Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination

N. D. Jeffery\textsuperscript{2} and W. F. Blakemore\textsuperscript{1}

\textsuperscript{1}MRC Cambridge Centre for Brain Repair and the Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, UK

Correspondence to: N. D. Jeffery, Department of Clinical Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, UK

\textbf{Summary}

Demyelinating lesions induced by intraspinal injection of gliotoxin have been studied for many years in order to gain insights into reasons for failure of remyelination and to improve understanding of the axonal conduction disorders in multiple sclerosis. Although the electrophysiological correlates of experimental demyelination and remyelination are well established, the behavioural effects have not been investigated. In this study we aimed to determine whether behavioural deficits could be detected during spinal cord demyelination, and furthermore, whether remyelination was associated with return of lost function. We used injections of the gliotoxin ethidium bromide into the dorsal funiculus of the cervical spinal cord of the rat to induce zones of demyelination and compared the effects on locomotion with those resulting from saline injections. The resulting locomotor deficits were quantified by analysis of foot placement during traverse of a horizontal 18 mm diameter wooden beam. Following ethidium bromide injection there was a decrease in security of foot placement, that recovered by ~5 weeks post-injection. In a second experiment, remyelination was prevented by exposure of the spinal cord to 40 Gy of X-irradiation. Behavioural deficits were induced as before, but the animals failed to recover throughout the duration of the experiment. Saline-injected animals in both experiments exhibited minimal deficits and quickly recovered. We conclude that demyelination produces detectable behavioural deficits which disappear following spontaneous remyelination.

\textbf{Keywords:} behaviour; experimental; demyelination; remyelination; spinal cord

\textbf{Abbreviation:} EB = ethidium bromide

\textbf{Introduction}

The induction of a zone of demyelination in experimental animals by intraspinal injection of a gliotoxin has been used as a means to investigate the interactions of glial cells during both spontaneous and transplant-induced remyelination (Blakemore \textit{et al.}, 1995). Exposure to X-irradiation prevents spontaneous repair (Blakemore and Patterson, 1978), thereby producing a permanent zone of demyelination analogous to a multiple sclerosis lesion. Remyelination of this lesion has been successfully achieved by transplantation of glial cells (reviewed by Franklin, 1993), suggesting the possibility of repair of multiple sclerosis lesions by glial cell transplants (Blakemore \textit{et al.}, 1996). However, before clinical application can be contemplated, the functional consequences of demyelination, remyelination and transplant-mediated remyelination must be investigated in experimental models. Although behavioural deficits have been measured in animals affected by experimental allergic encephalomyelitis (e.g. Matthaei \textit{et al.}, 1989), the effects of this disease are attributable not only to demyelination but also to inflammation. Therefore it is also desirable to have a means of assessing the functional consequences of a more clearly delineated demyelinated zone, such as that induced by local injection of a gliotoxin. The functional effects of a localized zone of demyelination or remyelination can potentially be detected by measurement of electrophysiological or behavioural parameters. Electrophysiological evaluation of central conduction during lysolecithin-induced demyelination and subsequent remyelination of the dorsal funiculus of the cat spinal cord has provided clear evidence that conduction block occurs during the period of demyelination but that secure con-
duction is restored following spontaneous remyelination (Smith et al., 1979, 1981). Schwann cell mediated central remyelination has been demonstrated to be as effective in restoration of function as that produced by the predominantly oligodendrocyte-mediated repair of the lysolecithin lesion (Felts and Smith, 1992). Transplanted glial cells have recently been shown to improve conduction velocity to near-normal values in myelin-deficient rats (Utzschnieder et al., 1994) and Schwann cell transplants can restore normal conduction velocity to axons previously demyelinated with ethidium bromide (EB) (Honmou et al., 1996). In contrast, the behavioural effects of local zones of demyelination and remyelination in experimental subjects have received little attention, although clinical signs in diseases such as multiple sclerosis are often attributed to demyelination of central axons (e.g. Smith, 1994). It is important that the behavioural effects of demyelination and remyelination should also be investigated because a demonstration that electrophysiological integrity is restored during remyelination does not necessarily imply that normal behavioural function will return.

