 3.48-7.36	5.21±1.25 p_c = NS	0.34
Latency to peak of positivity (ms)	11 2.25-3.34	2.65±0.37	8 2.38-3.32	2.72±0.34 p_c = NS	10 2.08-3.08	2.60±0.28 p_c = NS	0.29
Latency to peak of negativity (ms)	11 3.50-5.02	4.35±0.55	8 3.44-5.50	4.32±0.70 p_c = NS	10 2.86–5.70	4.50±0.76 p_c = NS	0.20

ANOVA = one-way analysis of variance; p_c = P value calculated in comparison with normal controls using ANOVA with Bonferroni correction for multiple comparisons; NS = not significant (P > 0.05).

M wave recordings
The compound muscle action potential in the proximal ventral tail muscles evoked by stimulation of the left ventral caudal trunk was examined in 11 normal rats (Table 1). The normal response consists of a biphasic wave (positive, negative).

Dorsal root recordings
Conduction from the left ventral caudal trunk to the proximal S2 dorsal root was studied in 10 normal rats (Fig. 3A and Table 2). The normal dorsal root response consists of a biphasic wave (positive, negative). The initial positivity is due to passive outward current driven by the approaching impulses, and the negativity is due to active inward current occurring during the rising phase of the action potential under the active recording electrode.

Dorsal root entry zone recordings
To assess conduction through the PNS afferent pathway, volume conductor recordings were made of the S2 DREZ response evoked by stimulation of the left ventral caudal trunk in 19 normal rats. The normal DREZ response consists of the biphasic (positive, negative) afferent volley potential followed by the N wave, a field potential due to synaptic currents in second order neurons in the dorsal horn excited mainly by low-threshold cutaneous afferents (Fig. 4A and Table 3).

Ventral root recordings
Conduction from the left ventral caudal trunk to the proximal S2 ventral root was assessed in six normal rats (Fig. 5A and Table 4).

Dorsal column recordings
Conduction in the lumbosacral dorsal columns was assessed in 10 normal rats (Fig. 6A and Table 5). The normal response consisted of a biphasic wave (positive, negative). Repetitive supramaximal dorsal column stimulation (10 Hz for 60 s) resulted in a mean amplitude reduction of $3.8 \pm 2.5\%$ *(n = 5)*.

Fig. 3 Volume conductor recordings of the maximal S2 dorsal root compound action potential evoked by stimulation of the ventral caudal trunk in a normal rat (A), in a rat with PLP-EAE **(B)** and in a rat with MBP-EAE (C). The onset of the stimulus is indicated by a vertical line.

Proteolipid protein-experimental autoimmune encephalomyelitis

Clinical findings
Neurophysiological studies were performed on 11 rats with PLP-EAE, 13-20 days after inoculation and 2-4 days after the onset of neurological signs. At the time of the neurophysiological studies, the rats had severe tail weakness or tail paralysis. Two rats also had hindlimb weakness. The mean total clinical score of rats with PLP-EAE at the time of neurophysiological study was 4.2 ± 1.2.

Table 2 Maximal S2 dorsal root compound action potential evoked by stimulation of the ventral caudal trunk

	Normal Controls		PLP-EAE		MBP-EAE		*F* value
	n Range	Mean ± SD	*n* Range	Mean ± SD	*n* Range	Mean ± SD	(ANOVA)
Peak-to-peak Amplitude (μV)	10 816-2060	1436±385	8 760-1680	1225±298 $p_p < 0.01$	8 220-1040	551±315 $p_c < 0.001$ $p_p < 0.01$	15.90
Conduction velocity of peak of negativity (m/s)	6* 46.6-48.5	47.5±0.7	8 46.7-49.4	48.2±1.0 p_c = NS	7* 37.4-48.9	41.5±3.9 $p_c < 0.05$ $p_p < 0.01$	17.27

P values calculated by using ANOVA with Bonferroni correction for multiple comparisons;
p_c = compared with normal controls; p_p = compared with rats with PLP-EAE; *conduction distance measured in only six normal controls and seven rats with MBP-EAE.

