

Involvement of the Dorsal Root Ganglion in Acute Experimental Allergic Encephalomyelitis in the Lewis Rat

A Histological and Electrophysiological Study

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SUMMARY

Histological and electrophysiological studies were performed in Lewis rats with acute experimental allergic encephalomyelitis (EAE) in order to determine the extent of dorsal root ganglion (DRG) involvement. Histological studies showed inflammation and demyelination in both the central nervous system (CNS) and peripheral nervous system (PNS). The DRG was the most affected region of the PNS and its involvement increased caudally. Nerve conduction abnormalities were demonstrated in the regions of the lumbar, sacral or coccygeal DRGs in some of the rats with EAE. However, the overall DRG involvement was much less severe, both histologically and functionally, than what we recently found in rabbits with EAE. Conduction through the lumbar dorsal root entry zone was normal. We conclude that lesions of the afferent pathway to the spinal cord do not contribute significantly to the disturbances of hindlimb motor function in Lewis rats with EAE.

Key words: *Conduction abnormalities, Demyelination, Dorsal root ganglion, Experimental allergic encephalomyelitis, Lewis rat, Nerve conduction, Pathophysiology*

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an auto-immune disease of the nervous system induced by inoculation with central nervous system (CNS) tissue, or CNS myelin basic protein, and adjuvants and is widely studied as a possible model of multiple sclerosis, a human CNS demyelinating disease of unknown aetiology (Raine 1984). It has been induced in many species, but most studies have been done on the small rodents - rats, mice and guinea-pigs. In these animals the cause of the neurological signs (limb ataxia, weakness and paralysis and tail paralysis) is unknown (Simmons et al. 1982). However, we recently demonstrated that, in rabbits with EAE, demyelination-induced conduction block in the peripheral nervous system (PNS), specifically the dorsal root ganglion (DRG), has a major role in the production of neurological signs (Pender and Sears 1982, 1984). The vulnerability of the DRG was attributed to the known deficiency in the blood-nerve barrier of the rabbit DRG (Waksman 1961) and possibly also to a particular susceptibility of the peripheral or central process of the DRG neurone to demyelination-induced conduction block in the vicinity of the branch point. As the DRGs of other mammals, including rats, mice and guinea-pigs, also have a deficient blood-nerve barrier

(Olsson 1971; Jacobs et al. 1976), the DRGs of these animals may also be vulnerable to EAE. The present paper describes the results of histological and electrophysiological studies designed to determine the extent of DRG involvement in Lewis rats with acute EAE.

MATERIALS AND METHODS

Animals

Lewis rats bred by the Animal Breeding Establishment of the John Curtin School of Medical Research (JC strain) were used. The rats were kept in cages of five and were fed rat and mouse cubes and water *ad libitum*.

Preparation of inoculum

The inoculum was a homogenate of equal volumes of a 30% suspension of guinea-pig spinal cord (the spinal roots having been stripped away) in 0.9% saline and a suspension of 4mg of killed and dried *Mycobacterium butyricum* (Difco)/ml of incomplete Freund's adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia).

Inoculation procedure and management of inoculated animals

Under ether anaesthesia 8-10-week-old rats were inoculated with 0.05 ml of inoculum in the footpad of each of the four feet. They were examined daily from the 7th day post-inoculation. Histological studies were carried out on 4 rats (2 male and 2 female) with EAE, 2-3 days after the onset of neurological signs. In terminal experiments electrophysiological studies were performed on 14 male Lewis rats with EAE, 0-7 days after the onset of neurological signs.

Controls

One normal 12-week-old male Lewis rat served as a control for the histological studies. Eight normal 10-12-week-old male Lewis rats were used as controls for the electrophysiological studies. As the histological and electrophysiological studies were performed on the animals with EAE about 2 weeks after inoculation, the control animals were the same age as the animals with EAE at the time of these studies.

