

# Dorsolateral cervical spinal injury differentially affects forelimb and hindlimb action in rats

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## Abstract

In experimental spinal injury studies, damage to the dorsal half of the spinal cord is common but the behavioural effects of damage to specific pathways in the dorsal cord have been less well investigated. We performed bilateral transection of the dorsolateral spinal funiculus (DLF) on 12 Long–Evans rats at the third cervical spinal segment. We quantified overground locomotion by measuring ground reaction forces, step timing and step distances as animals moved unrestrained. We also assessed skilled locomotion by measuring footslip errors made while the animals crossed horizontal ladders, and examined paw usage in a cylinder exploration task and during a skilled reaching task. Ground reaction forces revealed that rats with bilateral DLF lesions moved with a symmetrical gait, characterized mainly by altered forces exerted by the hindlimbs, delayed onset of hindlimb stance, and understepping of the hindlimbs relative to the forelimbs. These alterations in overground locomotion were subtle but were nevertheless consistent between animals and persisted throughout the 6-week recovery period. During ladder crossing, rats with DLF lesions made more footslip errors with the hindlimbs after surgery than before. Spontaneous forelimb usage during exploration was not affected by DLF axotomy but lesioned animals were less successful during skilled reaching. This is the first study which describes preferentially altered hindlimb use during overground locomotion after cervical DLF transections. We discuss these findings in relation to previous work and to the possible contributions of different ascending and descending pathways in the DLF to locomotion and skilled movements in rats.

## Introduction

Animal models of spinal cord injury play an essential role in the search for effective therapies to improve functional recovery. In most experimental spinal cord injuries, damage invariably occurs to axons travelling in the dorsal part of the spinal cord. This is largely due to the easier surgical access to the dorsal cord than the ventral cord, and to the dorsal location of several prominent descending pathways, most notably the corticospinal and rubrospinal tracts (Tetzlaff *et al.*, 1994; Kwon *et al.*, 2002; Raineteau *et al.*, 2002; Tobias *et al.*, 2003). These pathways have fairly circumscribed cell body locations and axonal trajectories, such that they lend themselves well to anatomical examination after injury. At the same time, it is important to understand the functional contributions of these and other pathways damaged in experimental lesions so that we can better assess the behavioural effects of experimental treatments designed to promote regeneration and/or plasticity.

One pathway in the dorsal spinal cord that has been the subject of much attention is the rubrospinal tract, a brainstem–spinal pathway that is present in all limbed terrestrial animals (Ten Donkelaar, 1988). In rats, axons of this pathway travel in a relatively circumscribed fashion in the dorsal part of the lateral spinal funiculus (DLF; Brown, 1974; Kuchler *et al.*, 2002). Virtually all of the axons in each tract

arise from the contralateral red nucleus (Brown, 1974). Nevertheless, most experimental lesions do not lesion the DLF specifically but involve much of the dorsal half of the spinal cord, including primary sensory axons in the dorsal columns and the corticospinal tract. The contribution of rubrospinal neurons to the functional deficits occurring after these lesions is therefore confounded by damage to other pathways.

While the behavioural contributions of rubrospinal neurons have not been specifically elucidated, their anatomy and physiology suggests that these cells might play a role in the control of skilled limb movements. In rats, rubrospinal neurons provide direct innervation of forelimb motoneurons, particularly those of the flexor muscles in the distal limb (Kuchler *et al.*, 2002). This pattern of connections supports the assertion that the rubrospinal pathway is important for control of skilled forelimb movements in experimental animals, such as during ladder locomotion and reaching for food (Whishaw *et al.*, 1992, 1998; Muir & Whishaw, 2000). Nevertheless, the presence of the rubrospinal pathway in those species which do not perform skilled forelimb movements (e.g. reptiles) suggests that these neurons might also contribute to more general limb usage such as during locomotion (Ten Donkelaar, 1988). In support of this, rubrospinal neurons are phasically active during regular locomotor movements in cats, increasing their discharge during obstacle avoidance (Lavoie & Drew, 2002). Our previous work has shown that unilateral ablation of the red nucleus or unilateral DLF lesions results in permanent asymmetries in the forces acting through the limbs (i.e. ground reaction forces) during overground locomotion in rats (Muir & Whishaw, 2000; Webb & Muir, 2003). This suggests that rubrospinal input normally plays a role

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during locomotion and is not solely used for skilled movements. These results differ from those found after lesions of the corticospinal tract, unilateral lesions of which do not cause permanent asymmetries during overground locomotion (Metz *et al.*, 1998; Muir & Whishaw, 1999a). Nevertheless, the locomotor asymmetries after unilateral DLF lesions are not specific to this injury. Many features of the locomotor pattern, such as decreased weightbearing by the ipsilateral hindlimb and abnormal propulsive forces during the contact time of the ipsilateral hindlimb, are similar to those of rats with other unilateral CNS lesions (Muir & Whishaw, 1999a,b, 2000; Webb & Muir, 2002, 2003, 2004). We therefore hypothesized that, if rubrospinal axons do play a role during ongoing locomotion, bilateral lesions of the DLF, containing the rubrospinal pathway, would also result in measurable changes in the locomotor pattern in rats. In the present study, we describe the locomotor and skilled limb deficits occurring after bilateral dorsolateral funicular lesions. We show that rats with DLF lesions adjust their overground locomotion in subtle but consistent ways that involve changes in the forces and step lengths produced by the hindlimbs, and in the timing between fore- and hindlimbs. These rats are more prone to hindlimb placement errors when crossing a ladder and are less skilled when retrieving food pellets with the forelimbs. We discuss these findings in relation to previous work and to the possible contributions of rubrospinal tract and other pathways travelling in the DLF.

## Methods

### Subjects

Twenty-three (23) female Long-Evans rats, weighing between 230 and 270 g, were used in this study. The animals were housed in pairs on wood-chip bedding in the University of Saskatchewan Animal Care facility. They were food restricted to 90% of *ad lib* weight by providing measured feeding once each day. All animals were examined by a veterinarian (A.W. or S.K.) daily and were cared for according to the standards outlined by the Canadian Council on Animal Care.