M wave recordings
The compound muscle action potential in proximal ventral tail muscles evoked by stimulation of the ventral caudal trunk was normal in rats with PLP-EAE (Table 1).
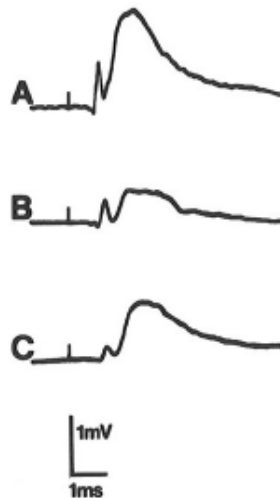
Dorsal root recordings
Conduction from the left ventral caudal trunk to the proximal S2 dorsal root was studied in eight animals with PLP-EAE. The mean peak-to-peak amplitude and the mean velocity of the peak of the negativity of the maximal dorsal root compound action potential were not significantly different from the corresponding values in normal controls (Fig. 3 and Table 2).
Dorsal root entry zone recordings
In rats with PLP-EAE, the mean peak-to-peak amplitude of the maximal afferent volley potential and the mean amplitude of the N wave peak were significantly reduced compared with the corresponding values in normal controls (Fig. 4 and Table 3). However, the mean conduction velocity of the peak of the negativity of the maximal afferent volley potential and the mean latency of the N wave were not significantly different from those in normal controls. In view of the normal amplitude of the S2 dorsal root compound action potential recorded 6-10 mm away, the reduction in the peak-to-peak amplitude of the S2 DREZ afferent volley potential without temporal dispersion and the reduction in the N wave amplitude indicate conduction block immediately distal to the recording site over the DREZ.

Ventral root recordings
Conduction from the left ventral caudal trunk to the proximal S2 ventral root was assessed in seven rats with PLP-EAE. The mean peak-to-peak amplitude and the mean velocity of the peak of the negativity of the maximal ventral root response were not significantly different from the corresponding values in normal controls (Fig. 5 and Table 4).

Fig. 4 Volume conductor recordings of the maximal S2 dorsal root entry zone afferent volley potential and N wave evoked by stimulation of the ventral caudal trunk in a normal control rat (**A**), in a rat with PLP-EAE (**B**) and in a rat with MBP-EAE (**C**).

Dorsal column recordings
Conduction in the lumbosacral dorsal columns was assessed in 11 rats with PLP-EAE. There was a marked reduction in the mean peak-to-peak amplitude of the maximal compound action potential compared with normal controls (Fig. 6 and Table 5). As there was no temporal dispersion, this reduction indicates either a failure of excitation at the stimulation site or conduction block in a high proportion of the dorsal column fibres. The mean latency to the peak of the negativity in rats with PLP-EAE was not significantly different from that in normal controls. As demyelinated fibres have an impaired ability to transmit trains of impulses (McDonald and Sears, 1970), the effect of repetitive supramaximal dorsal column stimulation

(10 Hz for 60 s) was assessed in four rats with PLP-EAE. Such stimulation resulted in a mean amplitude reduction of $11\pm 17\%$, which was not significantly different from that in normal controls. However, in two of these rats the reductions were 25 and 26%, respectively (>3 SDs beyond the normal mean) indicating rate-dependent conduction block rather than a failure of excitation.

Table 3 *Maximal S2 dorsal root entry zone response evoked by stimulation of ventral caudal trunk*

	Normal Controls		PLP-EAE		MBP-EAE		*F* value
	n Range	Mean ± SD	*n* Range	Mean ± SD	*n* Range	Mean ± SD	(ANOVA)
Peak-to-peak amplitude of afferent volley potential (µV)	19 600-1040	788±102	9 440-900	620±141 $p_c <0.01$	9 240-580	397±138 $p_c <0.001$ $p_p <0.01$	32.25
Conduction velocity of peak of negativity of afferent volley potential (m/s)	9* 49.1-54.0	51.0±2.2	8* 42.2-55.1	50.0±4.5 $p_c =$ NS	7* 42.2-49.1	44.0±3.4 $p_c < 0.01$ $p_p < 0.05$	9.06
Amplitude of N wave peak (mV)	18 1.08-2.90	1.84±0.52	8 0.28-1.88	1.01±0.54 $p_c < 0.01$	9 0.54-1.46	1.06±0.33 $p_c < 0.01$ $p_p =$ NS	12.30
Latency to peak of N wave (ms)	18 1.79-2.44	2.04±0.17	8 1.85-2.27	2.08±0.15 $p_c =$ NS	9 2.03-2.48	2.24±0.16 $p_c <0.05$ $p_p =$ NS	4.58

P values calculated using ANOVA with Bonferroni correction for multiple comparisons; p_c = compared with normal controls;
p_p = compared with rats with PLP-EAE; *conduction distance measured in only nine normal controls, eight rats with PLP-EAE and seven rats with MBP-EAE.