The dorsal funiculus of the rat spinal cord is commonly used for studies on local demyelination because it is easily accessible for injection and amenable to secondary interventions such as suppression of remyelination (Blakemore and Patterson, 1978) and transplantation (Blakemore et al., 1995). The white matter of the dorsal funiculus is well delineated thereby permitting accurate measurement of the dimensions of a lesioned zone, which is advantageous when attempting to relate the area of a lesion with the severity of behavioural deficits. However, the function of the white matter in the rodent dorsal funiculus is not well defined, thereby posing problems in design of appropriate behavioural tests. To date, behavioural testing following spinal cord injury has been used to detect and quantify the results of contusion injuries or partial myelotomy and has been concerned with relatively gross loss in function (Rivlin and Tator, 1977; Gale et al., 1985; Kerasidis et al., 1987; Kunkel-Bagden et al., 1993; Basso et al., 1995). Therefore, many tests which are normally applicable to functional grading of spinal cord injury may not be appropriate for quantification of deficits associated with lesions in the dorsal funiculus.

The largest part of the rat dorsal funiculus consists of ascending afferent fibres. The precise functional deficits which follow damage to these fibres in many species are poorly defined or understood (Wall, 1970; Eidelberg et al., 1976; Davidoff, 1989; Glendinning and Vierck, 1993), although they may form part of a system to ‘pre-programme’ the motor cortex (Dubrovsky et al., 1971; Melzack and Bridges, 1971; Melzack and Southmayd, 1974). The remainder of the rat dorsal funiculus—the corticospinal tract—is thought to be concerned with control of fine digital movements; disruption of this tract will cause reduction in efficient forepaw use, but gross locomotor dysfunction has not been reported (Castro, 1972; Kalil and Schneider, 1975; Reh and Kalil, 1982; Schrimster and Reier, 1993).

In this paper, we describe the use of a beam walking test for the detection and graded scoring of demyelinating and remyelinating lesions in the dorsal funiculus of the rat. Ethidium bromide was selected as the gliotoxic agent in these experiments because the demyelination it induces is delayed from the time of injection (Graça and Blakemore, 1986), thereby potentially more clearly separating the effects of demyelination from the effects of trauma inherent to the injection procedure. In order to define more clearly the effects of remyelination in this system, spontaneous remyelination was inhibited in a second group of rats by exposure of the lesioned spinal cord to 40 GY of X-irradiation. The EB injection was made in the spinal cord underlying the third cervical vertebra in this study. This site was chosen because, in pilot studies, it appeared that the locomotor deficits induced by spinal cord lesions at the third cervical vertebra were more severe and consistent in their effects than those induced by similar lesions elsewhere in the spinal cord.

Beam walking was selected for evaluation of deficits following demyelination because subjective alterations in narrow beam traverse have previously been reported following injury to the dorsal funiculus (Ganchrow et al., 1980; Bernstein and Goldberg, 1991) and because of its established use in detection and scoring of deficits in limb function associated with lesions of the cerebral cortex (Feeney et al., 1982; Dixon et al., 1987; Christie and Dalrymple-Alford, 1995). We have previously demonstrated the value of our beam walking test in behavioural scoring of a photochemically induced ablation of the dorsal funiculus of the rat thoracolumbar spinal cord (Jeffery et al., 1995).

**Material and methods**

**Rat training**

Fifty-five young adult female rats (37 Sprague–Dawley, 18 Lewis) were trained to traverse an elevated 2 m long horizontal 18 mm diameter wooden dowel and offered a reward (chocolate) on completion of the task. Each rat was trained for 5 min daily for 5–7 days; training was considered complete when each rat would reliably cross without stalling. Few footstep errors were made by this stage (see Results).

**Lesion induction**

Rats were anaesthetized with halothane and, under aseptic conditions, a dorsal laminectomy was made in the third cervical vertebra. To ensure stability of the vertebral column and spinal cord during the injection procedure, the large spinous process of the second cervical vertebra was grasped with mosquito forceps held in a micromanipulator. The exposed dura was cut and the pia punctured using a fine hypodermic needle. In 31 animals, demyelination was induced by injection 0.8 mm deep into the dorsal funiculus of 1 µl of 0.1% EB solution through a glass tipped Hamilton
syringe; in 24 control animals 1 µl saline was used in place of EB. The Hamilton syringe was also stabilized in a micromanipulator. Each rat received 10 mg/kg carprofen, given by subcutaneous injection for post-operative analgesia.