### Surgery

Rats were premedicated with atropine (MTC Pharmaceuticals, ON, Canada; 0.05 mg/kg s.c.) and buprenorphine (Buprenex; Reckitt and Colman Pharmaceuticals, VA, USA; 0.05 mg/kg s.c.) 30 min prior to induction of anaesthesia with sodium pentobarbital (Somnotol; MTC Pharmaceuticals; 35 mg/kg i.p.). Due to the introduction of new institutional protocols discouraging the use of pentobarbital, one group of animals ( $n = 6$ ) was premedicated with glycopyrrolate (Sabex, Quebec, Canada; 0.03 mg/mL) and anaesthetized with a mixture of medetomidine hydrochloride (Domitor; Novartis Animal Health, Ontario, Canada; 0.3 mg/kg), ketamine (Vetalar; Bioniche Animal Health, Ontario, Canada; 0.3 mg/kg) and fentanyl (Sabex; 50 µg/kg), injected i.p. After anaesthesia induction, dorsal laminectomy was performed under surgically sterile conditions as described previously (Webb & Muir, 2002). Briefly, the skin and muscles over the dorsal cervical spinal column were dissected and the dorsal spinal process and the dorsal spinal laminae of the third cervical vertebra were removed with fine ronguers. The dura was incised with fine scissors and the left and right dorsolateral funiculi were transected using a modified 25-gauge needle. A fat graft was placed over the laminectomy site and the muscles were closed with absorbable sutures. The skin incision was closed with staples or sutures which were removed 10 days after the surgery.

### Retrograde labelling and histology

After recording of behavioural data was completed, animals were anaesthetized as above and, under sterile surgical conditions, a dorsal laminectomy was performed on the 10th thoracic vertebra. Using a glass pipette (tip diameter 100–150 µm) attached to a Hamilton syringe, 0.2 µL of Fluorogold (Fluorochrome Inc., Englewood, CO, USA; 4% in sterile saline) was injected into each of the right and left sides of the thoracic spinal cord. The dorsal spinal musculature was sutured, the skin was closed with staples and animals were allowed to recover. After 72 h, animals were deeply anaesthetized with sodium pentobarbital (70 mg/kg i.p.) and transcardially perfused with 250 mL of heparinized 0.1 M phosphate-buffered saline followed by an equal volume of 4% paraformaldehyde. Cervical spinal cords were dissected and postfixed for 24 h in 4% paraformaldehyde, then processed and embedded in paraffin. Spinal cords were serially sectioned transversely at 8 µm throughout the injury site and mounted on slides coated with poly L-lysine. Slides were stained with Luxol fast blue and Cresyl violet and examined by light microscopy to determine the centre of the lesion for each rat. Brains were also dissected immediately after perfusion and were cryoprotected in 30% sucrose before being sectioned at 40 µm on a cryostat. Midbrain sections were examined under fluorescence microscopy for the presence of Fluorogold-labelled neuronal cell bodies in the red nucleus.

### Behavioural measurements

#### Overground locomotion

Rats were trained to locomote back and forth along a 2-m-long × 15-cm-wide runway for a food reward. As previously described (Webb & Muir, 2002), three force platforms measuring 10.5 × 11 cm each were located adjacent to each other in the floor of the runway so that the rats contacted the platforms with each pass along the runway. Ground reaction force data collection occurred when the rats moved in one direction only, and was triggered by the rat's body breaking an infrared beam which crossed the runway ahead of the first force platform. Each platform measured force in three directions: vertical, fore-aft (i.e. along the trajectory of the animals' movement), and medial-lateral. Forces were collected on a computer after analogue-to-digital conversion at a sampling frequency of 1000 Hz. Data were only saved from runs in which the rat maintained a constant speed over the platforms and paw placement was not on the edge of the platforms. Rats were videotaped at the same time (60 Hz sampling), and videotape frames were synchronized with force data using an LED timer which was triggered by the infrared beam.

Videotaped records were analysed frame-by-frame. As in previous studies, rats contacted each platform first with a forelimb and subsequently with the ipsilateral hindlimb. Videotape analysis of each pass was used to determine the approximate position and identity of paw placement on each platform (i.e. right or left) and to confirm that the rats maintained a constant speed over the platforms. Ground reaction forces from acceptable runs were digitally filtered using a low-pass recursive filter (half-power point 51.64 Hz) and normalized for body weight. Forces from each limb were averaged for each individual animal. The timing of limb overlap was determined from the force plate data (see below) and this timing was used to align the data from each limb appropriately to obtain the total ground reaction force for each animal for each run. Averaged data from each animal (10–12 passes per day) were used to produce group averages for presurgery and postsurgery groups for individual limbs and for the total ground reaction forces.

In addition to limb forces, data from the force platforms were used to produce limb timing and step distances for each pass. The point of force application on each plate was determined by the ratio of vertical force measured from one end of the force platform over the total force for each plate. Stride length and step lengths for each limb were determined for each pass by combining data from all three plates. Stride durations and the onset and end of the stance phases for each limb, as well as overlaps between limbs, were calculated for each pass. Because both length and timing measurements vary with speed of movement, distance measurements were normalized to stride length and timing measurements were normalized to stride duration. All data processing was performed using custom-written software (MS Visual Basic; Microsoft Corp).

#### *Skilled ladder locomotion*

Rats were trained to cross a horizontal ladder for a food reward. Two different ladders, requiring different levels of skill to cross, were used in this study. The first, easier, ladder was constructed of wooden dowels 6.0 mm in diameter, spaced 2.54 cm apart (Webb & Muir, 2003, 2004). The second, more difficult, ladder was constructed of metal rungs 2.0 mm in diameter, spaced 10 mm apart (Poulton & Muir, 2005). Each ladder was suspended above a 45° angled mirror so that rats could be videotaped simultaneously from both a lateral and a ventral view. Frame-by-frame videotape analysis was used to examine each pass. An LED timer in view of the camera was used to determine the speed of movement. Acceptable passes were those in which the animal moved at a moderate speed (50–80 cm/s) and in which the animal did not stop within, or just prior to entering or exiting, the field of view. For each acceptable pass, foot-fault scoring was performed on only one step for each limb to ensure that repeated samples from each limb remained independent of each other. Steps were scored as a correct step or an incorrect step. Incorrect steps consisted of steps which involved a total miss, deep slip, slight slip or replacement (Metz & Whishaw, 2002). Scores from 10 passes were sampled for each animal and averaged to provide a representative score for each limb from each animal for each test day. Postsurgical scores were expressed as a percentage of presurgical scores for the forelimbs and hindlimbs. Averaged percentages from each animal for each postsurgical test day were used to obtain group scores.