Histological studies of the lumbosacral spinal cord in PLP-EAE
Three rats with PLP-EAE were perfused with fixative after completion of the neurophysiological studies. Light microscopic histological examination of the lumbosacral dorsal columns in the region showing conduction abnormalities revealed meningeal, perivascular and parenchymal mononuclear cell infiltrates and primary demyelination (loss of the myelin sheath with axonal preservation), as we have recently reported in a detailed histological study of PLP-EAE in the Lewis rat (Chalk *et al.,* 1994). The proximal S2 dorsal and ventral roots were also examined histologically in one rat after completion of the neurophysiological study and were found to be normal, as we found to be the case throughout the PNS in our detailed histological study (Chalk *et al.,* 1994).
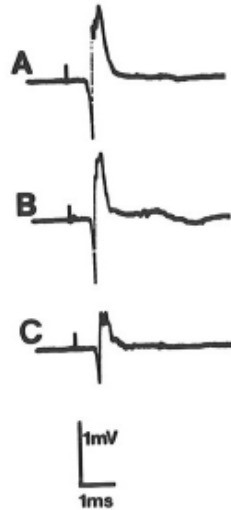
Fig. 5 Volume conductor recordings of the maximal S2 ventral root compound action potential evoked by stimulation of the ventral caudal trunk in a normal rat (**A**), in a rat with PLP-EAE (**B**) and in a rat with MBP-EAE (**C**).

Myelin basic protein-experimental autoimmune encephalomyelitis

Clinical findings

Neurophysiological studies were performed on 14 rats with MBP-EAE, 12-15 days after inoculation and 2-5 days after the onset of neurological signs. At the time of the neurophysiological studies 13 rats had tail paralysis and one had severe tail weakness. Eleven rats also had hindlimb weakness. The mean total clinical score of rats with MBP-EAE at the time of neurophysiological study was 5.9±1.6 which is greater than that in rats with PLP-EAE (P = 0.007).

M wave recordings

The compound muscle action potential in proximal ventral tail muscles evoked by stimulation of the ventral caudal trunk was normal in rats with MBP-EAE (Table 1).

Table 4 *Maximal S2 ventral root compound action potential evoked by stimulation of the ventral caudal trunk*

	Normal Controls		PLP-EAE		MBP-EAE		F value
	n Range	Mean ± SD	n Range	Mean ± SD	n Range	Mean ± SD	(ANOVA)
Peak-to-peak amplitude (μV)	6 1184-2300	1604±399	7 1060-2480	1604±582 p_c = NS	9 110-1130	534±351 p_c <0.01 p_p <0.01	15.16
Conduction velocity of peak of negativity (m/s)	6 46.0-51.6	47.6±2.1	7 44.2-51.4	48.1±2.3 p_c = NS	7* 20.4-48.9	39.3±9.3 p_c = NS p_p < 0.05	4.97

P values calculated using ANOVA with Bonferroni correction for multiple comparisons; p_c = compared with normal controls; p_p = compared with rats with PLP-EAE; *conduction distance measured in only seven rats.

Dorsal root recordings

Conduction from the left ventral caudal trunk to the proximal S2 dorsal root was studied in eight animals with MBP-EAE. There was a marked reduction in the mean peak-to-peak amplitude of the maximal dorsal root compound action potential compared with normal controls and rats with PLP-EAE (Fig. 3 and Table 2*).* The mean conduction velocity of the peak of the negativity in animals with MBP-EAE was also significantly less than that in normal controls and that in rats with PLP-EAE. As there was no temporal dispersion, the reduction in the amplitude of the dorsal root response in MBP-EAE indicates a failure of excitation at the stimulation site or conduction block in a high proportion of the afferent fibres between the ventral caudal trunk and the

proximal dorsal root. The reduction in the conduction velocity indicates conduction block or slowing in the fastest fibres.

