

# Compensatory Locomotor Adjustments of Rats with Cervical or Thoracic Spinal Cord Hemisections

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## ABSTRACT

The accurate measurement of behavioral compensation after CNS trauma, such as spinal cord injury, is important when assessing the functional effects of injury and treatment in animal models. We investigated the locomotor abilities of rats with unilateral thoracic or cervical spinal cord injuries using a locomotor rating (BBB) scale, reflex tests, and quantitative kinetic measurements. The BBB rating scale indicated that thoracic spinal hemisected (TH) rats had more severely affected hindlimbs compared to cervical spinal hemisected (CH) and sham-operated animals. Kinetic measurements revealed that CH and TH animals moved with different ground reaction force patterns, which nevertheless shared some similarities with each other and with the gait patterns of rats with different unilateral CNS lesions. Uninjured rats typically had an equal distribution of their body weight over the forelimbs and hind limbs, and used their forelimbs predominantly for braking while using their hind limbs mostly for propulsion. CH rats bore more weight on their hind limbs than their forelimbs, while TH animals bore more weight on their forelimbs than their hind limbs. Neither CH nor TH rats used the forelimb ipsilateral to the spinal hemisection for net braking or propulsion. The hindlimb contralateral to the hemisection was placed on the ground prematurely during the stride cycle for both CH and TH animals. The altered kinetics of the locomotor pattern in hemisected animals resulted in changes in the oscillations of total body potential and kinetic energies. These two forms of energy oscillate synchronously in intact locomoting rats, but were asynchronous during parts of the stride cycle in spinal hemisected animals. We conclude that rats develop a general compensatory response for unilateral CNS lesions, which may help stabilize the animal during locomotion.

**Key words:** gait; ground reaction forces; locomotion; motor control

## INTRODUCTION

**T**RAUMATIC SPINAL CORD INJURY afflicts 17–50 per million people annually (Chen et al., 1997; Hoque et al., 1999; Karamehmetoglu et al., 1997; National Spinal Cord Injury Statistical Center, 2000; Surkin et al., 2000). Al-

though the incidence is low, many people are afflicted at less than 40 years of age (Chen et al., 1997; Hoque et al., 1999; Karamehmetoglu et al., 1997; Surkin et al., 2000), and consequently the prevalence of spinal cord injury is high. There are approximately 250,000 people living with spinal cord injury in the United States alone (Ergas, 1985;

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National Spinal Cord Injury Statistical Center, 2000). Because of the high demand for a cure and for better treatments for spinal cord injury, there has been a great amount of effort to assess the benefits of treatments on behavioral motor recovery, including locomotor recovery. Most fundamental knowledge regarding the neural circuitry controlling overground locomotion has been obtained from a variety of cat preparations. Only recently has there been an interest for a more complete understanding and integration of knowledge concerning neural control mechanisms and responses to particular spinal cord injuries in locomoting rats—popular animals used by researchers studying spinal cord injury. Much of the knowledge gained from research in cat models of locomotion has been adopted and applied to spinal cord injury in the rat. These assumptions may not be valid for a variety of reasons, which include (1) phylogenetic differences in neuroanatomy and (2) morphometric differences that exist between rats and more cursorial animals such as cats. Consequently, there is a demand for novel findings regarding the locomotor abilities in spinal cord injured rats, which may provide valuable insight into the roles of different neuroanatomical pathways during overground locomotion in the rat and their response to spinal cord injury.

Terrestrial locomotion of quadrupedal species involves both a swing phase (when the foot is not in contact with the ground) and a stance phase (when the foot is in contact with the ground). There are very few studies investigating the compensations that rats make during the stance phase of the locomotor cycle (Kunkel-Bagden et al., 1992; Muir and Whishaw, 1999a, 2000). Importantly, the forces that the animal exerts on the ground during the stance phase provide unique, objective, and quantitative information regarding compensations that animals make for CNS injury (Muir and Whishaw, 1999a,b, 2000). Herein we describe, for the first time, compensations made during the stance phase of freely behaving unilateral cervical or thoracic spinal hemisectioned (CH or TH) rats, and compare them with sham-operated controls. We have previously demonstrated that rats make similar locomotor compensations for a variety of unilateral CNS injuries. We were interested in determining whether similar behavioral compensations would be made for spinal cord injury at different levels of the spinal cord, that is, thoracic versus cervical. The results indicate that behavioral compensations made following unilateral spinal cord injury are similar to other unilateral lesions produced in the CNS. These compensations may result from the constraints imposed upon terrestrial locomotion (Muir and Whishaw, 2000) or may be the consequence of innate, plastic changes that occur within the CNS. These results, taken together with previously published infor-

mation, lend novel insights into the response of rats to unilateral CNS lesions, which has direct impact on those investigating repair strategies for CNS, especially spinal cord, injuries. These results are interpreted and discussed in light of phylogenetic differences in neuroanatomy seen between rats and other species, the neurophysiological basis for locomotion, and what is known about locomotor changes following other unilateral CNS lesions in rats and cats.

## EXPERIMENTAL PROCEDURES

### *Animals*

Thirteen adult female Long-Evans rats (Charles River Canada, Quebec, Canada) weighing 250–320 g were used. Rats were fed restricted amounts of food to maintain constant body weights and to ensure they were motivated to perform the locomotor task. All rats were housed as pairs or trios in cages in the laboratory animal care facility, with 12-h light/12-h dark controlled lighting, at the Western College of Veterinary Medicine at the University of Saskatchewan. All animals were examined daily by a veterinarian and were cared for according to the standards set out by the Canadian Council on Animal Care.

### *Training*

Animals were trained to run the length of a 182 × 20 cm runway for a food reward once daily, 5 days per week for at least 2 weeks prior to surgery. Surgery was performed after all rats were proficient at completing the task. In addition, animals were habituated to an open field environment prior to behavioral assessments using the Basso, Beattie, and Bresnahan (BBB) scoring system (Basso et al., 1995).

### *Anesthesia and Analgesia*

Sham-operated control groups were comprised of two animals each. Five rats underwent left lateral cervical hemisection at vertebra C3. Four rats underwent left lateral thoracic spinal cord hemisection at vertebra level T9–10. All rats were premedicated 30 min prior to general anesthesia with 0.05 mg/kg buprenorphine hydrochloride (Buprenex, Reckitt & Colman Pharmaceuticals, VA) and 0.05 mg/kg atropine (MTC Pharmaceuticals, ON, Canada) administered subcutaneously. Preemptive analgesia allowed a surgical plane of anesthesia to be induced using 35–40 mg/kg sodium pentobarbital (Somnotol, MTC Pharmaceuticals) administered intraperitoneally (Roughan et al., 1999). Following induction of anesthesia, rats were kept warm on a recircu-

lating warm water blanket. Respiratory rates, heart rates, and pedal withdrawal responses were assessed periodically throughout the surgical procedure to ensure that a surgical plane of anesthesia was maintained. All rats were administered an additional dosage of buprenorphine (0.05 mg/kg) subcutaneously 12 h after the initial dose, thereby providing 24 h of analgesia.

### *Surgery*

All rats were administered 30 mg/kg methylprednisolone sodium succinate (MPSS; Solu-Medrol, Pharmacia & Upjohn Inc., ON, Canada) intraperitoneally upon induction of general anesthesia. MPSS was administered to prevent exacerbation of the mechanical injury from secondary injury mechanisms (Oudega et al., 1999; Webb and Muir, 2000). Rats were routinely surgically prepared and draped. Rats underwent either cervical or thoracic dorsal laminectomies at either vertebra C3 or T9/T10, respectively, using a Zeiss Stemi 2000C surgical microscope for magnification. Briefly, skin and superficial muscle were incised, and epaxial musculature was bluntly dissected from the appropriate dorsal spinous process and the lamina of the vertebrae. The dorsal spinous process was removed using a pair of microrongeurs. Laminectomy of the appropriate vertebra was performed using microrongeurs. A durotomy was made using microscissors, and the spinal cord was left laterally hemisected using a pair of microscissors. An autologous fat graft, obtained subcutaneously near the surgery site, was placed over the laminectomy site to prevent fibrous adhesions to the spinal cord and dura (Pospiech et al., 1995). Overlying muscles were closed using 3-0 braided polyglycolic acid (Dexon II, Sherwood Davis & Geck, St. Louis, MO) in a simple continuous pattern. Skin was closed using skin staples (Appose ULC, Davis & Geck). All rats had skin staples removed 10 days following surgery. Sham-operated animals (two cervical and two thoracic) were treated similarly except that no damage was done to the spinal cord.

### *Behavioral Assessments*

**BBB scoring.** A commonly used method for categorizing locomotor abilities, BBB scoring system (Basso et al., 1995), was employed as part of our methods to describe behavioral compensations. All rats were assessed weekly using the BBB scoring system as previously described (Basso et al., 1995). Ground reaction forces and proprioceptive/reflex tests were not performed until the rat scored at least 15 (for the lowest scoring hindlimb) on the BBB scoring system, with no further change in their score for at least 1 week. Rats also had to be able

to consistently locomote, within a runway, for food reward without dragging their paws. These criteria were met for all rats by 40 days following surgery.

**Reflex testing.** Several proprioceptive and reflex tests were performed by an observer blinded to whether an animal had undergone hemisection or not. Reflexes were tested following ground reaction force data collection for all rats. Reflex testing consisted of lateral tactile placing, hopping, and pedal withdrawal (Bradley et al., 1983; Kunkel-Bagden et al., 1992; Sechzer et al., 1984; Wolgin, 1985). Each limb of each rat was tested separately for each of the reflexes. Briefly, lateral tactile placing was assessed by covering the animal's eyes and moving the animal toward a table top until the lateral surface of the paw touched the edge of the table. A normal response for this reflex was to place the paw on the surface of the table. This test assesses sensory and motor function (Bradley et al., 1983; Metz et al., 2000a).

Hopping involved holding the animal above a hard surface and allowing only one paw to touch the surface and to bear weight. The animal was moved laterally to the weight-bearing paw. The normal response was to pick up and place the paw down again as the body passed over the paw. This test requires intact proprioception and motor function (Kunkel-Bagden et al., 1992; Little et al., 1988; Wolgin, 1985). This test was performed for each limb of each rat.