#### *Spontaneous forelimb use*

To assess whether rats would spontaneously use their forelimbs for support and balance during vertical exploration, rats were placed in a vertically orientated Plexiglass cylinder (46 cm high × 40 cm diameter) and videotaped from a ventral perspective through a clear glass floor for 5 min each (Webb & Muir, 2003, 2004; Webb *et al.*, 2003). Videotapes were viewed frame-by-frame and the number of times that the animals contacted the cylinder wall with the right forepaw, left forepaw or both were recorded separately. Averaged scores from each animal for each test day were used to obtain group scores.

#### *Skilled forelimb use*

Prior to surgery, six animals were trained to reach through a slot for a food pellet (Bio-Serve, Frenchtown, NJ, USA; 45 mg) as previously described (Whishaw *et al.*, 1998). Successful reaches were defined as those in which the animals picked up the pellet and brought it to their mouths without dropping it. After each reach, animals were trained to walk to the back of the reaching apparatus and then approach the reaching slot again so that each reach could be considered independent of the previous reach. Testing sessions

involved counting successful reaches from a total of 20 pellets. Animals were considered trained when their average percentage of successful reaches was at least 70% over three testing days (one session per day). An average of three testing days was used due to the intraindividual performance variability between days. Postsurgical testing sessions were similarly conducted over 3 days and the success score for each animal was the average of the three scores. Averaged success scores for each animal were used to obtain group scores. Additionally, the first five reaches in each trial were scored using the 10-component rating scale developed by Whishaw and colleagues (Whishaw *et al.*, 1993; McKenna & Whishaw, 1999). Briefly, each reach was divided into the following sequential components: limb lift, digits close, aim, advance, digits open, pronate, grasp, supination I (paw is withdrawn), supination II (paw is brought to mouth), release. Each component was rated on a three-point scale: 0, normal movement; 1, movement abnormal but present; 2, absent movement. In addition, the arpeggio movement occurring during pellet location, known to be affected by red nucleus ablation, was rated on the same scale (Whishaw *et al.*, 1998).

#### *Statistical analysis*

Groups were compared using repeated-measures ANOVA and *post hoc* Tukey analysis. Reaching scores were analysed using Friedman repeated-measures analysis on ranks and *post hoc* Tukey analysis. A *P*-value of <0.05 was considered significant (Sigmastat; SPSS Inc).

## **Results**

### *Histology*

All 23 animals had damage to the dorsolateral funiculi bilaterally, but not all had damage restricted to that region. For inclusion, the damage to either lateral funiculus could extend no further ventrally than a horizontal line level with the central canal, and there could be no damage to the dorsal funiculus on either side. Only 12 animals had lesions which satisfied these criteria and only these animals are discussed in the remainder of the analysis (Fig. 1). Retrograde labelling experiments in these animals demonstrated that, while many labelled cells were found throughout the brainstem, there were no labelled cells in either red nucleus, consistent with complete transection of rubrospinal axons in the dorsolateral funiculi of the spinal cord (data not shown).

### *Overground locomotion*

Rats moved overground (average speed of 72 cm/s) by bearing weight alternately on diagonal limb pairs (Fig. 2A). For each diagonal limb pair, the onset of the hindlimb contact slightly preceded that of the diagonal forelimb. There was no overlap between contact durations of the forelimbs. Measurement of step distances revealed that animals produced symmetrical step lengths with right and left limbs, and that each hindpaw was placed slightly ahead of the position of the ipsilateral forepaw (Fig. 3A). Ground reaction forces produced by all animals prior to surgery were identical to those from previous studies (Webb & Muir, 2002, 2003, 2004; Webb *et al.*, 2003; also Fig. 4A). Peak forces produced by the forelimbs were slightly higher than those produced by the hindlimbs. Forelimbs produced most of the braking forces while the hindlimbs produced most of the propulsive force. Lateral forces were small and laterally directed. Total ground reaction forces demonstrated a symmetric oscillation of vertical force

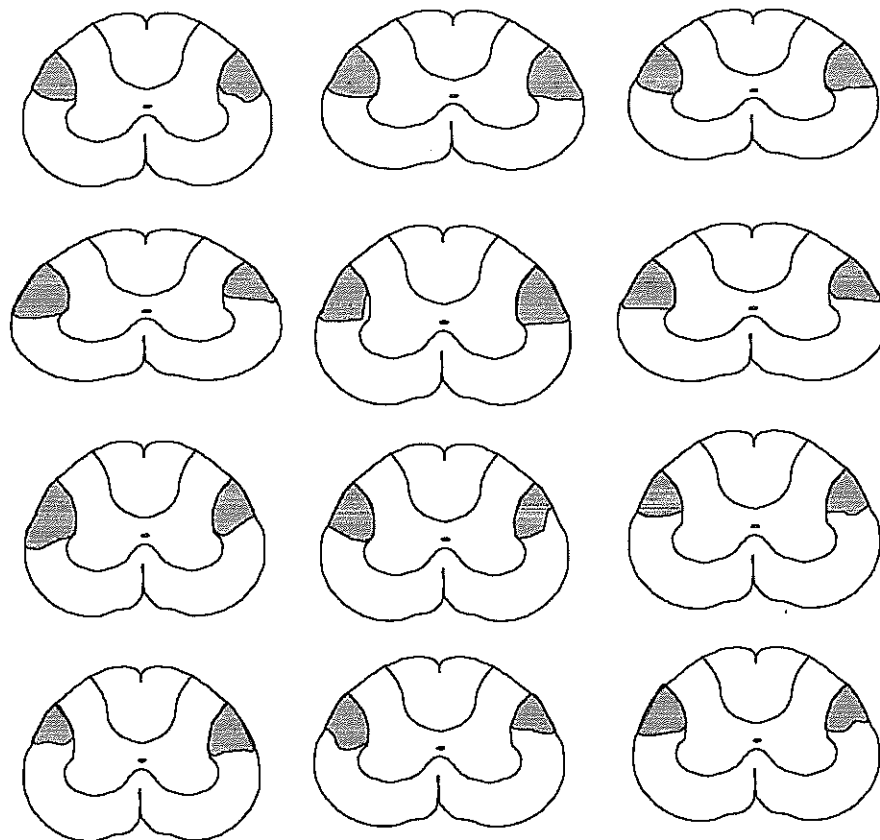


FIG. 1. Schematic drawings representing the lesion centre in the 12 animals used in this study. Animals not included in this study were those in which white matter damage extended into the dorsal funiculus, or extended in the lateral funiculi below a horizontal line level with the central canal.