Histological studies

Under ether anaesthesia the rats were perfused through the left ventricle with 0.9% saline, until the effluent was clear, and then with 120ml of 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The brain, spinal cord, dorsal and ventral roots, DRGs, spinal nerves and the sciatic and tail nerves were removed and immersed in fixative. After dehydration in ascending ethanols and clearing in chloroform, slices of the fixed brain tissue were embedded in paraffin wax, sectioned (4 μ m) and stained with haematoxylin and eosin. Tissues from the rest of the nervous system were cut into 1 mm thick slices and post-fixed with 2% osmium tetroxide. Most of the osmium-fixed specimens were dehydrated in ascending ethanols, embedded in HistoResin (LKB Bromma), sectioned (2 μ m) and stained with toluidine blue in phosphate buffer (pH 7.6) or with cresyl violet. Some were dehydrated in ascending ethanols and after a stage in absolute acetone were

embedded in Epok 812 (Ernest F. Fullam, Inc., Schenectady, NY), sectioned (1 μ m) and stained with toluidine blue.

Electrophysiological studies

(a) Preparation of animals for recording

Anaesthesia was induced with urethane (25% in 0.9% saline, intraperitoneal (i.p.), 5 ml/kg) and supplemented by pentobarbitone sodium (i.p., 12 mg/kg). The animals breathed spontaneously through a tracheostomy. At the beginning of each experiment 9 ml of Hartmann's solution (compound sodium lactate BP, Travenol) were given i.p.

(b) Experiments on the lumbar segments

A T₁₂-L₅ laminectomy was performed. The rat was mounted in an animal frame, and a metal box, through which water at 37°C was circulated, was placed under the animal. After the left hindlimb had been extended and supported in a horizontal position, the left sciatic nerve was exposed. Laminectomy and sciatic nerve pools were formed with the skin flaps. The dura was opened, the sciatic nerve in the mid-thigh was dissected free and the nervous tissues were covered in paraffin oil. A controlled radiant heat lamp maintained the laminectomy pool at 37°C. Under these conditions the sciatic nerve pool temperature was 36°-37°C and the rectal temperature 37°-38°C. The sciatic nerve in the mid-thigh was lifted away from the volume conductor and placed across a pair of platinum stimulating electrodes 3 mm apart. To avoid ischemia of the sciatic nerve, the nerve was left in continuity. Stimuli were 0.1 ms square-wave voltage pulses delivered at 1.0 Hz.

Volume conductor recordings were made over the left L4 and/or L5 dorsal root entry zones (DREZ(s)) and sometimes over the exposed L5 DRG and spinal nerve (or ventral primary ramus) 2-3 mm from the mid-point of the DRG (see Pender and Sears 1984). The active electrode was a 0.5 mm diameter silver ball electrode. The reference electrode was a platinum wire placed in the right paravertebral region at the level of the active electrode. Before recordings were made, any accumulated cerebrospinal fluid or other body fluid was aspirated. Short leads connected all recording electrodes to FET source-followers, thence to a preamplifier (band width limited to 5.3 - 10000 Hz) and thence for display on an oscilloscope. For all recordings, negativity at the active electrode gave an upward deflection on the oscilloscope. Oscilloscope traces were photographed for measurements. Conduction velocities were calculated after allowing for a utilization time of 0.1 ms (Blair and Erlanger 1936).

At the end of the experiment the dissection was extended to expose the entire length of the conduction pathway from the sciatic nerve to the relevant DREZ and to confirm the contribution of the spinal nerve, of each segment studied, to the sciatic nerve. The L4 and L5 spinal nerves always gave large contributions to the sciatic nerve, and the L3 and L6 spinal nerves gave small contributions. Conduction distance was measured as the length of a thread placed along the conduction pathway.

(c) Experiments on the sacrococcygeal segments

A lumbar-sacral-coccygeal laminectomy of variable extent was performed. After the filaments of the left sacral or coccygeal dorsal root to be studied had been carefully identified, they were tied together and cut immediately proximal to the tie. The distal cut end was lifted away from the volume conductor into oil and placed on a pair of platinum wire hook electrodes 3 mm apart. The exposed left ventral caudal trunk formed by the sacral and coccygeal ventral primary rami was freed just distal to its junction with the respective ventral primary ramus and was stimulated in continuity with a pair of platinum electrodes 2 mm apart. The stimulating and recording arrangements were otherwise the same as for the experiments on the lumbar region.