Pedal withdrawal responses were tested by manually applying pressure to a single digit on each limb. The normal response is flexion of all the joints of the limb. All animals were videotaped while performing the reflex tests, so as to provide a permanent record and in the event that further evaluation was required. Animals were scored qualitatively using a scale of 0–3, where 3 is an exaggerated response (e.g., hypermetria for hopping; clonic response for withdrawal); 2 is a normal response (compared to normal animals); 1 is a decreased/inconsistent response (increase in latency or number of times to complete withdrawal, placing, or hopping); and 0 is an absent response (unable to complete task).

Data was not normally distributed and was analyzed using analysis of variance on ranked data (SigmaStat, Chicago, IL).

**Ground reaction forces.** Measuring the forces exerted on the ground during locomotion has been shown to be a sensitive and reliable means by which one can assess the contribution of individual limbs to overground locomotion (Bertram et al., 1997; Cavagna et al., 1977; Clarke and Still, 1999; Muir and Wishaw, 1999a,b, 2000; Muir et al., 1998). This method has been employed as a method of determining the effects of several different unilateral

lesions of the central nervous system (Muir and Whishaw, 1999a,b, 2000; Muir et al., 1998).

Three force platforms were built based on the design of platforms used in previous work assessing gaits of locomoting chicks and rats (Muir and Whishaw, 1999a,b, 2000; Muir et al., 1996, 1998). The force platforms measured  $10.5 \times 11$  cm each, and were arranged in series embedded in the runway used for training the rats. Force platforms measured three-dimensional ground reaction forces (i.e., vertical, fore-aft, and mediolateral). Collection of ground reaction force data was triggered by the rat breaking an infrared beam (also triggering an LED timer) placed across the runway ahead of the force platforms. Ground reaction force data was amplified analogue-to-digital converted (RC Electronics, San Raphael, CA) at a sampling frequency of 1,000 Hz. Criteria for a successful run included (1) rat must have appeared to be traveling at a constant velocity as it passed over the force plates, (2) paw placement needed to be close to the center of each plate, and (3) at least two platforms and ideally three platforms must have been hit during the run. Data from successful runs was saved in ASCII format. During the data collection, animals were being videotaped from a lateral aspect (Panasonic AG-456U S-VHS, Secaucus, NJ) with a shutter speed of 1/500. Each animal was weighed after successfully completing 15 successful runs.

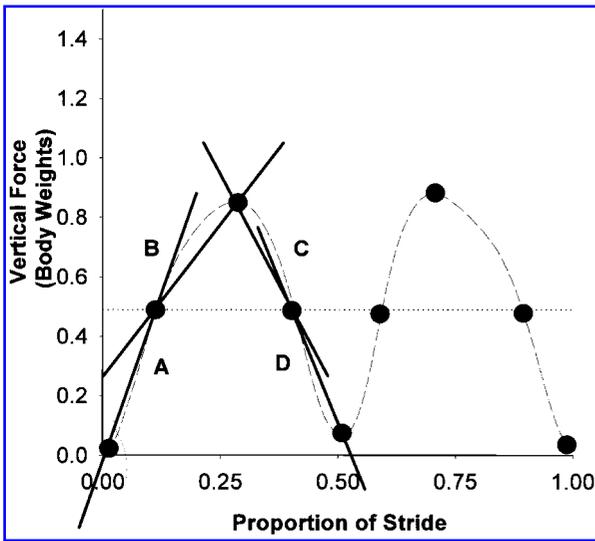
### Data Analysis

*Histologic evaluation of lesion site.* After all behavioral tests were completed, rats were deeply anesthetized with 70 mg/kg sodium pentobarbital (Somnitol, MTC Pharmaceuticals) administered intraperitoneally, and were transcardially perfused with 250 mL of heparinized 0.1 M PBS followed by an equal volume of 4% paraformaldehyde dissolved in 0.1 M PBS. Spinal cords were dissected and postfixed in 4% paraformaldehyde/0.1 M PBS for 24 h. Spinal cords were routinely processed and embedded in paraffin. Cords were serially and transversely sectioned at  $8 \mu\text{m}$  throughout the injury site and mounted on slides coated with poly-L-lysine and were stained with either crystal violet or luxol fast blue/crystal violet. All sections were examined by light microscopy to determine the maximum size of the lesion for each rat. Only rats having nearly complete histological disruption of the left lateral half of the spinal cord were included in this study. No rats were excluded from this study subsequent to histological evaluation.

*BBB scoring.* BBB scores were assigned to each animal based on previously published scoring criteria (Basso et al., 1995). Both right and left hind limbs were scored

independently. The Mann-Whitney rank sum test was performed to detect differences between groups. Inter-group differences in the length of time to reach a BBB score of 15 for the left (bad) hind limb were determined using single-factor analysis of variance. Differences were considered significant if  $p < 0.05$ .

*Ground reaction forces.* Single-frame video analysis was used to determine the order of limb pairs that hit each plate. For each successful run, animals moved across the plates so that ipsilateral limbs would hit a given plate. For example, if the left forelimb hit plate one, followed by the left hind limb, then the right limbs would hit plate two, and the left limbs would finally hit plate three. For successful runs, ground reaction force data was imported into spread sheets and filtered using a low-pass recursive filter (SPSS, Chicago, IL). Ground reaction forces were normalized for the animal's body weight and transformed to yield a proportion of body weight over a normalized proportion of stride using custom written software (SPSS). Ground reaction forces and velocity (obtained from the LED timer) were averaged for each individual animal using custom written software (Microsoft Visual Basic, Microsoft Corp., San Francisco, CA). Data was separated into either fast (50–100 cm/sec) or slow ( $< 50$  cm/sec) speeds. Ground reaction forces were kept separate for both the right and left limb pairs. Averages and standard errors for left limb, right limb, and total ground reaction forces, and mean overlap between right-left and left-right limb pairs for each group were calculated using custom written software (Microsoft Visual Basic, Microsoft Corp.). Data from limb pairs were aligned appropriately using mean overlap data. Total ground reaction force data was obtained by summing the correctly aligned left and right limb pairs. Group data was considered valid if there was less than or equal to 2% net acceleration or deceleration per stride (Farley et al., 1993). Components of the ground reaction force data analyzed consisted of (1) rate of increases and decreases in limb loading for individual limbs; (2) peak vertical, propulsive, and braking forces for individual limbs and diagonal limb pairs; (3) vertical impulse for individual limbs, and (4) net fore-aft impulse for individual limbs. Briefly, rates of limb loading and unloading for individual limbs were determined by measuring slopes for four components of the vertical force/time curve (Fig. 1). Slopes were calculated for initial limb loading, that is, from 0 to 0.5 body weights (BW; Fig. 1A), for limb loading after 0.5 BW to peak vertical force (Fig. 1B), limb unloading from peak vertical force to 0.5 BW (Fig. 1C), and limb unloading from 0.5 BW to 0 (Fig. 1D). Peak forces generated by individual limbs and diagonal limb pairs were taken directly from the force/time curves for



**FIG. 1.** Model for calculating rates of limb loading and unloading. Diagram showing calculation of rates of loading and unloading of limbs using vertical ground reaction forces. (A) Initial rate of loading = slope of straight line from 0.0–0.5 body weights. (B) Final rate of loading = slope of straight line from 0.5 to peak body weight support. (C) Initial rate of unloading = slope of straight line from peak body weight support to 0.5 body weights. (D) Final rate of unloading = slope of straight line from 0.5–0.0 body weights.

the vertical and fore-aft curves. Vertical impulse was determined by calculating the area under the vertical force/proportion of stride curve. Net fore-aft impulse was determined by summing the area under the curve in the braking direction with the area under the curve for the propulsive direction, thereby yielding the net fore-aft impulse, which indicates the relative contribution of the limb for propulsing or braking. Comparisons between groups were made using single factor analysis of variance (SigmaStat). Post hoc analysis for differences between groups was performed utilizing Bonferroni's *t* test (SigmaStat). Parameters compared were considered significant if  $p < 0.05$ .

Averaged total vertical and fore-aft ground reaction forces were used to determine potential and kinetic energy fluctuations throughout a stride, respectively. Potential and kinetic energy changes were calculated using custom written software (Visual Basic, Microsoft Corp.) after the method of Cavagna (1975). Briefly, total vertical forces and fore-aft forces (expressed in body weights) were converted to acceleration ( $\text{m/sec}^2$ ). Acceleration due to gravity ( $9.8 \text{ m/sec}^2$ ) was subtracted from total vertical acceleration, and the difference was then integrated twice with respect to time to yield vertical displacement of the body's center of mass. The integration constant for the

first integration of total vertical force was assumed to be zero because the average change in height of the body's center of gravity is zero over a number of steps while moving over a horizontal surface. Potential energy changes were calculated over time using the following equation:

$$E_p = mgP$$

where  $E_p$  is potential energy (J),  $m$  is body mass (kg),  $g$  is acceleration due to gravity ( $\text{m/sec}^2$ ), and  $P$  is vertical displacement (m) of the body's center of mass.

Velocity in the forward direction was calculated by integrating total fore-aft acceleration once with respect to time using the mean group velocity as the integration constant. Kinetic energy in the forward direction was then calculated over time using the following equation:

$$E_k = 1/2 mv^2$$

where  $m$  is body mass (kg), and  $v$  is velocity in the forward direction ( $\text{m/sec}$ ).

Kinetic and potential energies were indirectly derived from ground reaction force data and, as such, were not analyzed statistically. These energy changes were calculated and examined to provide descriptive information of the movement of the center of mass for each group of animals during locomotion.