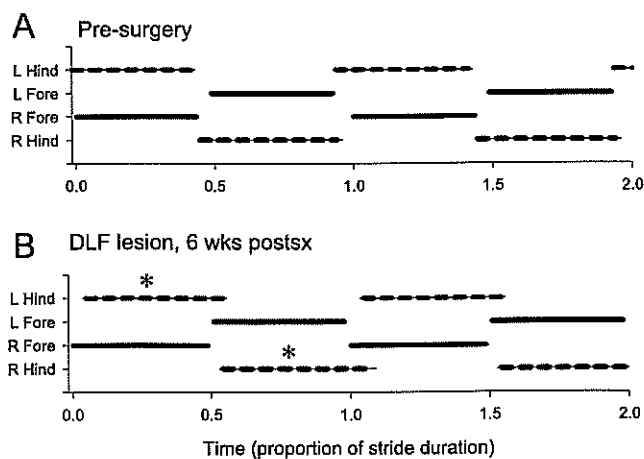


FIG. 2. Limb contact timing for animals (A, presurgery) before and (B) 6 weeks after DLF lesions;  $n = 7$ . Solid (forelimbs) and broken (hindlimbs) lines represent the portion of the stride when the limb was in contact with the ground. Both presurgical and DLF-lesioned animals moved at a trot, weightbearing on diagonal limbs. In DLF-lesioned animals, however, both onset and end times for the hindlimbs were delayed within the stride (\*). This resulted in times when three limbs were simultaneously in contact with the ground during the transition between diagonal limb pairs (i.e. at  $\sim 0.5$ , 1.0 and 1.5 proportion of stride units).

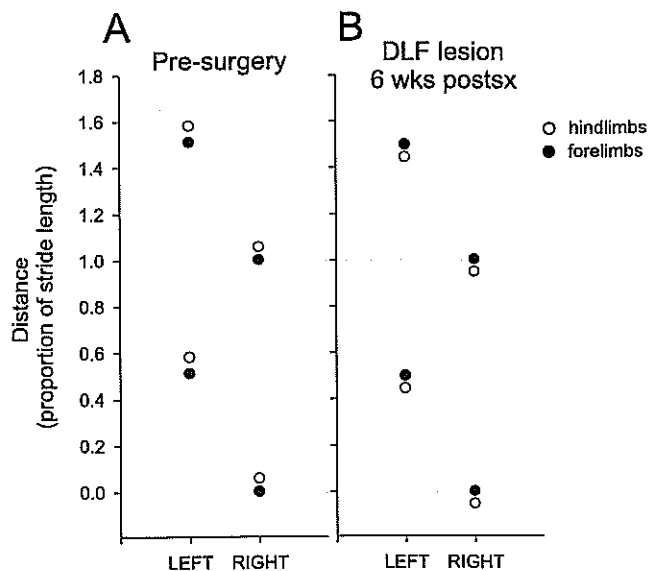


FIG. 3. Foot placement patterns for (A) presurgical and (B) DLF-lesioned animals;  $n = 7$ . Prior to surgery, rats placed their hindlimbs (○) ahead of the position of the previously placed ipsilateral forelimbs (●). DLF-lesioned animals placed their hindlimbs caudal to the position of the previously placed ipsilateral forelimbs.

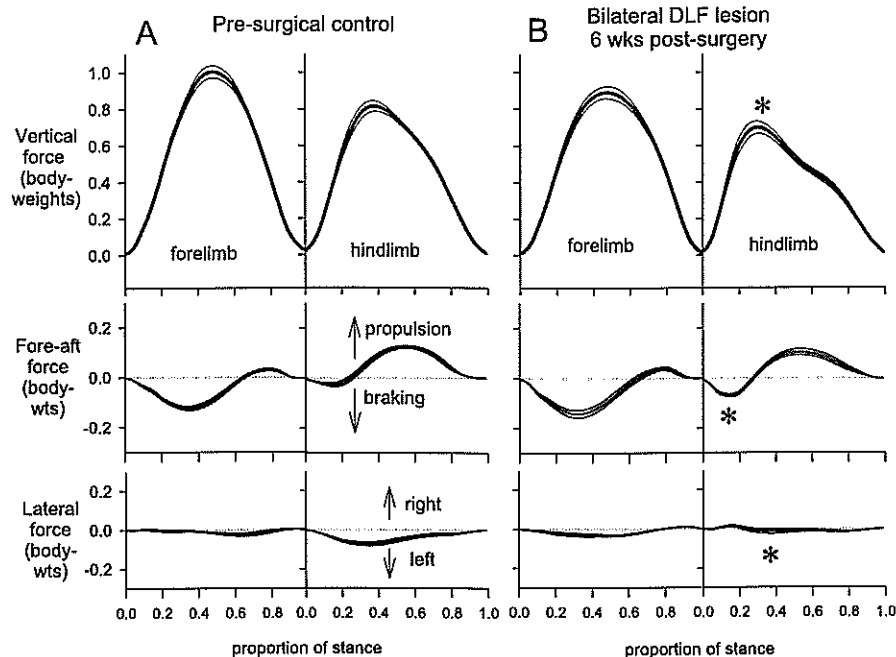


FIG. 4. Ground reaction forces produced by the forelimbs and hindlimbs for (A) presurgical and (B) DLF-lesioned animals;  $n = 7$ . For both time points, only forces for the left limbs are shown for clarity, as animals moved symmetrically throughout the study. Six weeks after surgery, DLF-lesioned animals moved with ground reaction forces similar to those produced prior to surgery, except that less weight was borne on the hindlimbs (reduced vertical forces), and the hindlimbs generated more braking and smaller lateral forces ( $*P < 0.05$  compared with pre-surgical values).