RESULTS

Clinical findings

Tail weakness was the first neurological sign and commenced 8-14 days after inoculation. This was followed by flaccid tail paralysis, symmetrical hindlimb weakness and sometimes hindlimb paralysis. Forelimb weakness occurred occasionally. Most rats survived the acute episode and by 20 days after inoculation were clinically normal apart from mild tail weakness.

Histological findings

Paraffin sections of the brain showed perivascular cuffing and infiltration with mononuclear cells in the brainstem (Fig. 1) and cerebellum, and minimal if any involvement of the cerebrum. Epok 812 and HistoResin semi-thin sections stained with toluidine blue showed inflammatory demyelinating lesions in the spinal cord (Fig. 2) and PNS. In the spinal cord there was perivascular cuffing and infiltration with mononuclear cells and meningeal and subpial infiltration with these cells. Demyelination was present in the regions of mononuclear infiltration. Both the grey and white matter were involved. The dorsal root entry and ventral root exit zones were sites of predilection. The lumbar, sacral and coccygeal segments of the spinal cord were more severely involved than the rest of the cord. In the PNS there were perivascular inflammatory demyelinating lesions which were most prominent in the DRGs (Figs. 3 and 4). The dorsal and ventral roots were involved to a lesser extent and there was minimal if any involvement of the spinal nerves and peripheral nerves. The DRG lesions were similar to those in the CNS, there being perivascular cuffing and para-adventitial infiltration with mononuclear cells and demyelination in the regions of mononuclear infiltration (Figs. 3 and 4). Intracellular myelin debris was present in the lesions (Fig. 4). Axons and DRG neuronal cell bodies were normal. The DRG involvement increased caudally, it being mild to moderate in the cervical, thoracic and lumbar segments and moderate to severe in the sacral and coccygeal segments.

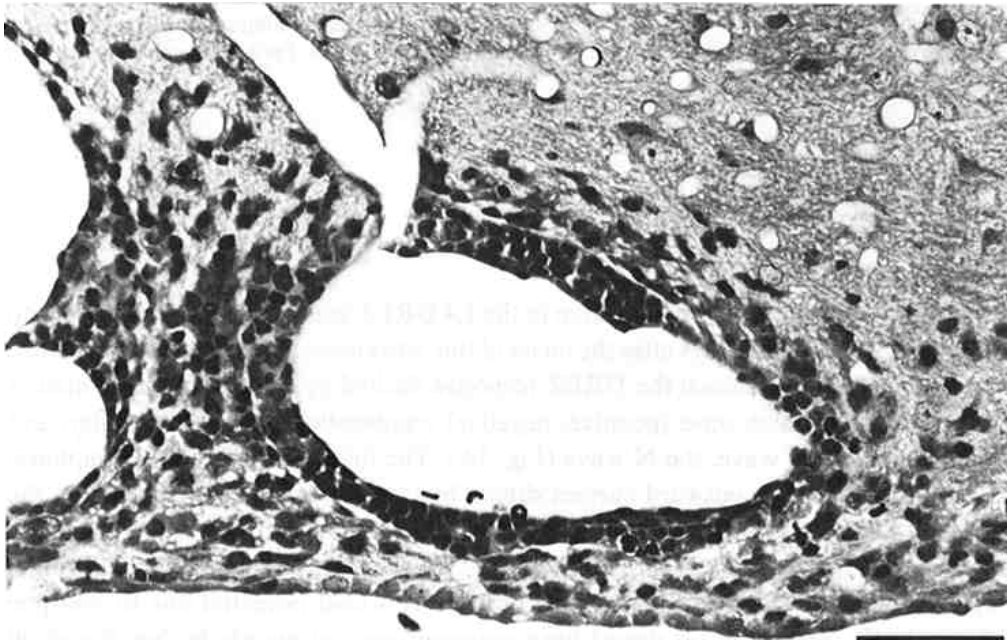


Fig. 1. Transverse section through the brainstem of a rat with EAE, 3 days after the onset of neurological signs. There is perivascular cuffing and parenchymal infiltration with mononuclear cells. Paraffin section stained with haematoxylin and eosin. Bar = 50 μ m.