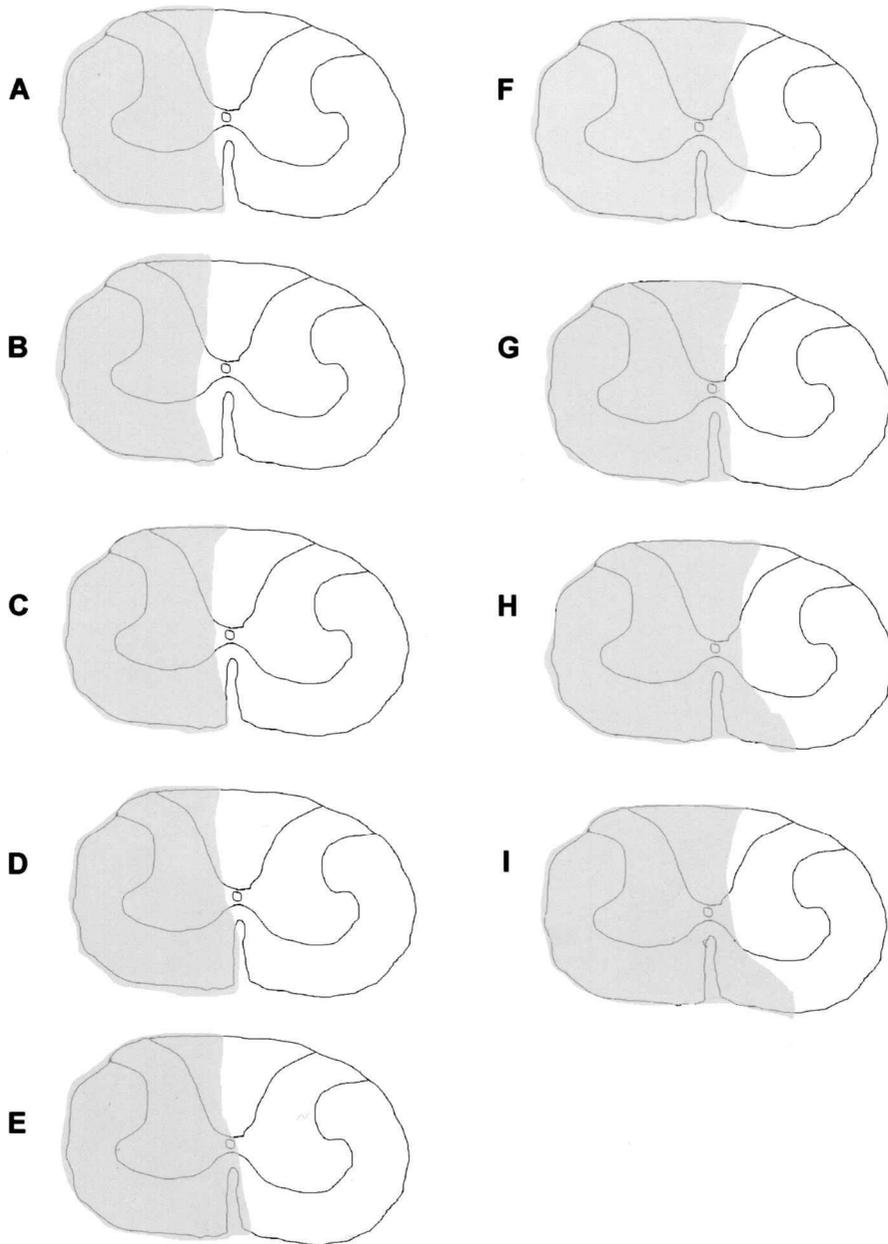
## RESULTS

### Histology

Sham-operated controls had no evidence of intraparenchymal inflammation or tissue destruction and had very few dural adhesions present. Cervical spinal hemisections (CH; Fig. 2A–E) typically spared a small amount of the ipsilateral dorsal and ventral funiculi. Thoracic spinal cord hemisections (TH; Fig. 2F–I) were 100% complete and included some of the contralateral dorsal and ventral funiculi. The overlying dura was adherent to the spinal cord throughout the level of the injury site for both cervical and thoracic spinal hemisectioned animals. All animals with spinal hemisection were included in the study.

### BBB Scores

*Left hindlimb.* Three CH rats achieved a BBB score of at least 15 for the left (bad) hind limb within the first week postoperatively. All CH rats ( $n = 5$ ) had achieved and surpassed a score of 15 by 2 weeks postoperatively. Only two TH animals had achieved a score of at least 15 by 2 weeks postoperatively, one TH rat achieved a score of at least 15 by 3 weeks postoperatively, and the final



**FIG. 2.** Completeness of cervical and thoracic spinal hemisections. Schematic drawings depicting lesion epicenters for five cervical (A–E) and four thoracic (F–I) spinal hemisected rats. Cervical (A–E) spinal hemisections typically had some sparing of the left (ipsilateral) dorsal and ventral funiculi. Thoracic (F–I) spinal hemisections were complete and included variable amounts of right (contralateral) dorsal and ventral funiculi.

TH rat achieved a score of 15 during the 6th postoperative week. No TH rats received a score higher than 16 for the duration of the study. Although, statistically, TH rats did not take longer to recover compared to CH rats, they did take longer than sham-operated controls ( $F_{(2,10)} = 4.9$ ;  $p = 0.033$ ). CH rats did not take statistically longer to recover compared to sham-operated con-

trol rats. Statistical power was lacking when comparing CH rats with TH rats; therefore, one cannot discount the possibility that CH rats generally take less time to reach a BBB score of 15 compared to TH rats. Because of our small sample sizes, frequency of BBB score determination, and the relatively speedy rate of recovery for CH rats, we were unable to conclude that CH rats recover

more of their hind limb locomotor abilities in a shorter amount of time compared to TH animals. Future studies investigating the rapidity of recovery for CH and TH rats will need to score animals daily using the BBB scoring system, as recovery is generally very rapid with these partial lesions.

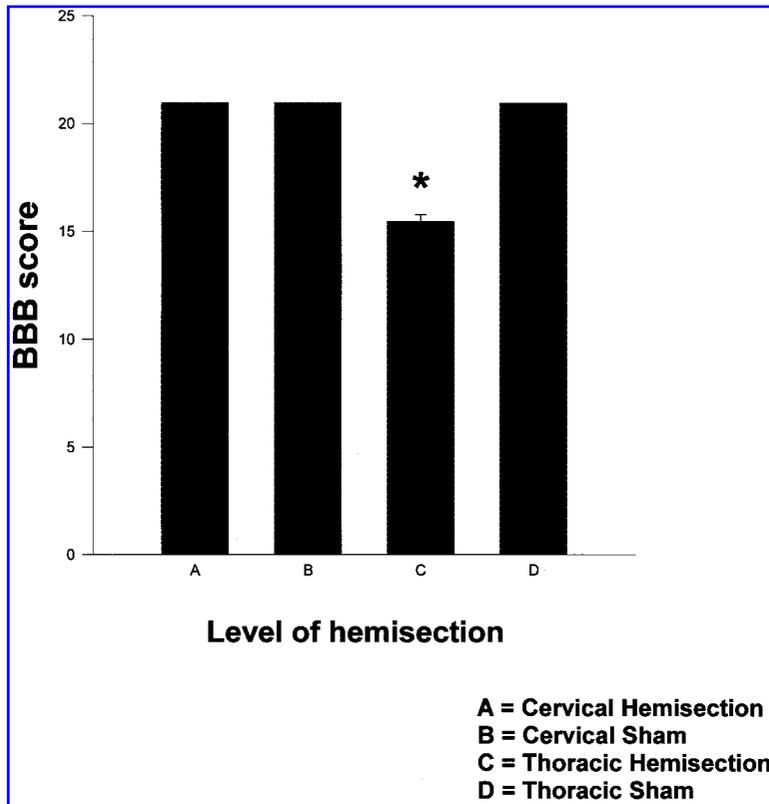
*Right hindlimb.* The right (good) hind limb received a score of 21 for all CH animals and for three of the four TH animals at postsurgical day 40, while one TH rat received a score of 16 for the right (good) hind limb.

*General.* There were no significant differences between BBB scores received by sham and CH groups (Fig. 3). CH animals achieved a score of 21 for both hind limbs by 40 days postoperatively. Several behaviors were altered in CH animals that were noted during BBB scoring but could not be documented using the BBB scoring system itself. CH animals held their left (bad) forelimb flexed during rest and appeared to not fully extend it during locomotion or at rest. Generally, CH rats would lie in sternal recumbancy or stood on four limbs (or occasionally on three limbs, with the affected forelimb being

raised off the ground). These rats commonly held the paw of the affected (left) forelimb closed during locomotion while in the open field. Occasionally, their paws appeared to open during the stance phase of locomotion. CH rats would preferentially balance themselves with their right forelimb during rearing-up on the side of the open field. CH animals groomed with only the right (good) forelimb, whereas TH and sham animals did not appear to have any forelimb preference for grooming. TH animals would stand on all four limbs while at rest and would rear up onto the side of the open field, appearing to put more weight on the right side of their bodies. TH rats did not show any preference for balancing themselves with their right or left forelimbs while rearing-up on the side of the open field. TH animals BBB scores were significantly decreased ( $H = 11.6; 2 df; p = 0.01$ ) compared to sham (Fig. 3).

*Description of Locomoting Rats During the Runway Task*

Careful review of videotaped records showed that sham-operated rats did not have any asymmetries be-



**FIG. 3.** The BBB scoring system differentiated thoracic but not cervical hemisected animals from sham-operated control animals. BBB score versus level of hemisection, at 40 days postoperative. Only rats with thoracic hemisections ( $n = 4$ ) were significantly different from control animals ( $n = 4$ ). Mean BBB scores  $\pm$  SE;  $*p < 0.05$ .

tween their forelimbs or hindlimbs locomoting for a food reward while in the runway. CH animals, however, consistently placed their left (bad) forelimb down almost perpendicular with their bodies at the onset of stance. That is, CH animals did not extend their left (bad) shoulder compared to sham-operated animals. Consequently, the left (bad) forelimb step length appeared to be shorter than that for the right limb. CH animals did, however, open the paw of the left forelimb prior to the onset of stance during the runway task and consistently used plantigrade paw placement during runway locomotion with paw drag. This is in contrast to their behavior during the BBB scoring sessions.