To ensure that the type of injection received by each rat was unknown to the scorer of the behavioural tests the EB-injected rats were mixed with the saline-injected rats. Rats were then taken at random from the mixed group, numbered, housed individually and offered standard rat pellets and water ad libitum for the remainder of the experiment.

**Induction of non-repairing demyelinating lesion**

Two days following intraspinal injection, 21 rats, 13 of which had received EB injection and eight of which had received saline, were re-anasthetized by intraperitoneal injection of diazepam (2.5 mg/kg) plus a fentanyl citrate (0.0785 mg/kg)/ fluanisone (2.5 mg/kg) mixture (Hypnorm: Janssen, Oxford, UK). Each received a dose of 40 Gy of X-irradiation (255 kV; filter: 0.3 mm Sn, 0.5 mm Cu, 1.5 mm Al) to the cervical spinal cord. This procedure has previously been demonstrated to prevent the spontaneous remyelination that occurs following gliotoxin-induced demyelination (Blakemore and Patterson, 1978). Prior to radiation exposure, radiographs of the cervical area were taken with each rat positioned in lateral recumbency with the cervical spine under slight linear traction. By reference to these radiographs, precise positioning of the radiation field (4×2 cm) around the lesioned zone was possible and also prevented unnecessary exposure of adjacent vital structures.

**Behavioural data recording**

The central 1 m portion of the 2 m long 18 mm diameter beam was measured and the limits marked; a video camera positioned in the same horizontal plane was focused on this region. Videotape recordings were made preoperatively and then twice weekly postoperatively for up to 5 weeks. During each recording session each rat traversed the beam twice in each direction.

Behavioural scoring was carried out during slow motion or frame-by-frame replay on a standard domestic video cassette recorder (JVC HR-S4700-EK). For each rat, a score was given to every footstep of the hindlimb nearest to the observer as it crossed the beam each time, i.e. during left-to-right traverse the right hindpaw was evaluated and vice versa. Scores were given according to the following scheme: 0 = ‘normal’: foot positioned on top of the beam, no slippage; 1 = ‘minor error’: foot slip so that part of the foot was visible below the lower surface of beam, or the foot dragged along beam surface; 2 = ‘major error’: whole foot slipped below lower surface of the beam. The total score for each rat at each timepoint (i.e. the sum of errors for the four crossings) was recorded and used for subsequent statistical analysis. Forepaw errors were noticed occasionally but were not included.

**Histological preparation and examination**

At 28 days (n = 18), 35 days (n = 31) or 56 days (n = 6) following intraspinal injection each animal was deeply anaesthetized using sodium pentobarbital (160 mg/kg) and perfusion-fixed via the heart with 350 ml of 4% gluteraldehyde in phosphate buffer (pH 7.4). The cervical spinal cord was immediately removed and placed in 4% gluteraldehyde for 2 h. The lesioned zone was cut into 1 mm transverse slices and washed in phosphate buffer (pH 7.4). Slices were kept in order, post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol, then embedded in Taab resin following transitional stages in propylene oxide and resin. Sections (1 µm) were cut from each slice and stained with toluidine blue.

In sections containing demyelinated or remyelinated axons, lesion size was indirectly determined by measurement of the minimum undamaged cross-sectional area of corticospinal tract, gracile tract and total white matter in the dorsal funiculus. This analysis was carried out using a ‘Seescan’ image analysis system. Measurements were made in saline-injected individuals of the normal cross-sectional area of the corticospinal tract, gracile tract and total area of the dorsal funiculus, which then permitted calculation of the percentage area affected at the lesion epicentre (i.e. the tissue slice in which the lesion was at its maximum size) in EB-injected animals. A semi-quantitative score (0–3 at each end of the lesion) was attributed to the extent of Wallerian degeneration for each animal by examination of sections taken just cranial and just caudal to the limits of the demyelinated zone. The total score (i.e. combined cranial and caudal score) was compared with the animal’s behavioural score at various timepoints using scatterplots.