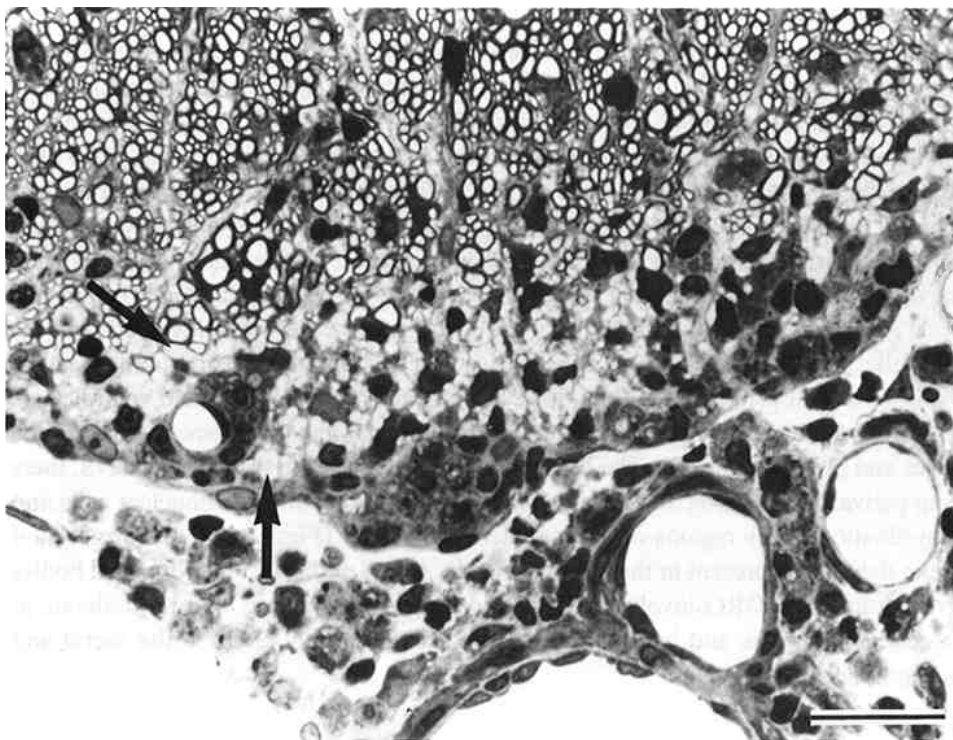


Fig. 2. Transverse section through a ventral column of the sacral spinal cord of a rat with EAE, 2 days after the onset of neurological signs. There is meningeal infiltration and subpial infiltration with mononuclear cells. Many demyelinated axons (arrows) can be seen in the subpial region. Epok 812 section stained with toluidine blue. Bar = 25 μ m.

Electrophysiological findings

(a) Lumbar segments

Conduction from the sciatic nerve to the L4 DREZ was studied in 7 animals with hindlimb weakness, 0-2 days after the onset of this weakness, and in 5 normal controls. In the normal control animal the DREZ response elicited by sciatic nerve stimulation consisted of a biphasic wave (positive, negative) representing the afferent volley, and a late slow negative wave, the N wave (Fig. 5A). The initial positivity in the biphasic wave 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. The negativity was always larger in amplitude than the initial positivity. The N wave is a field potential due to synaptic currents in the second order dorsal horn neurones excited mainly by low-threshold cutaneous afferents.

In the animals with EAE the responses were similar in configuration and in amplitude to those of the normal controls (Fig. 5B). However, as a group, the animals with EAE had slight reductions in the peak-to-peak amplitudes and in the conduction velocities of the biphasic wave (Table 1), indicating conduction abnormalities (block and/or slowing) in a small proportion of the large diameter myelinated fibres between the sciatic nerve and DREZ. The N wave latency and amplitude were normal (Table 1). The L5 DREZ response evoked by sciatic nerve stimulation was also studied in 4 of the animals with hindlimb weakness and in 3 of the normal controls. The findings were similar to those for the L4 DREZ responses (Fig. 6A and B).