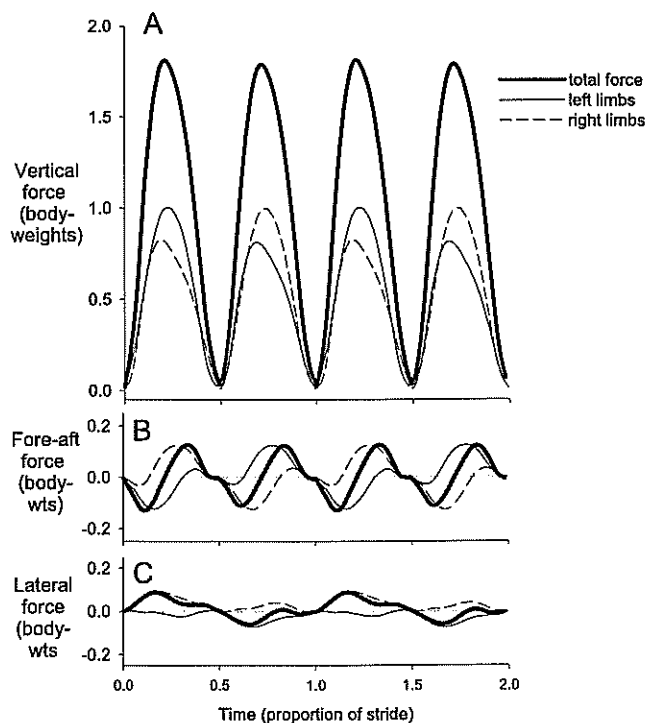


FIG. 5. Total ground reaction forces generated by presurgical animals;  $n = 7$ . (A) Vertical forces demonstrate the normal oscillation above and below 1.0 (i.e. body weight), as the animals trotted symmetrically overground. Alternation (B) between braking and propulsive forces, and (C) between right and left lateral forces, were the same as the forces produced by rats and other animals in previous studies.

around bodyweight and an equal alternation of braking and propulsive forces, demonstrating that animals were moving at a constant speed over the force platforms (Fig. 5).

Rats with DLF lesions moved very similarly to presurgical animals (average speed 69 cm/s) by 2 weeks postoperative, and their overall locomotor patterns did not change up to the 6th postoperative week, the last measurement period in this study. Even frame-by-frame examination of the 60-frame/s videotapes did not reveal differences in limb timing measurements. Nevertheless, more precise timing measurements obtained from the force platforms (sample frequency 1000 Hz) showed consistent differences from presurgical data (Fig. 2B). Most of the changes related to a delayed onset of the hindlimb during each stride cycle. Unlike presurgical animals, the hindlimbs of DLF animals consistently contacted the ground after the onset of the diagonal forelimb. For example, the time delay between the onset of the left forelimb and the onset of the right hindlimb was increased by an average of 0.06 s compared to pre-surgical animals, which was approximately 20% of the average stride duration ( $F = 8.020$ ,  $P = 0.001$ , see also Fig. 2). Contact durations for the hindlimbs were not different from presurgical values, however, such that the liftoff of the hindlimbs was also delayed during the stride. This resulted in an increase in limb timing overlap, such that there were two phases in each stride where three limbs were in ground contact simultaneously (e.g. at times 1.02–1.1 and 1.5–1.58 on the proportion-of-stride scale in Fig. 2B), an event which did not occur presurgically. Distance measurements also revealed a consistent difference in the position of the hindpaw in rats with DLF lesions (Fig. 3B;  $F = 33.031$ ,  $P < 0.001$ ). The hindpaws were placed behind the previous position of the ipsilateral forepaw. On average, the hindpaws were placed 2 cm behind their presurgical position. Changes in both timing and

distance measurements were present by 2 weeks postoperative and did not change by the 6th postoperative week.

Ground reaction forces produced by DLF-lesioned animals were very similar to those produced presurgery, except for small but significant changes in hindlimb forces (Fig. 4B). In particular, DLF-lesioned animals demonstrated reduced peak vertical force ( $F = 5.275$ ,  $P = 0.01$ ), increased braking force ( $F = 11.98$ ,  $P < 0.001$ ) and reduced lateral forces ( $F = 3.238$ ,  $P = 0.042$ ) produced by the hindlimbs (Fig. 4B). When individual limb forces were summed according to limb overlap to provide information on total ground reaction force, there were several differences between forces produced prior to surgery and those produced after surgery (Figs 5 and 6). Most significantly, vertical force never approached zero between diagonal limb pairs as closely as it did for presurgical animals. This is attributable to the increased overlap of the limb contact times seen in Fig. 2B. Total forces also revealed a reduction in the mediolateral forces produced by DLF-lesioned animals, indicating that these animals moved with less side-to-side motion than did animals prior to surgery (Figs 5 and 6).

#### Ladder locomotion

As previously found, intact rats moved across both ladders easily and made very few errors (slips or corrections) with the hindlimbs, although many did make a few minor corrections or slips with the forelimbs (Metz & Whishaw, 2002; Webb *et al.*, 2003). In contrast,

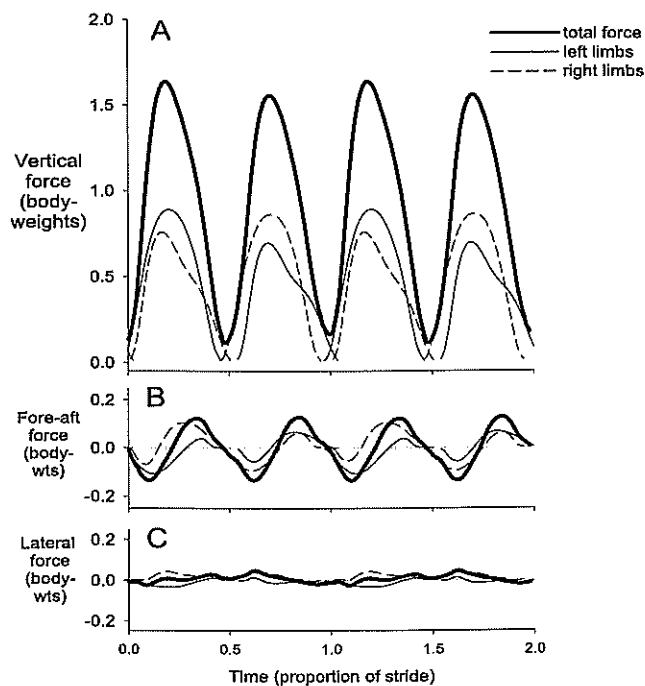


FIG. 6. Total ground reaction forces generated by DLF-lesioned animals;  $n = 7$ . (A) The increased overlap between limbs during the transitions between ground contact of diagonal limb pairs resulted in larger vertical forces at 0.5, 1.0 and 1.5 multiples-of-stride units, compared to corresponding times in Fig. 5A (i.e. minimum vertical forces remained well above zero in DLF-lesioned animals). (B) Values for alternating braking and propulsive forces indicated that DLF-lesioned animals moved at constant speeds, which were the same as for pre-surgical animals. (C) Reduced alternation between right and left lateral forces were the result of the smaller lateral forces generated by the hindlimbs in DLF-lesioned animals, as in Fig. 4B.