**Statistical analysis**

Rats were assigned to groups (EB; saline) following histological examination of their spinal cords and by whether they had received radiation or not (irradiated, non-irradiated). For each group the mean behaviour score and standard error of the mean was calculated and used to plot graphs of behaviour score versus time. Groups were initially compared by whether the hindpaw nearest to the behaviour score versus time. Groups were initially compared using ANOVA (Genstat), pre-operative scores were not included in this analysis. Post hoc Newman Keuls’s tests were used to analyse the significance of changes in scores at various timepoints. Results of both tests were taken to be significant if P < 0.05. This statistical analysis of behaviour was carried out on log-transformed data because the raw behavioural score data formed a skewed distribution. Significance of relationships between behavioural scores and lesion sizes was analysed by Spearman’s rank correlation test for non-parametric data.
Ethidium bromide injection causes deficits in behaviour function that spontaneously resolve

Both saline and EB-injected rats exhibited an immediate increase in error score following intraspinal injection, but this effect was more pronounced in the EB-injected group. Thereafter, the saline-injected group gradually improved such that scores returned to pre-injection values by about 25 days. In the EB-injected group, there was a slight improvement between 4 and 11 days after injection, a worsening between 11 and 14 days, and then a gradual improvement until the end of the experiment at 35 days. The mean score of this group had returned to within the pre-operative range by 35 days, although the saline-injected group still achieved a better mean score at this time (Fig. 2).

ANOVA revealed a highly significant difference between the EB-injected group and the saline-injected controls \[ F(1,32) = 23.21; P < 0.001 \] and also a significant change in scores of the combined groups with time \[ F(10,284) = 28.44; P < 0.001 \]. ANOVA also indicated that there was no effect of time on the difference in behaviour scores between the EB-injected group and the saline-injected throughout the experiment \[ \text{group} \times \text{time interaction}: F(10,284) = 1.11; P = 0.355 \], suggesting that the EB-injected animals did not achieve such complete recovery as those that had received saline, despite improvement in their mean score to within the preoperative range.

Post hoc testing demonstrated that the observed gradual recovery of the saline-injected group from 1 day onwards produced significant differences in scores between 1 and 11 days \( t = 5.76; P < 0.01 \) and between 11 and 35 days \( t = 4.03; P < 0.01 \). In contrast, in the EB-injected group, improvement in behaviour score did not occur uniformly from 1 day after injection. Instead, variations in behaviour score between 1 and 14 days did not reach statistical significance, but significant improvements in scores occurred between 14 and 21 days \( t = 5.12; P < 0.01 \) and between 21 and 35 days \( t = 7.2; P < 0.01 \).

Severity of behavioural deficit can be related to extent of lesion

The size of the lesion would be expected to influence the severity of behavioural deficits, so this relationship was initially investigated by examining scatterplots. The demyelinated proportion of each tract at the lesion epicentre was calculated for the EB-injected animals (Table 1), and plotted against behavioural scores at 14 days (the timepoint at which demyelination will be most predominant) (Fig. 3). This relationship was found to be significant \( r_s = 0.826; P < 0.01 \) by Spearman’s rank correlation test. Similar analysis at later timepoints (18 days and later) showed a loss of statistical significance in this relationship. Because the EB-mediated lesion also produces a variable degree of Wallerian degeneration in the spinal cord, we investigated its influence on behavioural scores. The scatterplot between Wallerian degeneration score and behaviour score at 4 days

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**Results**

**Non-irradiated (remyelinating) animals**

**Spontaneous remyelination occurs following EB-mediated demyelination**

At the end of the experiment, the EB-injected rats could be easily distinguished from the saline-injected rats by the presence of large remyelinated areas in the dorsal funiculus of the spinal cord (Fig. 1). The presence or absence of this type of lesion allowed each animal to be assigned to either the EB-injected or saline-injected group allowing analysis of the previously accumulated behavioural data.