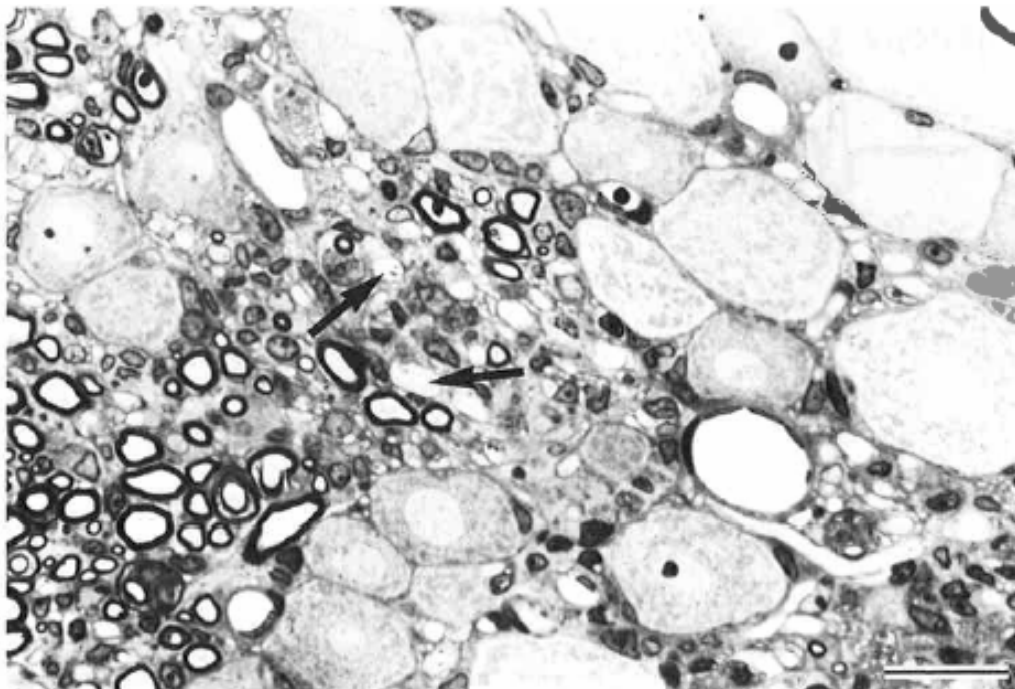


Fig. 3. Transverse section through a L5 dorsal root ganglion of a rat with EAE, 2 days after the onset of neurological signs. Demyelinated axons (arrows) are present in the regions of mononuclear infiltration. Epok 812 section stained with toluidine blue. Bar = 25 μ m.