Dorsal root entry zone recordings
Conduction from the ventral caudal trunk to the S2 DREZ was studied in nine animals with MBP-EAE. The mean peak-to-peak amplitude and the mean velocity of the peak of the negativity of the maximal afferent volley potential were significantly reduced compared with the corresponding values in normal controls and in rats with PLP-EAE (Fig. 4 and Table 3). The peak of the N wave was significantly decreased in amplitude and prolonged in latency in rats with MBP-EAE compared with normal controls but not compared with rats with PLP-EAE. These abnormalities in the DREZ recordings in MBP-EAE are mainly explained by the conduction abnormalities in the peripheral afferent pathway detected in the S2 dorsal root recordings *(see above),* but conduction abnormalities between the dorsal root and DREZ recording sites may also have contributed.

Ventral root recordings
Conduction from the left ventral caudal trunk to the proximal S2 ventral root was assessed in nine rats with MBP-EAE. The mean peak-to-peak amplitude of the maximal response was markedly reduced compared with that in normal controls and that in rats with PLP-EAE, indicating a failure of excitation at the stimulation site or conduction block in a high proportion of the efferent fibres between the ventral caudal trunk and the proximal ventral root (Fig. 5 and Table 4). The mean conduction velocity of the peak of the negativity was less than that in normal controls and that in rats with PLP-EAE, although the difference from normal controls was not statistically significant. However, in four rats with MBP-EAE the velocity was >3 SDs below the mean in normal controls, indicating conduction block or slowing in the fastest fibres.

Dorsal column recordings
Conduction in the lumbosacral dorsal columns was assessed in 11 rats with MBP-EAE. The mean peak-to-peak amplitude of the dorsal column compound action potential was markedly reduced compared with that in normal controls but was not significantly different from that in rats with PLP-EAE (Fig. 6 and Table 5). The latency to the peak of the negativity was increased in MBP-EAE compared with that in PLP-EAE and that in normal controls, although the latter difference was not statistically significant. As there was no temporal dispersion, the reduction in amplitude indicates a failure of excitation at the stimulation site or conduction block in a high proportion of the dorsal column fibres in MBP-EAE. The effects of repetitive supramaximal dorsal column stimulation (10 Hz for 60 s) were assessed in four rats with MBP-EAE. Such stimulation resulted in a mean amplitude increase of $0.7\pm6\%$, which was not significantly different from the effect in normal controls.
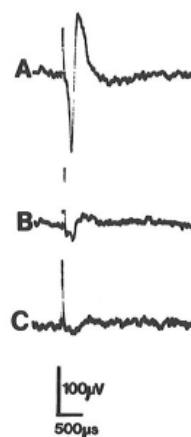

Fig. 6 Volume conductor recordings of the maximal sacral dorsal column compound action potential evoked by stimulation of the lumbar dorsal column in a normal rat (**A**), in a rat with PLP-EAE (**B**) and in a rat with MBP-EAE (**C**).

Table 5 *Maximal lumbosacral dorsal column compound action potential*

	Normal Controls		PLP-EAE		MBP-EAE		F value
	n Range	Mean ± SD	n Range	Mean ± SD	n Range	Mean ± SD	(ANOVA)
Peak-to-peak amplitude (µV)	10 154-316	228±55	11 0-308	92±91 $p_c < 0.01$	11 0-240	71±71 $p_c <0.001$ p_p = NS	13.55
Latency to peak of negativity (µs)	10 390-440	411±21	9* 380-445	403±22 p_c = NS	7[†] 390-480	441±35 p_c = NS $p_p < 0.05$	4.65