TH animals generally held their tails high in the air, except for one animal who held its tail parallel with the body and occasionally touched down on the ground with the end of its tail. As mentioned previously, only trotting data was collected for all rats for the assessment of ground reaction forces. One TH rat, however, occasionally used a pacing gait to locomote while in the runway (not used for forceplate analysis). A pace occurs when the ipsilateral limbs hit the ground at the same time. This gait is seen in more cursorial animals such as camels, horses, dogs, and cats, so it was quite unusual to observe pacing in this animal. There did not appear to be obvious asymmetries for the forelimbs in TH rats. The left (bad) hind limbs of these animals appeared to move in an exaggerated fashion. That is, the left hind limb would

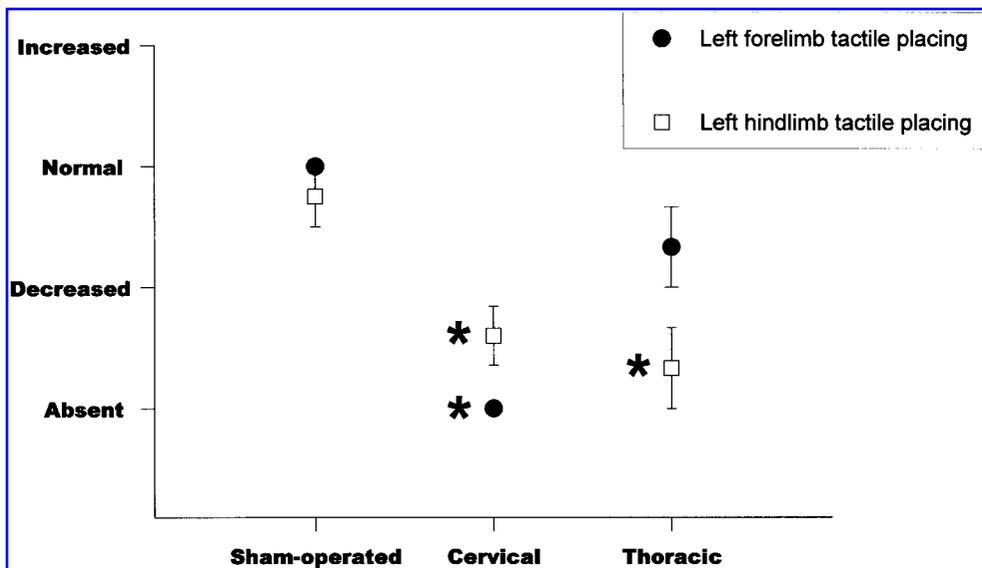
be lifted higher than the right limb, and the range of extension and flexion appeared increased in the left limb. None of the TH rats dragged their paws while locomoting in the runway.

### Reflex Testing

Of the reflexes assessed, only lateral tactile placing was able to discriminate CH and TH animals from control animals (Fig. 4). CH animals had impaired or absent lateral tactile placing responses in both the left fore (ipsilateral to lesion;  $H = 10.3; 2 \text{ df}; p < 0.001$ ) and left hind (ipsilateral to lesion;  $H = 6.65, 2 \text{ df}, p = 0.039$ ) limbs. TH animals had impaired or absent responses in the left hind limb only ( $H = 6.65; 2 \text{ df}; p = 0.039$ ). There were no differences between groups of animals with respect to pedal withdrawal responses and the hopping reactions.

### Ground Reaction Forces

*Control animals.* Prior to surgery, the ground reaction forces of the three groups of animals (CH, TH, and sham-operated) could not be distinguished from one another. Postsurgery, no differences were detected for cervical sham and thoracic sham-operated animals. Consequently, data for both cervical and thoracic sham animals were combined into a single control group. The most common gait employed by sham-operated rats before and after surgery was trotting. No walking gaits were observed



**FIG. 4.** Tactile placing was useful for determining the neuroanatomic location of the lesion. Tactile placing responses versus level of hemisection for the left limbs of either left cervical or left thoracic hemisectioned groups of rats. Responses of both the left forelimb and hind limb of cervical hemisectioned rats ( $n = 5$ ), and of only the left hind limb of thoracic hemisectioned rats ( $n = 4$ ) were significantly different from control animals ( $n = 4$ ). Mean  $\pm$  SE; \*  $= p < 0.05$ ). Tactile placing responses for right limbs did not differ from controls (not shown).

even at speeds less than 50 cm/sec. Analysis of vertical impulse for individual limbs revealed that trotting rats (Fig. 5A) bear approximately 0.25 body weights (BW) on each limb over one single trotting stride. Thus, 50% of their body weight is supported by the forelimbs, and 50% is supported by the hind limbs. These findings are also supported by the fact that peak vertical forces were not different for forelimbs compared to hind limbs. Analysis of net fore-aft impulse revealed that sham-operated rats use their forelimbs more for braking than propulsion and their hind limbs more for propulsion than braking over one stride (Fig. 5A).

*Cervical spinal hemisected animals.* Peak vertical forces generated by both forelimbs of CH animals were significantly smaller than control animals (Fig. 5B; left fore  $F_{(2,10)} = 9.5$ ;  $p < 0.005$ ; right fore  $F_{(2,10)} = 8.54$ ,  $p = 0.007$ ). CH rats initially unloaded their right forelimb more slowly until 0.5 BW was met ( $F_{(2,10)} = 8.76$ ,  $p = 0.006$ ; Fig. 1). These rats initially unloaded their right hind limb more slowly than sham-operated controls ( $F_{(2,10)} = 89.7$ ,  $p < 0.001$ ). The left (bad) forelimb was loaded normally ( $F_{(2,10)} = 3.35$ ,  $p = 0.08$ ). However, this limb was unloaded more slowly (initial phase  $F_{(2,10)} = 5.11$ ,  $p = 0.03$ ; and late phase  $F_{(2,10)} = 13.49$ ,  $p = 0.001$ ). The left (bad) hind limb was loaded more slowly throughout the loading period for CH animals (initial phase  $F_{(2,10)} = 5.43$ ,  $p = 0.025$ ; and late phase  $F_{(2,10)} = 4.95$ ,  $p = 0.032$ ). This same limb was unloaded more slowly only after 0.5 BW was met ( $F = 56.09$ ,  $df = 12$ ,  $p < 0.001$ ). CH animals were able to generate a significantly higher peak propulsive force with their left (bad) hind limb than the control animals' left hind limb ( $F_{(2,10)} = 11.79$ ,  $p < 0.005$ ). These rats generated a higher peak braking force with their right (good) forelimb compared to the forelimbs of control animals ( $F_{(2,10)} = 6.91$ ,  $p = 0.013$ ). CH animals essentially did not use their left (bad) forelimb for braking or propulsion. However, the right (good) forelimb was used significantly more for braking compared to controls ( $F_{(2,10)} = 10.62$ ,  $p = 0.003$ ), and the right (good) hind limb was used similar to controls ( $F_{(2,10)} = 2.02$ ,  $p = 0.18$ ).

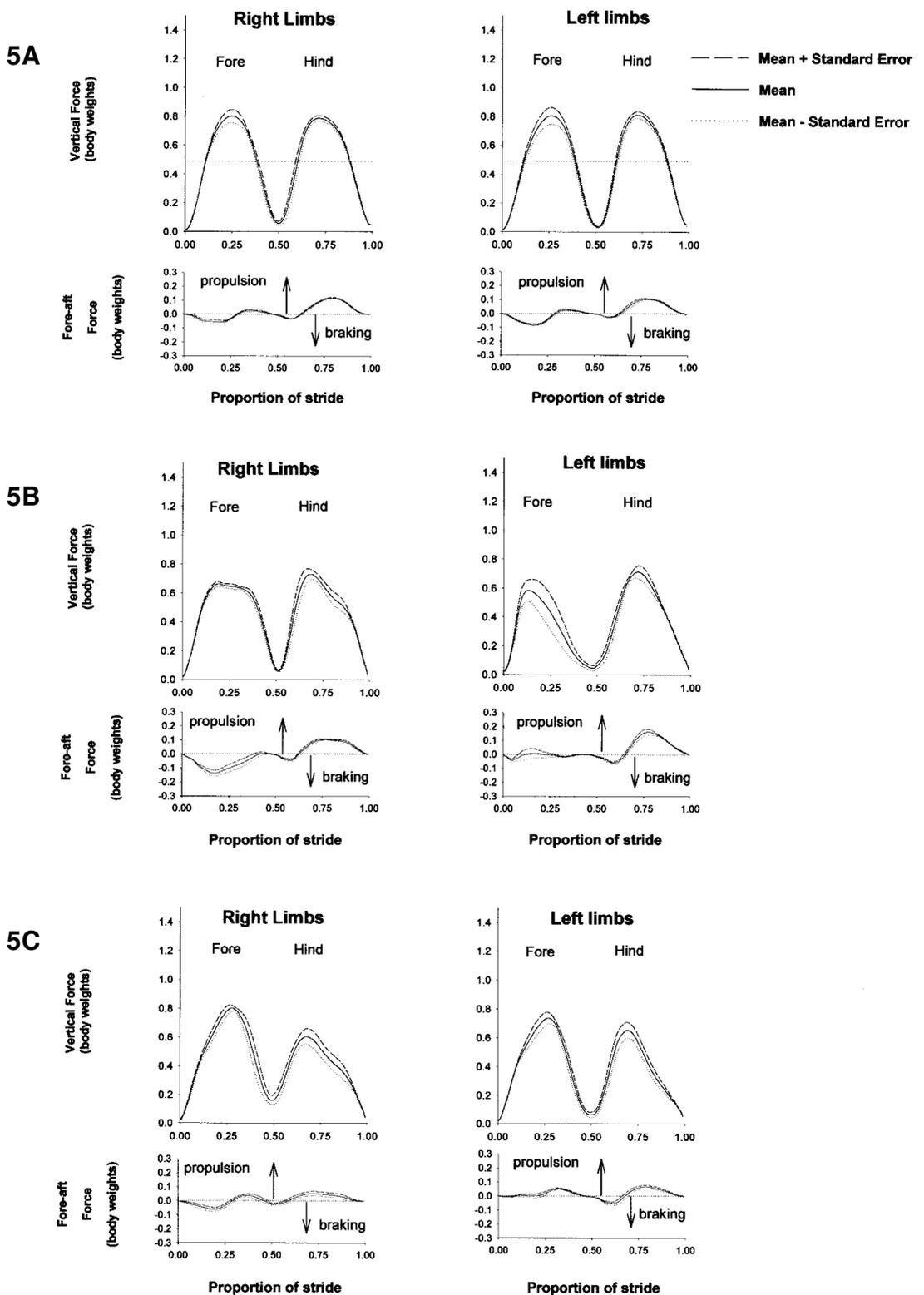
*Thoracic spinal hemisected animals.* TH animals generated significantly smaller peak vertical forces with the right hind limb, and nearly significant smaller forces with the left hind limb, compared to sham-operated control animals (left hind  $F_{(2,10)} = 3.57$ ,  $p = 0.067$ ; right hind  $F_{(2,10)} = 5.97$ ,  $p = 0.02$ ; Fig. 5C). However, analysis of total vertical forces revealed that the left fore/right hind (bad fore/good hind) had statistically smaller peak vertical forces compared to control animals ( $F_{(2,10)} = 4.7$ ,  $p =$

0.04; Fig. 6C). These animals initially loaded their right forelimb more slowly ( $F_{(2,10)} = 11.3$ ,  $p < .0003$ ) than controls and unloaded their right hind limb more slowly throughout the unloading period (initial phase  $F_{(2,10)} = 89.7$ ,  $p < 0.001$ ; and late phase  $F_{(2,10)} = 18.08$ ,  $p < 0.001$ ). The left forelimb was loaded similarly to controls. However, the left hind limb was loaded more slowly during the late phase of loading ( $F_{(2,10)} = 4.95$ ,  $p = 0.03$ ). The left hind limb was also unloaded more slowly during the late phase of unloading compared to controls ( $F_{(2,10)} = 56.09$ ,  $p < 0.001$ ; Fig. 5C). The peak propulsive force produced by TH rats was significantly reduced for the right (good) hind limb ( $F_{(2,10)} = 16.2$ ,  $p < 0.001$ ) but not the left (bad) hind limb compared to control animals. This group also had significantly reduced braking force generated by the left forelimb compared to forelimbs of the control animals ( $F_{(2,10)} = 19.14$ ,  $p < 0.001$ ; Fig. 5C). The left (bad) forelimb of TH animals was used more for propulsion than in control animals ( $F_{(2,10)} = 41.7$ ,  $p < 0.001$ ). They used the right (good) hind limb similar to control animals for braking and propulsion ( $F_{(2,10)} = 2.02$ ,  $p = 0.18$ ; Fig. 5C). However, these rats use the left (bad) hind limb significantly less for braking compared to control animals ( $F_{(2,10)} = 23.3$ ,  $p < 0.001$ ; Fig. 5C).