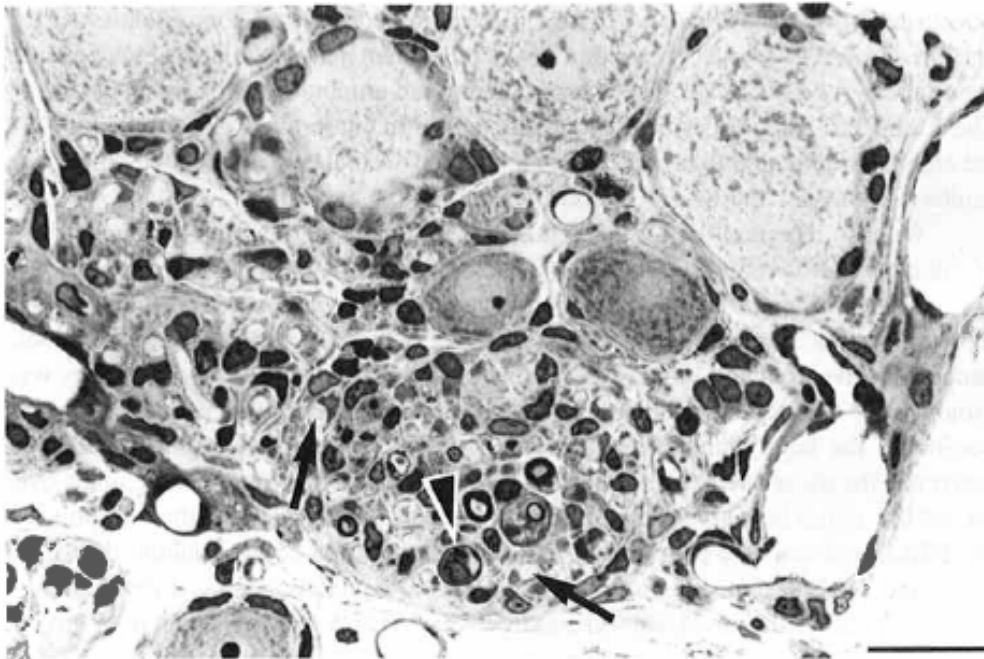


Fig. 4. Transverse section through a coccygeal dorsal root ganglion of a rat with EAE, 2 days after the onset of neurological signs. Demyelinated axons (arrows) are present in the regions of mononuclear infiltration. Intracellular myelin debris is also seen (arrowhead). Epok 812 section stained with toluidine blue. Bar = 25 μ m.

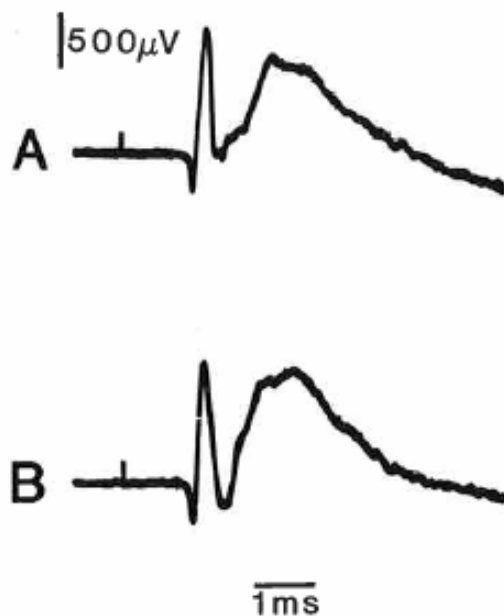


Fig. 5. Volume conductor recordings over the L4 dorsal root entry zone showing the maximum afferent volley evoked by sciatic nerve stimulation in a normal control rat (A) and in a rat with EAE (B). For these and all other recordings, negativity at the active electrode is represented by an upward deflection.

We also recorded the L4 and L5 DREZ response in 2 animals on the first day of full clinical recovery from hindlimb weakness. In one the L4 DREZ response was normal. In the other the L5 DREZ response was abnormal (Fig. 6C). The biphasic wave representing the afferent volley was considerably reduced in peak-to-peak amplitude, temporally dispersed and reduced in conduction velocity, and the N wave latency was prolonged (3.5 ms). These findings indicate conduction abnormalities in a high proportion of the large diameter fibres between the sciatic nerve and the DREZ. To determine the site of these abnormalities, volume conductor recordings were made over the left L5 spinal nerve or ventral primary ramus (2-3 mm distal to the mid-point of the DRG) and over the proximal L5 DRG. In the normal control animal the spinal nerve and DRG responses evoked by sciatic nerve stimulation were triphasic waves (positive, negative, positive) with the negativity having an amplitude equal to or greater than that of the initial positivity (Fig. 7A). In the animal with EAE the spinal nerve response was normal, but the DRG response showed a marked reduction in the amplitude of the negativity and some temporal dispersion of this negativity (Fig. 7B).