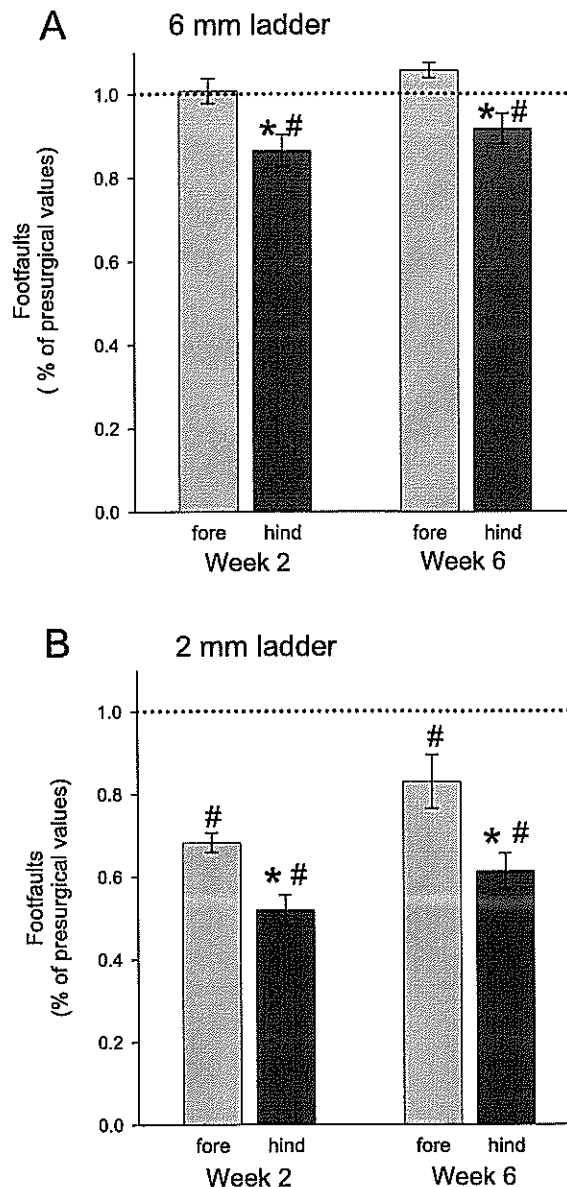


FIG. 7. Ladder footfaults produced by presurgical and DLF-lesioned animals at 2 and 6 weeks postsurgery. For both (A) the 6-mm-rung ( $n = 7$ ) and (B) the 2-mm-rung ( $n = 5$ ) ladders, the hindlimbs made more errors than the forelimbs at both time points after surgery (\* $P < 0.05$  vs. corresponding forelimb footfaults). On the 6-mm-rung (easy) ladder, only the hindlimbs made more footfaults than in presurgical performance. On the 2-mm-rung (difficult) ladder, animals made significantly more footfaults with both the forelimbs and the hindlimbs than in presurgical performance ( $^{\#}P < 0.05$  vs. 1.0).

DLF-lesioned animals made more footslip errors with the hindlimbs than with the forelimbs on both ladders (Fig. 7). On the 6-mm-rung (easier) ladder, there were no changes in the number of errors made by the forelimbs before or after surgery but the hindlimbs made significantly more errors than in presurgical performance (Fig. 7A;  $F = 7.234$ ,  $P = 0.002$ ). On the 2-mm-rung (difficult) ladder, DLF rats made more errors with both forelimbs and hindlimbs than in presurgical performance but again the hindlimbs were more affected than were the forelimbs (Fig. 7B;  $F = 9.207$ ,  $P = 0.001$ ).

### Spontaneous forelimb use

Spontaneous paw use during cylinder exploration was not affected by DLF lesions. There were no significant differences between forelimb contacts prior to surgery and forelimb contacts after surgery ( $P > 0.05$ , data not shown). These results differ from those of rats with unilateral DLF lesions, who used the forepaw ipsilateral to the lesion much less than the contralateral paw. (Webb & Muir, 2003)

### Skilled forelimb use

In contrast to spontaneous use, skilled paw use in the pellet-reaching task was affected by DLF transection. Animals were still able to reach for, and prehend, food pellets, but reaching success at all time points after surgery was lower than success prior to surgery ( $\sim 62$  compared to 82%; Fig. 8;  $F = 8.541$ ,  $P = 0.002$ ). Detailed analysis revealed that the supination movement which brings the food pellet to the mouth and the arpeggio movement were abnormal or missing in rats with DLF lesions, at all time points after surgery [Fig. 9;  $\chi^2 = 50.205$  (supination) and 51.26 (arpeggio),  $P < 0.001$ ].

### Discussion

This is the first study to describe altered hindlimb use during overground locomotion after bilateral cervical DLF transections in rats. The sensitivity and high sample rate of the ground reaction force measurements allowed us to detect small but consistent differences in locomotor patterns between intact and DLF-lesioned animals. Interestingly, all of the overground locomotor changes were associated with the hindlimbs. Hindlimb contact was delayed during the stride, and hindpaw placement occurred behind the ipsilateral forelimb

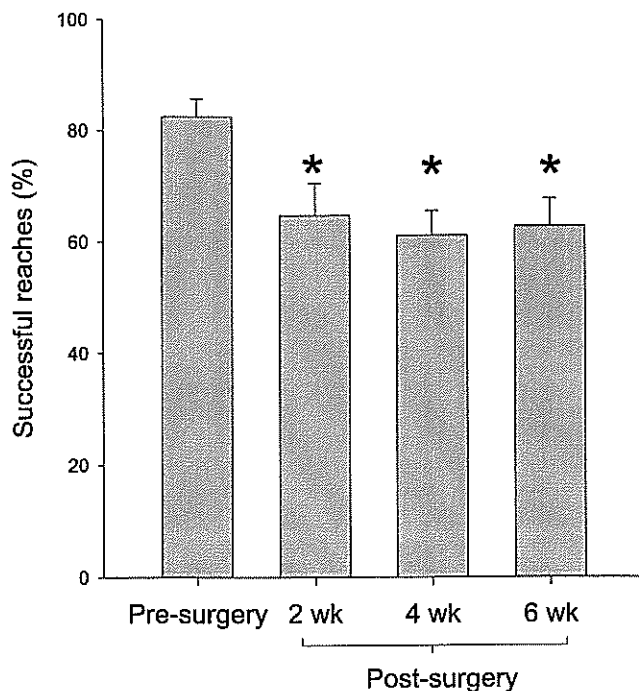


FIG. 8. Reaching success for presurgical and for DLF-lesioned animals at 2, 4 and 6 weeks postsurgery;  $n = 5$ . The performance of DLF-lesioned animals dropped to  $\sim 60$  from 80% prior to surgery, and did not change throughout the duration of the study ( $*P < 0.01$  vs. presurgical value). Bars represent group means  $\pm$  SEM.