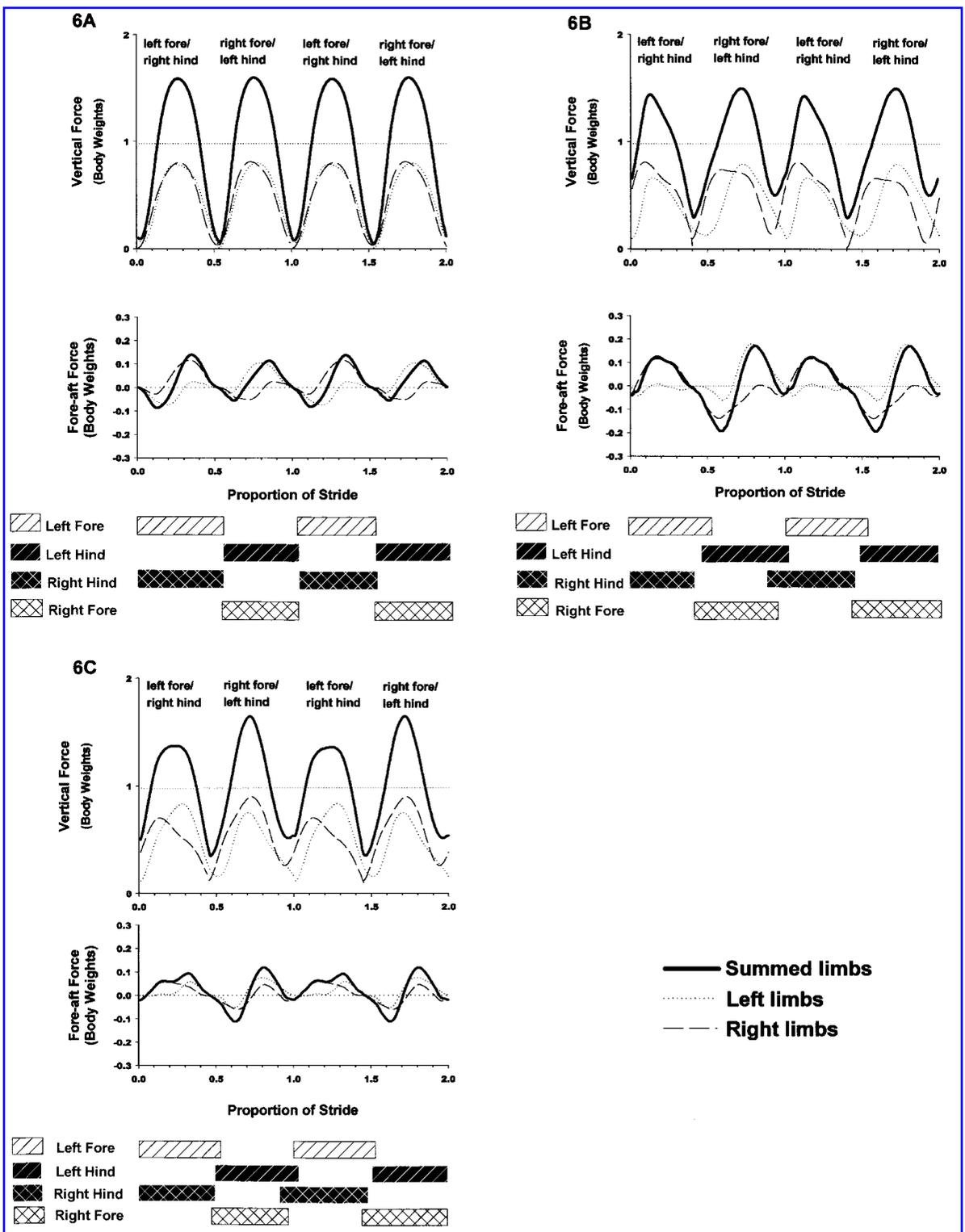
### *Energetics*

In the present study, kinetic and potential energy was determined from integrating the summed vertical and fore-aft ground reaction forces twice with respect to time so as to provide insight into the efficiency by which hemisected animals locomote. Potential energy changes indicate the vertical position of the body, whereas kinetic energy changes reflect the fore-aft movement of the body. During walking, kinetic and potential energies are approximately 180° out-of-phase with each other, such that energy could be exchanged between the two forms, thereby reducing total energy expenditure by as much as 70% (Cavagna et al., 1977). During normal trotting in quadrupeds, kinetic and potential energies are almost completely in-phase with each other and energy is conserved not by exchange between the two forms but by conversion to elastic energy, which is stored in tendons and muscles during stance (Cavagna et al., 1977).

*Control animals.* Unoperated and sham-operated rats demonstrate both footfall placement patterns and energetics, which confirm that they are in fact trotting (Fig. 7A). Diagonal limb pairs landed almost simultaneously on the ground together in an alternating pattern, and potential and kinetic energies were in-phase with each other (see bar diagram in Fig. 7A). Overall, kinetic and poten-



**FIG. 5.** Cervical and thoracic hemisected rats compensated similarly to one another during locomotion. Averaged ground reactions versus proportion of stride for individual limbs in the fore-aft and vertical directions. Solid lines represent mean curves for each group of animals. (A) Sham-operated animals ( $n = 4$ ) demonstrated no asymmetries in either the vertical or fore-aft direction between fore/hind limbs and between right and left sides. (B) Cervical hemisected rats ( $n = 5$ ) did not brake with their left forelimb and produced more vertical force with both hind limbs compared to forelimbs. (C) Thoracic hemisected animals ( $n = 4$ ) also did not brake with their left forelimb but produced higher peak vertical forces with both forelimbs compared to the hind limbs.

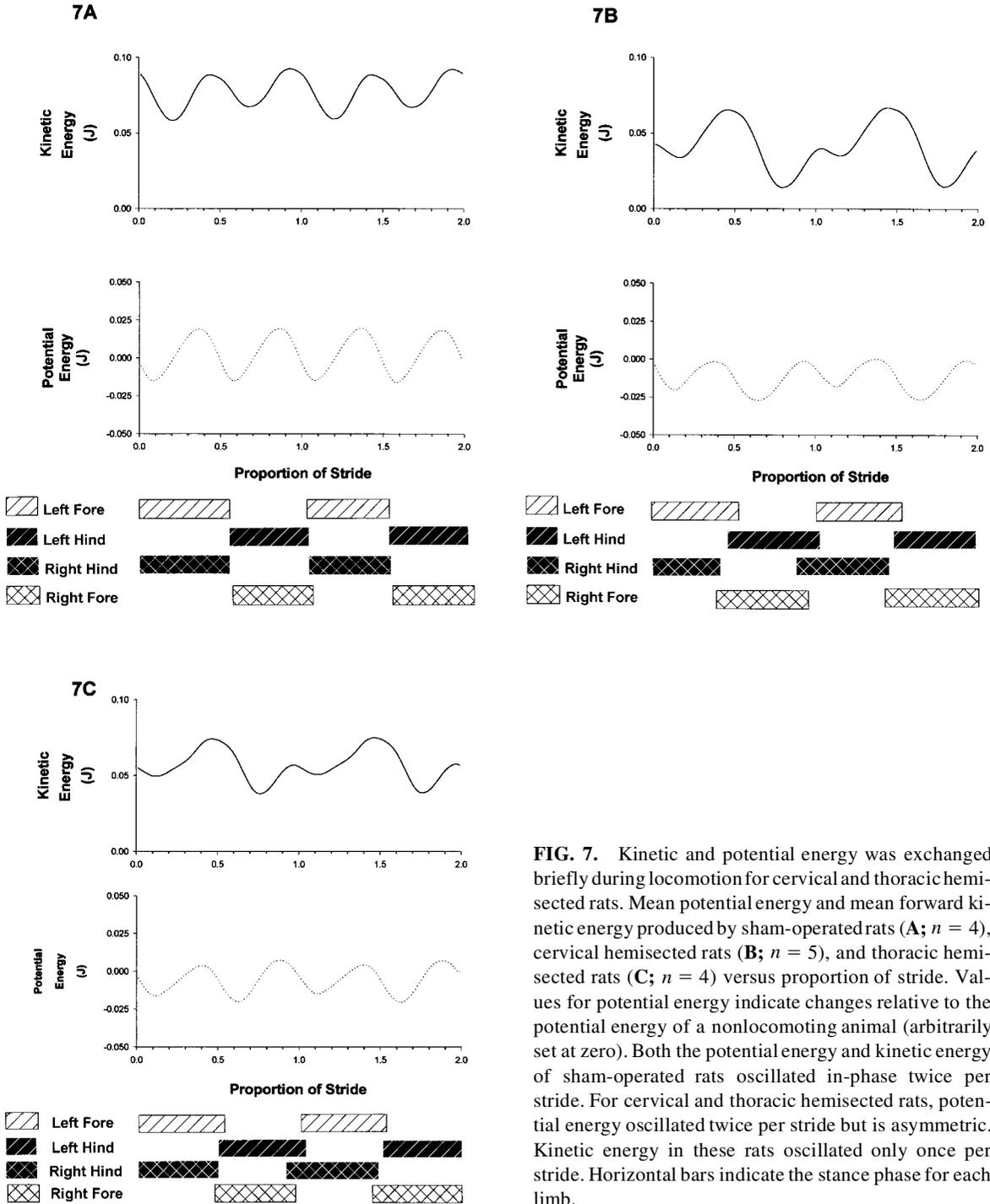


**FIG. 6.** Interlimb coordination produced differences between ground reaction forces generated by cervical and thoracic hemisected rats compared to control animals. Total ground reaction forces versus proportion of stride for sham-operated (**A**;  $n = 4$ ), cervical hemisected (**B**), and thoracic hemisected rats (**C**). Both cervical ( $n = 5$ ) and thoracic hemisected ( $n = 4$ ) rats do not use their left fore/right hind limbs for braking or propulsion (fore-aft force **B** and **C**). Thoracic hemisected animals produce less total vertical force on the left fore/right hind limb pair (vertical force **C**). Cervical and thoracic hemisected rats place their right hind limb down before their left fore (horizontal bars). Horizontal bars indicate the stance phase for each limb.

tial energies oscillated twice per stride, and there was little exchange between potential and kinetic energy.