TABLE 1

COMPARISON BETWEEN L4 DORSAL ROOT ENTRY ZONE RESPONSES TO SCIATIC NERVE STIMULATION IN NORMAL CONTROL RATS AND IN RATS WITH HINDLIMB WEAKNESS DUE TO EAE

	Controls (n = 5) ^a mean ± SD	EAE (n = 7) mean ± SD	Statistical significance of difference between groups (Student's one-tailed <i>t</i> -test)
<i>Maximum afferent volley</i>			
Peak-to-peak amplitude (μV)	1391 ± 478	996 ± 259	<i>P</i> = 0.05
Amplitude of negativity	3.0 ± 0.5	3.0 ± 0.6	NS ^b
Amplitude of positivity			
Cv ^c peak positivity (m/s)	63.6 ± 3.9	55.7 ± 2.1	<i>P</i> < 0.001
Cv onset negativity (m/s)	62.0 ± 4.1	52.7 ± 2.5	<i>P</i> < 0.001
Cv peak negativity (m/s)	52.7 ± 3.6	45.6 ± 1.9	<i>P</i> < 0.001
<i>Maximum N wave</i>			
Latency to peak (ms)	2.5 ± 0.2	2.7 ± 0.2	NS
Amplitude of peak (μV)	1663 ± 546	1524 ± 589	NS

^a n = 4 for maximum N wave measurements.

^b Not significant (*P* > 0.05).

^c Conduction velocity at 37°C.



Fig. 6. Volume conductor recordings over the L5 dorsal root entry zone showing the maximum afferent volley evoked by sciatic nerve stimulation in a normal rat (A) and in 2 rats with EAE (B and C). Note the different amplitude scales.

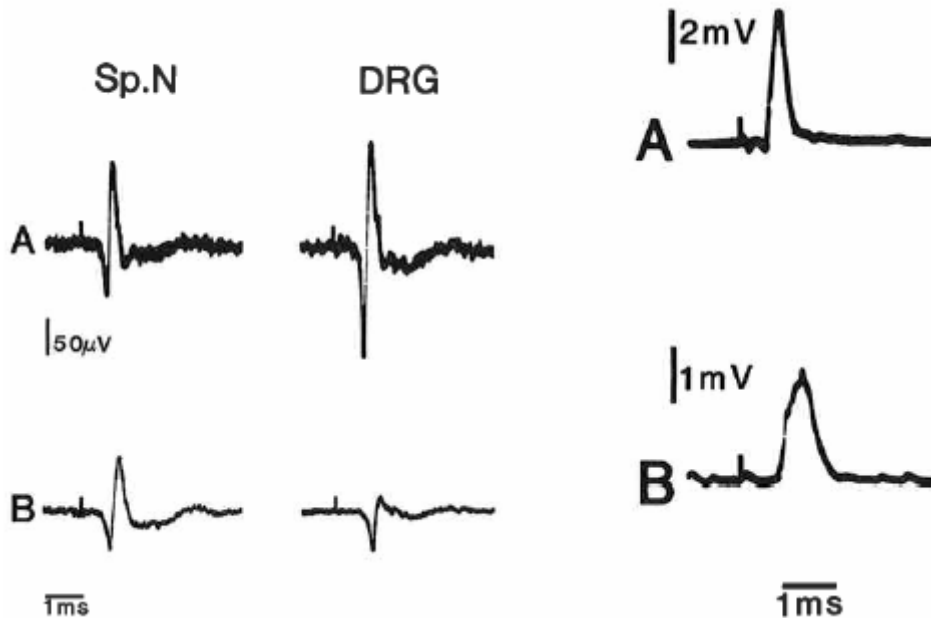


Fig. 7. Volume conductor recordings over the L5 spinal nerve (Sp. N) and dorsal root ganglion (DRG) of the response to sciatic nerve stimulation at the intensity giving the maximum spinal nerve response in a normal control rat (A) and in a rat with EAE (B).

Fig. 8. Recordings from the distal cut end of the first coccygeal dorsal root showing the maximum response to stimulation of the ventral caudal trunk in a normal control rat (A) and in a rat with EAE (B). The conduction distances were similar. Note the different amplitude scales.

This indicates conduction abnormalities (slowing and probably block) in a high proportion of the large diameter myelinated fibres in the DRG. The onset of neurological signs in this animal was later (14 days after inoculation) than in the others.