A detailed analysis of the lesions in the EB-injected animals showed that almost all of the demyelinated axons were remyelinated. In accordance with previous observations on the effects of intraspinal EB (Blakemore, 1982; Graça and Blakemore, 1986), Schwann cell remyelination predominated in the ascending tracts, while oligodendrocytes remyelinated the majority of previously demyelinated axons in the corticospinal tract. A large number of debris-filled macrophages was evident in the centre of the lesions. Wallerian degeneration of ascending and descending axons was apparent in all lesions and varied in extent, although in all EB-injected animals it was more extensive than in those that had received only saline. In all the saline-injected animals there was a small zone of axonal injury in the dorsal funiculus but there was no evidence of demyelination and remyelination.
Fig. 2 Relationship between time following intraspinal injection and behavioural score. Data points show means, error bars represent SEM.

Table 1 Lesion size in EB-injected rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Total WM</th>
<th>CST</th>
<th>GT</th>
<th>WD score</th>
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Size is expressed as percentage of normal tract area affected at lesion epicentre. WM = white matter; CST = corticospinal tract; GT = gracile tract; WD = Wallerian degeneration.

suggested a significant relationship (Fig. 4), however the nature of the data precluded reliable statistical testing.

**X-irradiated (non-remyelinating) animals**

**Irradiation prevents remyelination following ethidium bromide-mediated demyelination**

At the end of the experiment, histological examination of the spinal cords easily determined which animals had received EB, allowing assignation of each rat to a group as described above. In the EB-injected irradiated rats a zone of demyelinated axons was visible in the dorsal funiculus with a central core of macrophages containing myelin debris (Fig. 5). The cross-sectional area of the dorsal funiculus appeared greatly reduced, associated with the loss of a large quantity of myelin. In the saline-injected rats there was slightly more damage compared with the non-irradiated group described before. In four out of eight there was a narrow subpial rim of demyelinated axons and hypertrophied astrocytes in the white matter between the dorsal horns. In two animals there was a small number of demyelinated axons in the dorsal funiculus extending along the needle track.

**Inhibition of remyelination prevents recovery of lost function**

Once again, there was a marked deterioration in function within the first 24 h in both the saline- and the EB-injected rats. The irradiated saline-injected rats showed an improvement in score with time, somewhat more quickly than their non-irradiated counterparts. In contrast, the EB-injected irradiated rats, although initially showing an improvement in function by 7 days, deteriorated again by 11 days and thereafter exhibited little alteration in function (Fig. 6).

ANOVA confirmed that both EB \( F(1,19) = 46.29; P < 0.001 \) and time \( F(10,190) = 4.86; P < 0.001 \) significantly affected behaviour scores. *Post hoc* testing of the irradiated saline-injected group revealed significant improvement in scores between 1 and 14 days \( t = 4.46; P < 0.05 \) and then no significant alteration. Testing of the EB-injected rats revealed a significant improvement between 1 and 7 days \( t = 5.26; P < 0.01 \), but the deterioration between 7 and
11 days was not significant. However, in sharp contrast to the non-irradiated EB-injected rats, there was no significant alteration in behavioural score from 11 days onwards.

**Relationship between area of demyelination and severity of deficits cannot be detected**

The extent of the lesion at the epicentre in each of the EB-injected rats was measured as before (Table 2) and plotted against behavioural score at 14 days (Fig. 7). Spearman’s rank correlation test demonstrated that there was no significant relationship between these variables ($r_s = 0.394; P > 0.05$). Similar analysis using behavioural data at different timepoints also failed to find a significant relationship. A scatterplot of extent of Wallerian degeneration against severity of behavioural deficits at 4 days was less suggestive of a relationship than in the non-irradiated rats (Fig. 8).

**Comparison between the remyelinating and persistently demyelinated rats**

ANOVA was used to compare the behaviour scores of irradiated rats of one group with their non-irradiated counterparts. Statistical comparison between these groups is important because it is not possible to undertake an experiment to compare directly behaviour in irradiated and non-irradiated EB-injected rats because of the visible skin lesions that result from exposure to the X-irradiation—a difference which could potentially introduce bias into the behaviour scoring.
Table 2  Lesion size in EB-injected, irradiated rats

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Size is expressed as percentage of normal tract area affected at lesion epicentre.