*Cervical spinal hemisected animals.* CH animals displayed slightly abnormal trotting footfall patterns and energetically appeared to have a gait somewhere between

walking and trotting. The right (good) forelimb hit the ground slightly before the left (bad) hind limb, and then the right (good) hind limb hit the ground while the left (bad) hind was on the ground and before the left (bad) forelimb contacted the ground (see bar diagram in Fig. 7B). The potential and kinetic energies of this group of



**FIG. 7.** Kinetic and potential energy was exchanged briefly during locomotion for cervical and thoracic hemisected rats. Mean potential energy and mean forward kinetic energy produced by sham-operated rats (**A**;  $n = 4$ ), cervical hemisected rats (**B**;  $n = 5$ ), and thoracic hemisected rats (**C**;  $n = 4$ ) versus proportion of stride. Values for potential energy indicate changes relative to the potential energy of a nonlocomoting animal (arbitrarily set at zero). Both the potential energy and kinetic energy of sham-operated rats oscillated in-phase twice per stride. For cervical and thoracic hemisected rats, potential energy oscillated twice per stride but is asymmetric. Kinetic energy in these rats oscillated only once per stride. Horizontal bars indicate the stance phase for each limb.

rats were not completely in-phase with each other. There were two instances during the stride where kinetic and potential energies were out of phase with each other. The first instance occurred at 0.65–0.8 proportion of the stride (Fig. 7B). As the potential energy rose, kinetic energy fell, indicating that the rats were rising and slowing down during this portion of the stride. The second instance occurred at 0.9–1.1 proportion of stride, when the right (good) hind limb was being placed on the ground just before the left forelimb contacted the ground (Fig. 7B). During this phase, kinetic energy rose and potential energy fell, implying that the rats were falling onto their left (bad) forelimb and were speeding up. Potential energy oscillated twice per stride, whereas kinetic energy oscillated less than twice per stride (Fig. 7B).

*Thoracic spinal hemisected animals.* Interestingly, TH rats demonstrated footfall and energetic patterns similar to those of CH rats. This group of animals also had two phases during the stride where there was an exchange between kinetic and potential energies. Kinetic and potential energies were being exchanged at 0.65–0.8 proportion of the stride (Fig. 7B). As potential energy rose, kinetic energy fell. Again, this indicated that the center of mass was rising and slowing down at this point in the stride, similar to that of the CH group. There was also a brief period of time when the right (good) hind limb was on the ground where kinetic and potential energies were out-of-phase with each other, indicating that an exchange between potential and kinetic energy occurs. However, as in the CH rats, this energy exchange lasted only until the left (bad) forelimb was placed on the ground (i.e., 0.9–1.1 proportion of stride). Similar to the CH group of animals, these animals were also falling down and speeding up while landing on the left forelimb (Fig. 7C).

## DISCUSSION

We have demonstrated that, overall, CH and TH rats make similar behavioural compensations during locomotion. In particular, the forelimb ipsilateral to either lesion produced no effective braking or propulsive forces, and the hind limb contralateral to the lesion contacted the ground early in the stride cycle compared to sham-operated controls. Nevertheless, kinetic measurements revealed some differences between the two lesion models: for example, CH rats bore more weight on their hind limbs, whereas TH rats bore more weight on their forelimbs. Interestingly, the BBB hind limb locomotor rating scale revealed that the hind limbs of CH animals were much less impaired than those of TH animals, and in fact

could not be distinguished from those of sham-operated animals after 40 days postsurgery. CH rats also displayed deficits in tactile placing of the ipsilateral hind limb as well as the ipsilateral forelimb, whereas TH rats had tactile placing deficits only in the ipsilateral hind limb.

### *BBB Scoring*

The BBB scores that TH animals obtained for the limb ipsilateral to the hemisection was similar to that for other Long-Evans rats examined at 35 days following thoracic spinal hemisection (Mills et al., 2001). The similarity between CH and TH rats revealed by ground reaction force measurements was not detected using the BBB locomotor rating scale. Also interesting is our finding that the BBB scoring system did not distinguish CH animals from sham-operated control animals 40 days after surgery. This probably was the result of the fact that we used the BBB scoring system for a lesion for which it was not originally designed. The BBB scoring system was designed for contusion models of thoracic spinal cord injury in rats (Basso et al., 1995) and not for cervical hemisection models of spinal cord injury. The BBB scoring system evaluates the hind limbs, not the forelimbs, of rats during locomotion. The BBB scoring system did, however, determine that TH rats had more severely altered use of their hind limbs than did CH animals. This may be, in part, due to the relative sparing of white matter tracts in CH animals and the involvement of additional contralateral spinal tracts in TH rats. Additionally, the left hind limb of TH animals may have received less supraspinal input compared to the left hind limb of CH animals, simply because of the differences in the distance between the lesion and the left hind limb motoneuron pools in these two groups of animals. In short, these results emphasize the importance of using methods for assessing behavioral recovery only in experimental models where they have been shown to be useful.

An additional observation was that the BBB scoring system did not assess the maximum behavioral abilities of rats following spinal cord injury. In this study, rats receiving 15 on the BBB scale, that is, occasional toe clearance, did not drag their paws while moving along the runway for a food reward. This finding emphasizes the difference in motor performance when animals are carrying out a task in which they are motivated, that is, locomoting in the runway, compared to when they are not, that is, open field (exploratory) locomotion. This is important and relevant when assessing the maximum beneficial effects of therapies for spinal cord injuries and when addressing discrepancies in the results obtained between laboratories.

### *Tactile placing*

Both CH and TH animals demonstrated absent tactile placing responses on the side ipsilateral to the lesion. These findings are consistent with those of others (Bradley et al., 1983; Kunkel-Bagden et al., 1992). Absence of the placing response for a given limb was related to the level of the lesion. CH animals lacked tactile placing in both limbs ipsilateral to the hemisection, whereas TH animals lacked tactile placing only in the hind limb ipsilateral to the lesion. The exact neural control pathways for the tactile placing response are still unknown. There is some evidence that the reflex is (1) cortically mediated (Bard, 1933; Barth and Stanfield, 1990; Bregman and Goldberger, 1983a,b,c; Brooks, 1933; Donatelle, 1977; Zappella, 1966); (2) spinally mediated (Forsberg et al., 1974); and (3) dependent upon white matter tracts running within the ventrolateral spinal cord (Metz et al., 2000a).

The discrepancies in neural control of the tactile placing response may be explained by the phylogenetic differences in neuroanatomy. Cerebral cortical inputs may be more important in primates, as corticospinal tract axons make more projections to ventral gray matter in primates compared to other mammals (Armand, 1982). For example, humans and monkeys have more projections within the gray matter containing motoneurons for limb muscles than rats, and marsupials have no projections to these areas whatsoever (Kuypers, 1982). It should be mentioned, however, that marsupials inconsistently demonstrate tactile placing (Cassidy et al., 1994), which might imply that a corticospinal tract is indeed necessary for tactile placing.

Taken together, the findings of studies investigating the neural control of tactile placing imply that the absence of tactile placing in our CH and TH rats is most likely due to (1) the disruption of tracts running in the ventral portion of the hemisected spinal cord and/or (2) the disruption of the corticospinal tracts (Kunkel-Bagden et al., 1992). Variable amounts of the major dorsal corticospinal tract were spared in our CH rats. This implies that the dorsal corticospinal tract is not solely responsible for tactile placing in CH rats.

### *Ground Reaction Forces*

Limb timing and ground reaction force data were similar between CH and TH rats but were different compared to sham-operated control animals. The most obvious similarities between CH and TH animals were seen in the fore-aft ground reaction force pattern, which was, in part, due to abnormal interlimb coordination (Fig. 6B,C). In both TH and CH animals, the right (good) hind limb hit the ground during left (bad) hind limb stance but prior to left (bad) forelimb stance. In intact animals, the left fore-

limb normally contacted the ground at the same time as the right hind limb. In addition, the left (bad) forelimb was not used for braking in either the TH or CH groups. Normally, rats use their forelimbs primarily for braking and their hindlimbs for net propulsion (Fig. 6A).

It is interesting that CH and TH animals use their left forelimb similarly. Reasons for these similarities differ between the two groups. CH animals lack a large amount of supraspinal input to the motor neurons controlling the left forelimb. For example, the forelimb impairment may be the result of an interruption of the vestibulospinal tract in CH rats. The vestibulospinal tract is known to have a net excitatory effect on limb extensors (Orlovsky, 1972). Animals with lesions of their vestibulospinal tract walk with a crouched gait and slightly abnormal interlimb coordination (Matsuyama and Drew, 2000). Additionally, the ascending proprioceptive and exteroceptive input from this limb was interrupted, as the ipsilateral dorsal column and spinocerebellar tracts were disrupted. Possible reasons explaining why CH animals used their left (bad) forelimb less for braking could be that (1) these animals lacked important proprioceptive or exteroceptive tracts or (2) because they lacked motor control and/or significant inputs necessary for initiation of this limb's central pattern generator. The left forelimb must therefore rely on afferent input from the limb to stimulate the local central pattern generator and/or rely on input from the central pattern generator of the contralateral forelimb.