(b) Sacral and coccygeal segments

To study conduction through the sacral and coccygeal DRGs, we recorded the response from the distal cut end of a left sacral or coccygeal dorsal root close to the respective DRG when the ventral caudal trunk was stimulated close to this DRG. Recordings were made from one or two of the S2, S3, S4 and coccygeal 1 (Co1) dorsal roots in 5 animals with tail paralysis due to EAE, 0-3 days after the onset of tail weakness (total of 9 roots) and in 3 normal control animals (total of 5 roots). In 4 of the animals with EAE abnormal responses were obtained. In 1 of these animals the Co 1 response was abnormal (Fig. 8) but the S3 response was normal. The abnormalities consisted of a reduction in maximum amplitude, reduced conduction velocities of the onset and peak of the response and temporal dispersion (for example, Fig. 8) and indicate conduction slowing and possibly block in myelinated fibres in the regions of the sacral and coccygeal DRGs. In the other animal with EAE both the S4 and Co 1 dorsal root responses were normal.

DISCUSSION

The histological studies showed perivascular inflammatory demyelinating lesions in both the CNS and PNS, confirming the results of previous studies (Levine and Wenk 1963; Piliero and Cremonese 1973; McFarlin et al. 1974; Dal Canto et al. 1977). The DRG was the most affected region of the PNS.

Electrophysiological studies demonstrated conduction abnormalities in only a small proportion of the large diameter myelinated fibres between the sciatic nerve and the lumbar dorsal root entry zones in animals with hindlimb weakness. In view of the histological findings it is likely that these abnormalities were due to demyelination and were mainly localized in the DRG. This localization was confirmed in one animal that had recovered from hindlimb weakness and in which conduction abnormalities were demonstrated in a high proportion of the large diameter fibres in the L5 DRG. In several animals with tail paralysis, conduction abnormalities were demonstrated in the region of sacral or coccygeal DRGs. Demyelination in the dorsal roots may have contributed to some of the functional abnormalities in the lumbar, sacral and coccygeal segments. Conduction through the lumbar dorsal root entry zone was normal. From these studies it is apparent that lesions of the afferent pathway to the spinal cord do not contribute significantly to the hindlimb motor dysfunction in Lewis rats with acute EAE. Recent studies indicate that demyelination-induced conduction block at the ventral root exit zone of the spinal cord is an important cause of hindlimb weakness in Lewis rats with EAE (Pender, submitted for publication).

The DRG involvement in Lewis rats with EAE is much less severe, both histologically and functionally, than what we recently found in rabbits with EAE (Pender and Sears 1982, 1984). This difference is unlikely to be due to interspecies

variation in the blood-nerve barrier of the DRG, as this barrier is deficient in both the rat and the rabbit (Waksman 1961; Olsson 1971; Jacobs et al. 1976). The involvement of the PNS in rabbits with EAE induced by inoculation with rabbit spinal cord is explained by the finding that the amino acid sequences of myelin basic protein (MBP) from the rabbit CNS and of the P1 myelin basic protein (P1 protein) from the rabbit PNS are identical (Brostoff and Eylar 1972). In rats also, the P1 protein from the PNS is very similar to CNS MBP (Greenfield et al. 1973). Nevertheless, differences in the structures of guinea-pig CNS MBP and rat PNS P1 protein or differences in the amount of P1 protein in the DRG in rats and rabbits may account for the less severe DRG involvement in rats with EAE.

The greater histological involvement of the more caudal ganglia in rats with EAE could be accounted for if the blood flow per DRG neurone increased caudally to meet the presumably greater metabolic demands of neurones supporting larger areas of membrane, the length of the central processes (Waibl 1973) and peripheral processes increasing caudally. This would increase the chance of circulating sensitized lymphocytes encountering the target tissue. Alternatively the greater involvement of the more caudal ganglia could be related to the higher proportion of small diameter myelinated fibres in these ganglia (compare Figs. 3 and 4), as there is evidence that smaller diameter fibres are more susceptible to demyelination (Brown et al. 1980; Lafontaine et al. 1982; Saida et al. 1983).

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