P values calculated using ANOVA with Bonferroni correction for multiple comparisons; p_c = compared with normal controls; p_p = compared with rats with PLP-EAE; *response absent in two rats; [†]response absent in four rats.
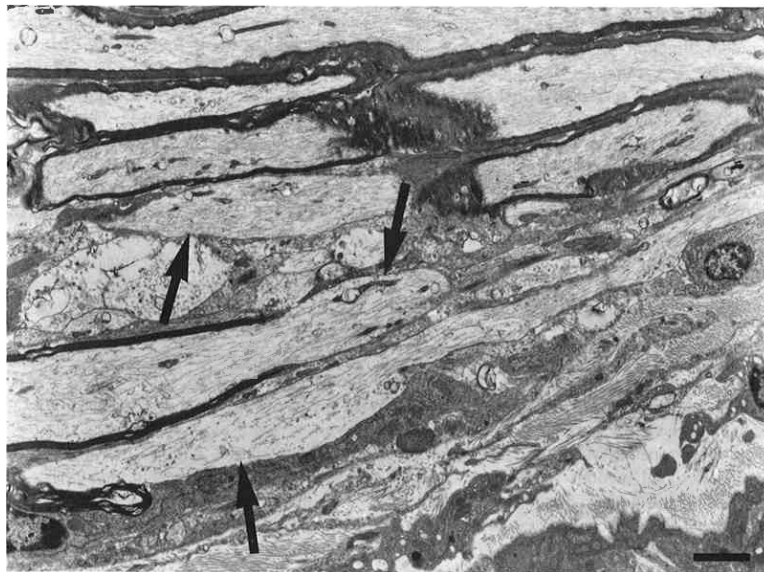
Fig. 7 Electron micrograph of a longitudinal section through the dorsal column of the S2-3 spinal cord of a rat with MBP-EAE that had been studied neurophysiologically and found to have evidence of dorsal column conduction block. Demyelinated axons (arrows) can be seen. Bar = 2 µm.

Histological studies of the lumbosacral spinal cord in MBP-EAE
Light microscopic histological examination of the lumbosacral spinal cord from one rat with MBP-EAE that was perfused with fixative at the completion of the neurophysiological studies revealed meningeal, subpial and perivascular infiltrates of mononuclear cells and some primary demyelination in the dorsal columns in the region showing conduction abnormalities. There was little demyelination in transverse sections but more prominent demyelination in longitudinal sections. Electron microscopy confirmed the presence of primary demyelination (Fig. 7).

Discussion
In this study we found that conduction abnormalities were restricted to the CNS in acute PLP-EAE, but were present in the CNS and proximal PNS in acute MBP-EAE. In the CNS there was a marked reduction in the peak-to-peak amplitude, without temporal dispersion, of the dorsal column compound action potential in PLP-EAE and MBP-EAE. This could be due to a failure of excitation at the stimulation site caused by axonal degeneration or due to conduction block caused by demyelination. Further reduction of the amplitude by repetitive supramaximal stimulation in two rats with PLP-EAE indicated that demyelination-induced conduction block rather than axonal degeneration was responsible for the conduction abnormalities. Moreover, the finding of conduction slowing in the dorsal columns at 1-2 weeks and restoration of conduction at 4 weeks after clinical recovery from MBP-EAE (J. B. Chalk, P. A. McCombe and M. P. Pender, unpublished data) also indicates that

demyelination is responsible for the conduction abnormalities. This conclusion is supported by the fact that primary demyelination is the main neuropathological finding in the spinal cord in these forms of EAE, whereas axonal degeneration is an infrequent finding (Pender, 1988*b*, 1989; Chalk *et al.,* 1994). In transverse sections of the spinal cord, primary demyelination can be readily identified in PLP-EAE but is mainly limited to the CNS parts of the ventral root exit and dorsal root entry zones in MBP-EAE. However, in the present study, longitudinal sections revealed significant primary demyelination in the dorsal columns of the spinal cord in a rat with dorsal column conduction abnormalities due to MBP-EAE (Fig. 7). In addition to demyelination, structurally minor yet functionally significant changes in the myelin sheath, such as disruption of the paranodal axoglial junctions (Hirano and Dembitzer 1978), may also contribute to the CNS conduction block in MBP-EAE.