Fig. 5  Transverse section through a portion of dorsal funiculus in an EB-injected, irradiated rat. Large numbers of demyelinated axons are present (d), abutted by areas of unlesioned white matter (w). Debris-laden macrophages are also visible (m). Toluidine blue stain. The bar represents 50 μm.

Fig. 6  Relationship between time following intraspinal injection and behavioural score for irradiated rats. Data points show means, error bars represent SEM.

To enable meaningful statistical comparisons between groups of EB-injected rats to be made in this study it is important to demonstrate first that the saline injected animals are not affected by exposure to irradiation. When comparing the saline-injected groups, irradiation did not have an overall effect \( F(1,22) = 1.74; P = 0.201 \), and although there were observable differences at three timepoints these were inconsistent in magnitude and direction.

The effect of irradiation on the EB-injected animals was markedly different. Although there was no overall difference between these groups \( F(1,29) = 2.74; P = 0.109 \), there was a highly significant time/group interaction \( F(10,270) = 11.4; P < 0.001 \), demonstrating that the effects of radiation become significant at particular timepoints. Post hoc analysis revealed that these differences between groups occurred from 25 days postoperatively until the end of the experiment (day 25: \( t = 4.45; P < 0.01 \); day 28: \( t = 4.4; P < 0.01 \); day 32: \( t = 4.52; P < 0.01 \); day 35: \( t = 6.51; P < 0.01 \)), thereby confirming that irradiation (and by association, persistent demyelination) prevents recovery of function in EB-injected rats.

To examine the possibility that differences in lesion size may be the reason for differences in recovery between X-irradiated and non-irradiated EB-injected animals, the lesion sizes were compared. On average, lesion area was slightly larger in the persistently demyelinated group, but extent of Wallerian degeneration was very similar (Tables 1 and 2).
This difference in lesion size is not responsible for differences in recovery between groups because exclusion of the behaviour scores associated with the four smallest lesions in the non-irradiated group does not alter the significance of differences between the irradiated and non-irradiated EB-injected groups (calculations not shown).

**Discussion**

The results clearly demonstrate that injection of EB into the dorsal funiculus of the spinal cord causes a detectable and significant decrease in locomotor ability measured on this beam walking test. This lost ability is restored by remyelination but persists when X-irradiation is used to suppress remyelination.

In our hands, injection of EB into the spinal cord causes immediate oedema and axonal damage, the severity of which can be related to the concentration of EB injected (Graça, 1986). Although glial cells show signs of toxicity from 24 h post-injection, large scale demyelination does not occur until between 7 and 10 days, reaching its full extent at 14 days. From this time remyelination predominates and nearly all axons are remyelinated by 4 weeks post-injection (Blakemore, 1982; Graça and Blakemore, 1986). Therefore we conclude that, in the non-irradiated rats, the initial loss of function is associated with acute effects of the toxin and trauma inherent...
to the injection procedure, while the second period of functional deterioration (between 11 and 14 days) and the subsequent recovery are associated in time with demyelination and remyelination, respectively. (It is of interest that, in the irradiated group, the second period of deterioration appeared to occur slightly earlier, perhaps because the interaction of irradiation with the effects of EB causes earlier destruction of the myelin sheath.) In the irradiated group, recovery of behaviour does not occur and is accompanied by failure of remyelination, thereby strongly suggesting that remyelination is responsible for the recovery of function observed in the non-irradiated rats. Electrophysiological studies also provide supportive evidence for the significance of remyelination in restoring function. In other lesions, such as those resulting from injection of lysolecithin into the cat or rat dorsal funiculus (Smith et al., 1979, 1981; Yezierski et al., 1992) or EB (at lower concentration than we used) into the rat dorsal funiculus (Felts and Smith, 1992), return of secure conduction occurs at the same time as remyelination.