It has been shown that the red nucleus plays an important role for normal locomotion in rats (Muir and Whishaw, 2000). Similar to the findings presented here, rats with unilateral red nucleus ablation use their contralateral (bad) forelimb less for braking than control animals (Muir and Whishaw, 2000). In the present study, the left (bad) forelimb/right (good) hind limbs were not used together for braking but were used predominantly for propulsion. This occurred because the left forelimb was not used for braking. In unilateral red nucleus lesioned rats, however, the equivalent limb pair was not used for braking or propulsion because the timing of the forces generated by each limb negated one another (Muir and Whishaw, 2000). Rats have many projections from the red nucleus to cervical and lumbar enlargements (Huisman et al., 1982). Disruption of descending inputs from the red nucleus may account for some of the similarities seen in braking/propulsion in CH and red nucleus lesioned rats. Nevertheless, because the timing of forces differs between red nucleus lesioned rats and CH rats, there must be other pathways that are important and/or working in concert to account for the differences in these two groups of animals.

Although rats can recover complete locomotor abilities following corticospinal tract lesions (Muir and

Whishaw, 1999a), this recovery may be the consequence of plastic changes occurring within the CNS. Rats with unilateral corticospinal tract lesions do not recover immediately following surgery. Two days after surgery, ground reaction force patterns are very similar to both CH and TH rats (Muir and Whishaw, 1999a). As was observed in CH rats in the present study, the bad forelimb and good hind limb of acutely corticospinal injured rats are together used predominantly for propulsion and not for braking (Muir and Whishaw, 1999a) and the impaired forelimb is used less for braking compared to control animals. This indicates that the corticospinal tract may be used in locomotion, but in cases where the corticospinal tract is the only injured tract, rats are able to regain full locomotor recovery. This indicates that the corticospinal tract is not absolutely necessary for overground locomotion in rats. However, this does not rule out the possibility that descending corticospinal input is necessary and important for locomotion when other tracts have been abolished. There was histological evidence that the major corticospinal tract was injured, with variable sparing between TH and CH rats. The right (contralateral to hemisection) corticospinal tract was partially injured in the TH rats, whereas the CH rats sometimes had an incomplete injury of the left (ipsilateral to hemisection) major corticospinal tract. Direct corticospinal input would be more important in the cervical injured group, because corticospinal fibers terminate only on motor neurons in cervicothoracic spinal cord in the rat (Armand, 1982). In light of the substantial amount of sparing of the corticospinal tract in our CH rats, the dorsal corticospinal tract probably cannot account for the deficits seen in our animals.

TH rats use the left (bad) forelimb less for braking than do control animals (Fig. 5C). Why do TH animals use this limb much the same as CH rats? Descending inputs onto hind limb motor neurons are affected, but this does not account for the similar ground reaction force pattern seen for the left (bad) forelimb. Propriospinal inputs from the ipsilateral (bad) hind limb are interrupted following TH. Long ascending propriospinal neurons project from the lumbar spinal cord and connect the cervical and lumbar enlargements; they may influence central pattern generating networks (English et al., 1985). Long ascending propriospinal neurons are thought to play a role in interlimb coordination (English, 1989); however, it is not definitely known how these neurons contribute to interlimb coordination. It has been hypothesized that they probably synapse on high cervical interneurons and/or indirectly onto motor neurons of the ipsilateral forelimb (English et al., 1985). It may be that propriospinal neurons relay information, directly and/or indirectly, about the position of the hind limbs to the forelimbs. Although

the specific role of propriospinal fibers was not assessed in this study, TH has interrupted the connection from the left hind limb to the ipsilateral forelimb, which could prevent local spinal networks from generating normal ground reaction force timing. Abnormal limb timing could then prevent normal braking as the animal moves down onto the left forelimb during locomotion.

### *Energetics*

We found that TH animals prematurely placed their right (good) hind limb on the ground, similar to CH animals. This may be a general compensatory response used by both groups of animals in an attempt to stabilize the body and conserve energy during the transition period when the left (bad) forelimb is being placed on the ground. Kinetic and potential energy exchange occurs briefly during the placement of the right (good) hind limb (Fig. 7B,C). We also found that potential and kinetic energy was exchanged during 0.65–0.8 proportion of stride (Fig. 7B,C). In this instance, the animals are rising up and slowing down. The left (bad) hind limb and right (good) forelimb are on the ground during this exchange between energies. The rats may be slowing down and raising their bodies in an attempt to place their right (good) hind limb up and under their body in anticipation of having to fall quickly onto their left (bad) forelimb. This energy exchange is also seen in normal walking gaits, in both quadrupeds and bipeds, and can help to recover up to 70% of the energy in a given stride, thereby leaving only 30% to be generated by muscles (Cavagna et al., 1977).

It is also interesting to consider the changes in the position of the body center of gravity between each group of animals. CH animals bore more weight on their hind limbs compared to controls, and TH animals bore more weight on their forelimbs compared to control animals. This indicates that the center of gravity of CH rats was more caudally located, whereas the center of gravity of TH rats was located more cranially. These findings may be a reflection of the relative impairments of the fore and hind limbs in CH and TH animals. Gross observations made during BBB scoring indicated that, in CH animals, the left forelimb was more severely affected than the hind limbs. Because BBB scores were significantly lower in the TH rats, these animals had more severely affected left hind limbs compared to CH rats. CH animals may have shifted their weight caudally in an attempt to use their most severely impaired limb most effectively.

### *Methodological Considerations*

A variety of spinal cord injury rat models exist and are used for many purposes (Basso et al., 1996; Black et al.,

1986, 1988; Khan and Griebel, 1983; Kunkel-Bagden et al., 1992; Metz et al., 2000a,b; Muir et al., 1998). The various paradigms used for spinal cord injury investigations involve either complete or partial injuries. Partial spinal cord injury models lend insight into the importance of different neuroanatomical structures for regulating, modifying, and controlling terrestrial locomotion (for a review, see Rossignol et al., 1999), which may provide information useful for studying regeneration within the spinal cord. It has been suggested that bilateral lesions might produce symmetrical changes, making it difficult to detect specific alterations in an animal's gait (Muir and Whishaw, 2000). Unilateral lesion paradigms may be more sensitive for detecting differences from normal, symmetrical locomotion (Muir and Whishaw, 2000). Few studies have investigated the effects of unilateral spinal cord injuries on overground locomotion (Bregman and Goldberger, 1983a,b,c; Kato, 1992; Muir et al., 1998; Soblosky et al., 2001). This may result from the difficulty of detecting quantitative differences following such lesions and because many animals recover a substantial amount of their locomotor abilities following unilateral spinal cord injury (Bregman and Goldberger, 1983b; Muir et al., 1998). Using a variety of behavioral testing procedures makes it possible to more completely describe the locomotor abilities of animals having undergone partial spinal cord injury.

It has been recommended that a variety of tests should be used when assessing the locomotor abilities of rats following spinal cord injury (Metz et al., 2000a; Muir and Webb, 2000). Each method used to assess locomotor recovery in rats has its own innate limitations and benefits (Muir and Webb, 2000). The results we obtained from CH and TH rats demonstrated the limitations and benefits of various testing procedures and demonstrate how the benefits of one testing procedure can be used to compensate for the limitations of another. For example, the BBB scoring system provides qualitative kinematic data that was used to indicate the motor capabilities of the hind limbs of both CH and TH animals, and allowed us to screen animals before proceeding to ground reaction force determination. In addition, the information obtained from each testing procedure provides useful and unique information about the locomotor abilities of spinal cord injured animals. By employing various methods of assessing behavioral recovery, it was possible to maximize the information we obtained concerning the locomotor abilities of spinal cord injured rats following either CH or TH.

Our study provides obvious examples of how employing different methods of assessing motor recovery following spinal cord injury is able to maximize the description of the motor recovery and how one testing procedure complements another. The BBB locomotor rating

scale was originally designed to assess the progression of hind limb functional recovery following thoracic spinal cord injury in rats. Many studies, however, have used the final score at the end of the study to assess therapeutic benefits of a given treatment (Kamencic et al., 2001; Yu et al., 2001). In our study, animals had to have regained a satisfactory amount of recovery following spinal hemisection in order for ground reaction force determination to be performed (a limitation of ground reaction force determination). In this instance, the BBB locomotor rating scale acted as a screen by which animals could be tested using ground reaction force determination and provided a general description of how the animals behaved from simple observations. Ground reaction force determination, although unable to be used during the recovery period, provided specific, objective, quantitative information regarding the locomotor adjustments rats made during stance.

In conclusion, it appears that a general compensatory response is adopted for unilateral lesions at different levels of the spinal cord. This response also shares similarities with locomotor behavior observed after unilateral red nucleus ablation and corticospinal tract lesions in rats. The function of this compensation is to possibly provide extra stabilization of the animal during locomotion, which also brings about an exchange between potential and kinetic energies. The similarity in ground reaction force patterns seen between the two groups of animals in this study is probably the result of an interruption of descending supraspinal input for both groups, and of disruption of the ascending and descending propriospinal neurons connecting the fore- and hindlimbs in TH animals. Our findings provide novel and useful information regarding the locomotor abilities of rats following unilateral CH or TH. These findings provide insight into the neural control of interlimb coordination, which may not otherwise be detected in rats using more conventional methods.

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## REFERENCES

- ARMAND, J. (1982). The origin, course and terminations of corticospinal fibers in various mammals. *Prog. Brain Res.* **57**, 329–360.