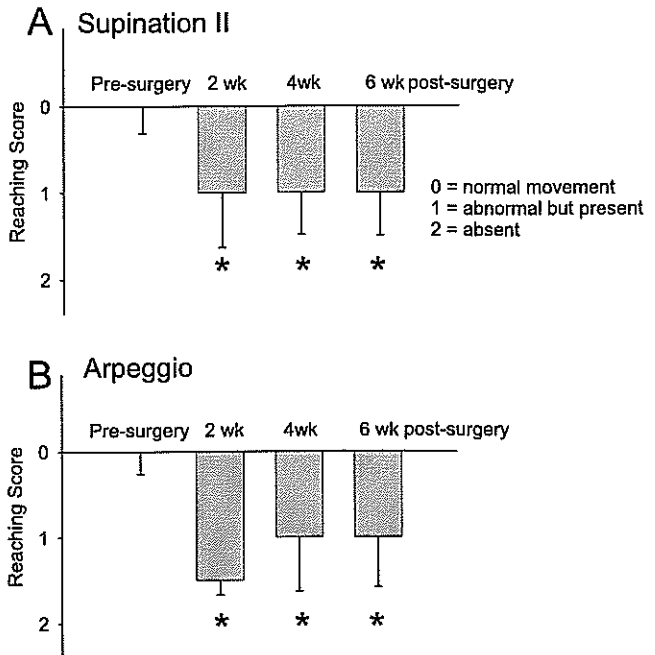


FIG. 9. Reaching scores for presurgical and DLF-lesioned animals at 2, 4 and 6 weeks postsurgery;  $n = 5$ . DLF-lesioned animals did not demonstrate normal supination (A) or arpeggio (B) movements (see text for explanation) while reaching for food pellets at any time postsurgery ( $*P < 0.001$  vs. presurgical values). Bars represent median value  $\pm$  95% confidence limits.

position. Ground reaction forces exerted by the hindlimbs were subtly altered after bilateral DLF transection. During skilled locomotion, DLF-lesioned animals made more errors with the hindlimbs than the forelimbs on ladders with different degrees of difficulty. During a test of skilled forelimb function, DLF rats displayed abnormal reaching movements and were less able to retrieve food pellets compared to their performance prior to surgery. Together, these results suggest a role for DLF pathways in the control of hindlimb function during overground and skilled locomotor tasks, and also support previous evidence for the contribution of the red nucleus and rubrospinal tract to skilled forelimb movements.

Although this is the first study to examine locomotor changes after bilateral cervical DLF lesions, the same lesions at the thoracic level in rats and cats have also resulted in altered hindlimb locomotion. Bilateral lesions of the thoracic DLF in rats caused altered interlimb coordination during overground locomotion (Hendriks *et al.*, 2006). These rats also made more hindlimb errors on a skilled locomotor task (traversing a horizontal rope) after thoracic DLF lesions, although many differences appear to have been related to differences in speed of movement between pre- and postoperative time points (Hendriks *et al.*, 2006). In the present study, animals were moving at similar velocities pre- and postinjury and all stride variables were normalized to either stride length or stride duration. In cats, bilateral DLF lesions at the thoracic level also resulted in hindlimb deficits during level locomotion, although these lesions include the main corticospinal tract in this species (Jiang & Drew, 1996).

While it is perhaps not surprising that thoracic-level lesions affect hindlimb locomotor action in rats and cats, the specific effect of cervical lesions on hindlimb function in the present study are somewhat unexpected. Examination of the anatomy of the cervical DLF reveals the presence of several ascending and descending pathways, including the dorsal spinocerebellar tract, the rubrospinal

tract, the lateral corticospinal tract and reticulospinal tract (Matsushita & Gao, 1997; Matsushita & Xiong, 1997; Matsushita, 1999; Houle & Jin, 2001a; Kuchler *et al.*, 2002; Bareyre *et al.*, 2005; Ballermann & Fouad, 2006). Of these, only damage to the dorsal spinocerebellar tract might immediately account for the selective effect of cervical DLF lesions on the hindlimbs. This pathway transmits proprioceptive information, including information about locomotor limb coordination, from the lower thoracic and lumbosacral spinal cord to the cerebellum (Poppele *et al.*, 2003). These axons travel in the lateral funiculus and undergo a dorsal shift to the DLF during their rostral ascent (Snyder *et al.*, 1978; Yamada *et al.*, 1991; Xu & Grant, 1994; Matsushita & Gao, 1997; Matsushita & Xiong, 1997; Terman *et al.*, 1998; Matsushita, 1999). Spinocerebellar input from the forelimbs, however, is located more laterally and ventrally in the lateral funiculus, along with additional spinocerebellar input from the hindlimbs, and thus would be less damaged by DLF lesions (Xu & Grant, 1994). Reduced proprioceptive input specifically from the hindlimbs in DLF rats could preferentially affect hindlimb control during overground locomotion (Figs 2–4 and 6) and could also explain why the hindlimbs were consistently more affected than the forelimbs during skilled ladder locomotion (Fig. 7).

Of the remaining known pathways in the DLF, the rubrospinal tract is the most prominent. DLF axotomy will arguably have its greatest effect on the function of this pathway, in that all rubrospinal axons are axotomized by DLF lesions. It is possible that loss of rubrospinal input to the spinal cord contributed to locomotor changes seen in the present study. Although much of what is known regarding rubrospinal anatomy and function focuses on forelimb control, there is evidence that rubrospinal input also contributes to hindlimb function. The rubrospinal tract projects to all levels of the cord in rats, including the lumbar cord, with neurons somatotopically arranged within the red nucleus (Murray & Gurule, 1979; Shieh *et al.*, 1983; Jankowska, 1988; Antal *et al.*, 1992; Schwartz *et al.*, 2005). In cats, rubrospinal neurons have been shown to be phasically modulated with the swing phase of the hindlimbs during locomotion and some rubrospinal neurons have receptive fields which are limited to the hindlimbs (Lavoie & Drew, 2002; Orlovsky, 1972). The same research also suggests a role for rubrospinal neurons in interlimb control, in addition to distal control of forelimb musculature (Lavoie & Drew, 2002).