Although we believe that the behavioural recovery observed in the non-irradiated rats is accounted for by remyelination, there are alternative means by which recovery of function could occur following demyelination. For instance, in experimental animals, return of conduction through persistently demyelinated axons can occur in the absence of remyelination (Bostock and Sears, 1976; Waxman, 1988), probably by redistribution of axonal sodium channels (Black et al., 1991). Alternatively, it is also possible that behavioural recovery could be associated with other adaptive mechanisms in the CNS, such as compensation by undamaged pathways (Helgren and Goldberger, 1993; Harris et al., 1994), that can occur following any injury that disrupts conduction through particular tracts. Therefore the observation that X-irradiation following EB injection prevents recovery could potentially be interpreted in two possible ways; either that irradiation of axons (either those inside or outside the lesion) prevents their ability to adapt to the presence of the lesion, or that permanent loss of glial cells prevents recovery of conduction in demyelinated axons.

In examining the first of these possibilities, the effects of irradiation on behavioural recovery following spinal cord injury have not been investigated, but there is little reason to consider it detrimental to the axons themselves. Mastaglia et al. (1976) found no observable behavioural deficits in rats which had received single doses of 40 Gy of X-irradiation to the uninjured cervical spinal cord until >8 months later and histological evidence of axonal injury was limited to finding occasional fibres undergoing Wallerian degeneration. There are other observations suggesting that X-irradiation does little harm to axons. For example, lesioned dorsal root axons are able to regrow into the spinal cord after they have been irradiated (Sims and Gilmore, 1994), and the recovery of normal conduction following transplantation of Schwann cells into the X-irradiated EB lesion (Honmou et al., 1996) suggests that the ability to redistribute sodium channels is also unaffected. In addition, in our experiments not only was there apparently uninhibited recovery of function in the (albeit mildly affected) X-irradiated, saline-injected animals but we also observed that the irradiated EB-injected rats exhibited significant recovery of function between 1 and 7 days, in contrast to the non-irradiated group. This observation suggests that irradiation may even have a beneficial effect following spinal cord trauma; a point of view which has previously been espoused by Kalderon et al. (1990) regarding injury elsewhere in the CNS.

With regard to the effects of irradiation on the glial cell population, it can be concluded that the detrimental effect of irradiation in our experiment results only from its combination with injection of EB—a combination that causes permanent demyelination and loss of both oligodendrocytes and astrocytes from the area of demyelination. It has been demonstrated that redistribution of sodium channels occurs following demyelination and appears to be directed by axonal association with glial cells (Smith et al., 1982; Dugandzija-Novakovic et al., 1995). In our experiments, it is not possible to distinguish whether persistent axonal conduction block results solely from demyelination or through any effects of glial cell depletion on the ability to redistribute sodium channels.

If demyelination were responsible for the observed behavioural deficits it would be expected that the severity of loss of function could be correlated with the extent of demyelination. A significant correlation was found between these variables at 14 days in the non-irradiated rats, which coincides with the period during which demyelination is most prevalent. Furthermore, as expected, if demyelination were the cause of loss of function, this relationship is then lost during the period when remyelination occurs. In the irradiated rats the relationship between the size of the lesion and the severity of the behavioural deficits was not statistically significant. This apparent anomaly can be explained by comparing the range of lesion size between the irradiated and non-irradiated rats. In the irradiated group there was a smaller range of lesion size compared with the non-irradiated rats, especially within the corticospinal and gracile tracts (Tables 1 and 2). It is to be expected that hindlimb performance on the beam walking test is primarily dependent on adequate function of either or both of these tracts. Therefore in the irradiated group in which there was a high proportion of ‘complete’ lesions (i.e. little variability in lesion size), it could be expected that a correlation of lesion size with deficits may not be readily apparent. The relationship between extent of Wallerian degeneration and severity of behavioural deficits at 4 days suggests that axonal injury plays a significant role in the genesis of the early decline in behavioural function on which the effects of demyelination will then be superimposed. The observation that the non-irradiated EB-injected animals do not recover to quite the same extent as their saline-injected counterparts suggests that axonal injury, which is more extensive in EB-injected individuals, may also play a role in determining the severity of deficits remaining at 35 days. It is yet to be determined if the CNS responds
in the same manner to a demyelinating lesion as it does to axotomy.

**Conclusion**

These results demonstrate that demyelinating lesions in experimental animals result in loss of function that can be restored by remyelination. It will now be possible to use the current lesion and scoring system to determine whether the anatomical repair achieved by transplantation of glial cells into areas of persistent demyelination also leads to behavioural recovery.

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