- BARD, P. (1933). Studies on the cerebral cortex I. Localized control of placing and hopping reactions in the cat and their normal management by small cortical remnant. *Arch. Neurol. Psychiatry* **30**, 40–74.
- BARTH, T.M., and STANFIELD, B.B. (1990). The recovery of forelimb-placing behavior in rats with neonatal unilateral cortical damage involves the remaining hemisphere. *J. Neurosci.* **10**, 3449–3459.
- BASSO, D.M., BEATTIE, M.S., and BRESNAHAN, J.C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J. Neurotrauma* **12**, 1–21.
- BASSO, D.M., BEATTIE, M.S., and BRESNAHAN, J.C. (1996). Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp. Neurol.* **139**, 244–256.
- BERTRAM, J.E.A., LEE, D.V., TODHUNTER, R.J., et al. (1997). Multiple force platform analysis of the canine trot: a new approach to assessing basic characteristics of locomotion. *Vet. Comp. Orthop. Traumatol.* **10**, 160–169.
- BLACK, P., MARKOWITZ, R.S., COOPER, V., et al. (1986). Models of spinal cord injury: Part 1. Static load technique. *Neurosurgery* **19**, 752–762.
- BLACK, P., MARKOWITZ, R.S., DAMJANOV, I., et al. (1988). Models of spinal cord injury: Part 3. Dynamic load technique. *Neurosurgery* **22**, 51–60.
- BRADLEY, N.S., SMITH, J.L., and VILLABLANCA, J.R. (1983). Absence of hind limb tactile placing in spinal cats and kittens. *Exp. Neurol.* **82**, 73–88.
- BREGMAN, B.S., and GOLDBERGER, M.E. (1983a). Infant lesion effect: I. Development of motor behavior following neonatal spinal cord damage in cats. *Brain Res.* **285**, 103–117.
- BREGMAN, B.S., and GOLDBERGER, M.E. (1983b). Infant lesion effect: II. Sparing and recovery of function after spinal cord damage in newborn and adult cats. *Brain Res.* **285**, 119–135.
- BREGMAN, B.S., and GOLDBERGER, M.E. (1983c). Infant lesion effect: III. Anatomical correlates of sparing and recovery of function after spinal cord damage in newborn and adult cats. *Brain Res.* **285**, 137–154.
- BROOKS, C.M. (1933). Studies on the cerebral cortex. II. Localized representation of hopping and placing reactions in the rat. *Am. J. Physiol.* **105**, 162–171.
- CASSIDY, G., BOUDRIAS, D., PFLIEGER, J.F., et al. (1994). The development of sensorimotor reflexes in the Brazilian opossum *Monodelphis domestica*. *Brain Behav. Evol.* **43**, 244–253.
- CAVAGNA, G.A. (1975). Force platforms as ergometers. *J. Appl. Physiol.* **39**, 174–179.
- CAVAGNA, G.A., HEGLUND, N.C., and TAYLOR, C.R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243–R261.
- CHEN, H.Y., CHIU, W.T., CHEN, S.S., et al. (1997). A nationwide epidemiological study of spinal cord injuries in Taiwan from July 1992 to June 1996. *Neurol. Res.* **19**, 617–622.
- CLARKE, K.A., and STILL, J. (1999). Gait analysis in the mouse. *Physiol. Behav.* **66**, 723–729.
- DONATELLE, J.M. (1977). Growth of the corticospinal tract and the development of placing reactions in the postnatal rat. *J. Comp. Neurol.* **175**, 207–231.
- ENGLISH, A.W. (1989). Interlimb coordination during locomotion. *Am. Zool.* **29**, 255–266.
- ENGLISH, A.W., TIGGES, J., and LENNARD, P.R. (1985). Anatomical organization of long ascending propriospinal neurons in the cat spinal cord. *J. Comp. Neurol.* **240**, 349–358.
- ERGAS, Z. (1985). Spinal cord injury in the United States: a statistical update. *Cent. Nerv. Syst. Trauma* **2**, 19–32.
- FARLEY, C.T., GLASHEEN, J., and McMAHON, T.A. (1993). Running springs: speed and animal size. *J. Exp. Biol.* **185**, 71–86.
- FORSSBERG, H., GRILLNER, S., and SJOSTROM, A. (1974). Tactile placing reactions in chronic spinal kittens. *Acta Physiol. Scand.* **92**, 114–120.
- HOQUE, M.F., GRANGEON, C., and REED, K. (1999). Spinal cord lesions in Bangladesh: an epidemiological study. *Spinal Cord* **37**, 858–861.
- HUISMAN, A.M., KUYPERS, H.G., and VERBURGH, C.A. (1982). Differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in cat and monkey. *Prog. Brain Res.* **57**, 185–217.
- KAMENCIC, H., GRIEBEL, R. W., LYON, A. W., et al. (2001). Promoting glutathione synthesis after spinal cord trauma decreases secondary damage and promotes retention of function. *FASEB J.* **15**, 243–250.
- KARAMEHMETOGLU, S.S., NAS, K., KARACAN, I., et al. (1997). Traumatic spinal cord injuries in southeast Turkey: an epidemiological study. *Spinal Cord* **35**, 531–533.
- KATO, M. (1992). Walking of cats on a grid: performance of locomotor task in spinal intact and hemisectioned cats. *Neurosci. Lett.* **145**, 129–132.
- KHAN, M., and GRIEBEL, R. (1983). Acute spinal cord injury in the rat: comparison of three experimental techniques. *Can. J. Neurol. Sci.* **10**, 161–165.
- KUNKEL-BAGDEN, E., DAI, H.N., and BREGMAN, B.S. (1992). Recovery of function after spinal cord hemisection in newborn and adult rats: differential effects on reflex and locomotor function. *Exp. Neurol.* **116**, 40–51.
- KUYPERS, H.G. (1982). A new look at the organization of the motor system. *Prog. Brain Res.* **57**, 381–403.

- LITTLE, J.W., HARRIS, R.M., and SOHLBERG, R.C. (1988). Locomotor recovery following subtotal spinal cord lesions in a rat model. *Neurosci. Lett.* **87**, 189–194.
- MATSUYAMA, K., and DREW, T. (2000). Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. I. Walking on a level surface. *J. Neurophysiol.* **84**, 2237–2256.
- METZ, G.A., CURT, A., VAN DE, M.H., et al. (2000b). Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. *J. Neurotrauma* **17**, 1–17.
- METZ, G.A., MERKLER, D., DIETZ, V., et al. (2000a). Efficient testing of motor function in spinal cord injured rats. *Brain Res.* **883**, 165–177.
- MILLS, C.D., HAINS, B.C., JOHNSON, K.M., et al. (2001). Strain and model differences in behavioral outcomes after spinal cord injury in rat. *J. Neurotrauma* **18**, 743–756.
- MUIR, G.D., GOSLINE, J.M., and STEEVES, J.D. (1996). Ontogeny of bipedal locomotion: walking and running in the chick. *J. Physiol. (Lond.)* **493**, Pt 2, 589–601.
- MUIR, G.D., KATZ, S.L., GOSLINE, J.M., et al. (1998). Asymmetric bipedal locomotion—an adaptive response to incomplete spinal injury in the chick. *Exp. Brain Res.* **122**, 275–282.
- MUIR, G.D., and WEBB, A.A. (2000). Mini-review: assessment of behavioural recovery following spinal cord injury in rats. *Eur. J. Neurosci.* **12**, 3079–3086.
- MUIR, G.D., and WHISHAW, I.Q. (1999a). Complete locomotor recovery following corticospinal tract lesions: measurement of ground reaction forces during overground locomotion in rats. *Behav. Brain Res.* **103**, 45–53.
- MUIR, G.D., and WHISHAW, I.Q. (1999b). Ground reaction forces in locomoting hemi-parkinsonian rats: a definitive test for impairments and compensations. *Exp. Brain Res.* **126**, 307–314.
- MUIR, G.D., and WHISHAW, I.Q. (2000). Red nucleus lesions impair overground locomotion in rats: a kinetic analysis. *Eur. J. Neurosci.* **12**, 1113–1122.
- NATIONAL SPINAL CORD INJURY STATISTICAL CENTER. (2000). Spinal cord injury: facts and figures at a glance. *J. Spinal Cord Med.* **23**, 51–53.
- ORLOVSKY, G.N. (1972). The effect of different descending systems on flexor and extensor activity during locomotion. *Brain Res.* **40**, 359–371.
- OUDEGA, M., VARGAS, C.G., WEBER, A.B., et al. (1999). Long-term effects of methylprednisolone following transection of adult rat spinal cord. *Eur. J. Neurosci.* **11**, 2453–2464.
- POSPIECH, J., PAJONK, F., and STOLKE, D. (1995). Epidural scar tissue formation after spinal surgery: an experimental study. *Eur. Spine J.* **4**, 213–219.
- ROSSIGNOL, S., DREW, T., BRUSTEIN, E., et al. (1999). Locomotor performance and adaptation after partial or complete spinal cord lesions in the cat. *Prog. Brain Res.* **123**, 349–365.
- ROUGHAN, J.V., OJEDA, O.B., and FLECKNELL, P.A. (1999). The influence of pre-anaesthetic administration of buprenorphine on the anaesthetic effects of ketamine/medetomidine and pentobarbitone in rats and the consequences of repeated anaesthesia. *Lab. Anim.* **33**, 234–242.
- SECHZER, J.A., FOLSTEIN, S.E., GEIGER, E.H., et al. (1984). Development and maturation of postural reflexes in normal kittens. *Exp. Neurol.* **86**, 493–505.
- SOBLOSKY, J.S., SONG, J., and DINH, D.H. (2001). Graded unilateral cervical spinal cord injury in the rat: evaluation of forelimb recovery and histological effects. *Behav. Brain Res.* **119**, 1–13.
- SURKIN, J., GILBERT, B. J., HARKEY, H. L., III, et al. (2000). Spinal cord injury in Mississippi. Findings and evaluation, 1992–1994. *Spine* **25**, 716–721.
- WEBB, A.A., and MUIR, G.D. (2000). The blood-brain barrier and its role in inflammation. *J. Vet. Intern. Med.* **14**, 399–411.
- WOLGIN, D.L. (1985). Forelimb placing and hopping reflexes in haloperidol- and morphine-treated cataleptic rats. *Behav. Neurosci.* **99**, 423–435.
- YU, C.G., JAGID, J., RUENES, G., et al. (2001). Detrimental effects of systemic hyperthermia on locomotor function and histopathological outcome after traumatic spinal cord injury in the rat. *Neurosurgery* **49**, 152–158.
- ZAPPELLA, M. (1966). The placing reaction in the first year of life. *Dev. Med. Child Neurol.* **8**, 393–401.

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3. Gillian D. Muir, Aubrey A. Webb, Srikanth Kanagal, Laura Taylor. 2007. Dorsolateral cervical spinal injury differentially affects forelimb and hindlimb action in rats. *European Journal of Neuroscience* **25**:5, 1501-1510. [[CrossRef](#)]
4. Melanie L. McEwen , Joe E. Springer . 2006. Quantification of Locomotor Recovery following Spinal Cord Contusion in Adult RatsQuantification of Locomotor Recovery following Spinal Cord Contusion in Adult Rats. *Journal of Neurotrauma* **23**:11, 1632-1653. [[Abstract](#)] [[PDF](#)] [[PDF Plus](#)]
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12. Aubrey A. Webb, Gillian D. Muir. 2003. Unilateral dorsal column and rubrospinal tract injuries affect overground locomotion in the unrestrained rat. *European Journal of Neuroscience* **18**:2, 412-422. [[CrossRef](#)]