Lesion studies involving the red nucleus could provide some insight into the possible contribution of rubrospinal input to motor behaviour in the rat. Unfortunately, virtually all studies have been limited to unilateral lesions (Kennedy & Humphrey, 1987; Whishaw *et al.*, 1992; Whishaw & Gorny, 1996; Muir & Whishaw, 2000). We have previously investigated the locomotor effects of unilateral red nucleus lesions, which removes both rubrospinal and rubro-olivary projections, and found alterations in both fore- and hindlimb forces and timing during overground locomotion (Muir & Whishaw, 2000). Interestingly, these changes were primarily associated with the forelimb from which rubrospinal input was removed and with the hindlimb which retained its rubrospinal innervation (Muir & Whishaw, 2000). There were no changes associated with the hindlimb from which rubrospinal input was removed (Muir & Whishaw, 2000). These results then do not directly support a role for rubrospinal axotomy in production of hindlimb changes in the present study. It should be noted, however, that those compensatory changes which occurred after unilateral red nucleus lesions were very similar to those seen after unilateral lesions of several different and unrelated CNS regions, e.g. cervical spinal dorsal column transection, ventrolateral funicular lesions or striatal dopamine depletion (Muir & Whishaw, 1999a,b; Webb & Muir, 2003, 2004, 2005). The similarities between

the locomotor changes seen with these different unilateral CNS lesions led us to propose that rats develop a common compensatory response to unilateral CNS damage that might mask deficits that are specific to the loss of each different pathway (Muir & Whishaw, 2000; Webb & Muir, 2003, 2005).

In addition to rubrospinal axons, the lateral corticospinal tract and reticulospinal tract were also axotomized by DLF transection in the present study. The connections of either of these DLF pathways might be involved in motor control, among other functions. Damage to lateral corticospinal axons in the present study is not a probable cause of the overground locomotor changes seen, however. The lateral corticospinal pathway composes a small percentage of corticospinal input to the cord in rats and, in fact, removal of all corticospinal input by transection of the medullary pyramids has essentially no lasting effects on overground locomotion (Metz *et al.*, 1998; Muir & Whishaw, 1999a). Nevertheless, both corticospinal and rubrospinal inputs have been implicated in control of skilled movements, and loss of these inputs might contribute to the increased footfall errors for both fore- and hindlimbs which persist in the 2-mm-rung ladder task in DLF-lesioned rats (Fig. 7B). Some reticulospinal pathways, alternatively, have been shown to be involved in the ongoing control of locomotion. Spinal projections from numerous reticular nuclei are present diffusely throughout the dorsolateral, lateral and ventral funiculi (Houle & Jin, 2001b; Ballermann & Fouad, 2006). Specific behavioural functions have not been ascribed to reticular axons in particular funiculi, although it is well known that reticulospinal axons in the ventrolateral funiculus have several functions, including initiation of brainstem-stimulated locomotion in decerebrate animals and transmission of alternating rhythmic stimulation to phrenic motoneurons (Steeves & Jordan, 1980; Dick *et al.*, 1988). It is possible that reticulospinal axons in the DLF might be involved in ongoing control of locomotor movements as well although, similar to the rubro- and corticospinal tracts, there is no evidence available on the differential influence of this input to the forelimbs compared to the hindlimbs (Houle & Jin, 2001a; Loy *et al.*, 2002a,b).

Comparisons with our previous work suggest that rats make different compensations for unilateral lesions compared to bilateral lesions of the same pathway. As mentioned previously, we have shown that unilateral DLF lesions produce clear ipsilateral forelimb deficits during locomotion (Webb & Muir, 2003). These forelimb deficits were also apparent during ladder locomotion on a 6-mm-rung (easy) ladder, as well as during the spontaneous cylinder exploration task (Webb & Muir, 2003). In contrast, forelimb changes were not present after bilateral DLF lesions on any of these tasks in the present study, and only became apparent when rats were required to walk on a 2-mm-rung (difficult) ladder (Fig. 7B). It is possible that forelimb deficits were present after bilateral DLF lesions but were completely masked through altered hindlimb action during overground and easy-ladder locomotion. Nevertheless, the method by which animals compensate for bilateral lesions during relatively unchallenging tasks, including locomotion and cylinder exploration, is clearly qualitatively different than that for unilateral lesions, and this needs to be kept in mind when attempting to interpret these changes as being reflective of the function of particular pathways.

The present results also support well established findings that DLF pathways, particularly the rubrospinal tract and possibly the lateral corticospinal tract, are important for control of skilled forelimb movements. Bilateral lesions of the dorsolateral funiculi in rats resulted in reaching deficits which persist for at least 4 weeks postlesion (Schrimsher & Reier, 1993). In a different study, unilateral DLF lesions also resulted in deficits in reaching success which



eventually recovered, although components of the reaching movement, including supination and arpeggio as seen in the present study, were persistently abnormal (Fig. 9; also McKenna & Whishaw, 1999). Lesions of the red nucleus, the source of neurons for the rubrospinal tract, result in movement deficits during reaching in rats, again including absence of the arpeggio movement (Whishaw *et al.*, 1998). In cats, lesions of the dorsolateral funiculus which spared some fibres of the rubrospinal tract resulted in a more rapid recovery of reaching than did lesions which completely destroyed the rubrospinal tract (Pettersson, 1999). Anatomical and physiological evidence also supports the contribution of the rubrospinal tract toward control of skilled forelimb movement. The rubrospinal tract forms direct motoneuronal contacts with forelimb motoneurons, although most contacts are probably with interneurons (Brown, 1974; Kuchler *et al.*, 2002). These direct connections were seen with motoneurons innervating muscles of the intermediate and distal limb and not with proximally located limb muscles. Similarly, electrophysiological evidence supports a closer connection between rubrospinal axons and distal forelimb muscles than more proximal muscles (Kuchler *et al.*, 2002).

In conclusion, bilateral DLF transection at the cervical level produces forelimb deficits during skilled movements, but also produces previously undescribed changes in hindlimb action during overground and skilled ladder locomotion. While previous evidence suggests that loss of the rubrospinal tract is the probable cause of the forelimb deficits, the hindlimb deficits could most directly arise from damage to ascending proprioceptive pathways in the DLF, and possibly from loss of descending rubro- and reticulospinal tracts. The sensitivity of ground reaction force recordings suggests that these measurements would be a good functional locomotor assay for animals with partial spinal injuries, particularly when these injuries include the DLF. Research which focuses on regeneration of pathways located in the DLF stands to benefit from functional assessments which include ground reaction force analysis.

## Abbreviation

DLF, dorsal part of the lateral spinal funiculus.

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