In PLP-EAE, in contrast to the severe conduction abnormalities in the CNS, there was normal conduction through the PNS, namely the sacral dorsal and ventral roots and the peripheral nerve. The abnormalities in the DREZ recordings in PLP-EAE can be accounted for by conduction block in the oligodendrocyte-myelinated portion of the DREZ that extends into the proximal dorsal root (the central tissue projection). The neurological sign of tail weakness in rats with PLP-EAE is most likely due to demyelination-induced conduction block at the oligodendrocyte-myelinated region of the ventral root exit zone. This region is a site of predilection for primary demyelination in PLP-EAE (Chalk *et al.,* 1994) and is also a major site of demyelination and conduction block causing weakness in whole spinal cord-induced EAE (Pender, 1986*a*, 1988*a*). Our finding that nerve conduction abnormalities are restricted to the CNS in PLPEAE is consistent with the results of histological studies showing restriction of inflammation and demyelination to the CNS in PLP-EAE (Yamamura *et al.,* 1986; Chalk *et al.,* 1994). In other species, such as the rabbit (Williams *et al.,* 1982; Cambi *et al.,* 1983), guinea-pig (Yoshimura *et* al., 1985) and mouse (Satoh *et al.,* 1987; Trotter *et al.,* 1987; Tuohy *et al.,* 1988; van der Veen *et al.,* 1989) limited histological studies of the PNS have been normal in PLP-EAE. The electrophysiological and histological findings are also consistent with biochemical studies showing restriction of PLP to the CNS (Lees and Macklin, 1988).

In MBP-EAE, in contrast to PLP-EAE, there were major conduction abnormalities in the proximal PNS. The involvement of the PNS in MBP-EAE but not in PLP-EAE cannot be explained by the generally more severe clinical involvement in MBP-EAE, as the PNS was spared in rats with severe clinical disease (tail paralysis and hindlimb weakness) due to PLP-EAE but not in rats with milder clinical disease (tail paralysis without hindlimb weakness) due to MBP-EAE. The reduced amplitude, without temporal dispersion, of the sacral ventral root compound action potential in the presence of a normal compound muscle action potential elicited by stimulation of the ventral caudal trunk at the same site indicates that conduction block is present in the sacral ventral roots in MBP-EAE. It is highly likely that the reduced amplitude of the sacral dorsal root response is also due to conduction block. Conduction block in the dorsal and ventral routs in MBP-EAE is readily explained by the primary demyelination in these roots (Pender, 1986*b*, 1987, 1988*b,c*; Chalk *et al.,* 1994). This conclusion is supported by the complete restoration of conduction in the sacral ventral and dorsal roots at the time of PNS remyelination and clinical recovery (J. B. Chalk, P. A. McCombe and M. P. Pender, unpublished data). Conduction in the peripheral nerve itself (from the ventral caudal trunk to the tail muscles) was normal in MBP-EAE, as we have previously shown in the sciatic nerve (Pender, 1988*b*). In contrast to PLP-EAE, the neurological signs of MBP-EAE can be explained mainly by demyelination-induced conduction block in the spinal roots in the PNS, as we have previously reported (Pender, 1988*b,c*; Pender *et al.,* 1989). The involvement of the PNS in MBP-EAE is explained by the presence of MBP in the PNS (Brostoff and Eylar, 1972; Greenfield *et al.,* 1973).

Our findings of restriction of functional abnormalities to the CNS in PLP-EAE, but not in MBP-EAE, may have implications for the human inflammatory demyelinating diseases, including multiple sclerosis. Abnormal auto-reactivity to PLP and MBP has been incriminated in multiple sclerosis. Multiple sclerosis is classically regarded as a disease restricted to the CNS, although the PNS may be involved in at least some cases (Waxman, 1993). Restriction of autoimmune demyelination to the CNS in multiple sclerosis would suggest that PLP, or another antigen confined to the CNS such as myelin/oligodendrocyte glycoprotein (Gardinier *et al.,* 1992), is the target antigen. Involvement of the

PNS as well as the CNS in multiple sclerosis might occur when the immune attack is directed at MBP or other antigens, such as galactocerebroside, which are present in both CNS and PNS myelin. Interestingly, PNS disease and antibodies to MBP occur in some individuals immunized with rabies vaccine containing CNS tissue (Hemachudha *et al.*, 1987, 1988).

In conclusion, we have shown that nerve conduction abnormalities are restricted to the CNS in PLP-EAE but not in MBP-EAE in the Lewis rat. These findings may have implications for multiple sclerosis and other human inflammatory demyelinating diseases.

Acknowledgements

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