Interlimb Coordination After a Thoracic Hemisection in Rats

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Abstract
An important role for sensory input in the motor performance of spinal cord injured (SCI) humans and animals has been established. For example, loading of the hind limbs during assisted locomotion on a treadmill improves the stepping ability of SCI patients and of complete spinal cord transected cats and rats. In our laboratories we are now using robotic devices to control and modulate the amount and timing of loading of the hind limbs during treadmill locomotion of SCI humans and animals. In addition, these robotic devices are being used to provide a quantitative kinematic assessment of performance during test trials of stepping ability. Recently, we have been studying the effects of spinal cord hemisection on interlimb coordination using these procedures. Our initial studies include adult male rats hemisected at a mid-thoracic level and maintained for ~4 weeks. When the robots were used to provide a load level of 25% of body weight, there was an asymmetry in the hind limbs during bipedal stepping. The cycle periods were shorter for the limb on the non-hemisected vs. the hemisected side and the number of steps performed during 30 sec on the treadmill was greater for the limb on the non-hemisected vs. hemisected side. Furthermore, the cycle periods and number of steps taken by the limb on the non-hemisected side during the test period were similar to the pre-hemisection values for the same rats. When the rats were allowed to step bipedally with weight bearing, but without the robotic arm, the stepping was symmetrical. In a second experiment a smaller group of rats was tested for symmetry of the two hind limbs using the robotic device. These rats were able to step symmetrically even with the robotic arms attached. Combined, these results demonstrate that even when there is asymmetrical descending input to a normal spinal cord, symmetrical stepping can be achieved. Furthermore, these results suggest that the normality of the symmetry following a hemisection can be a function of the difficulty of the task imposed on the rat.

Key words: asymmetrical gait, locomotion, rats, robotic testing, spinal cord hemisection.
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cord segments. In the present study, we compared the symmetry of treadmill stepping in two groups of rats before and after a hemisection at a mid-thoracic level. The results suggest that asymmetric descending input can disrupt the symmetry of stepping that is intrinsic to the lumbosacral spinal cord, particularly when hemisected rats are performing a difficult motor task.

Materials and Methods
We performed two experiments. In the first experiment (Exp1) we studied hind limb coordination before and 32 days after a hemisection at a mid-thoracic level in rats having a mean body weight of ~500 g (n = 7). In the second experiment (Exp2) we examined hind limb coordination 3 and 28 days after a mid-thoracic hemisection in rats weighing ~350 g (n = 5). All spinal cord surgeries were performed under aseptic conditions and all procedures used in these studies were approved by the Animal Use Committee at UCLA and followed the American Physiological Society Animal Care Guidelines. Adult male rats were deeply anesthetized with ketamine hydrochloride (70 mg/kg body weight)

Figure 1. Hind limb locomotor performance of a representative rat pre-hemisection (Exp1). Scatterplots in (A) show the step cycle trajectories in the left and right hind limbs as measured by a robotic arm attached, but functioning in a passive mode, to the ankle of each limb. Corresponding horizontal (thick lines) and vertical (thin lines) position and velocity data over time are shown (B and C). Dots above the velocity traces indicate robotically-detected step cycles. Plots of the number of steps performed by the combination of the two hind limbs during 30 sec of testing for all rats (n = 7) are shown (D). These data are from treadmill tests performed at 11 cm/sec with a load equivalent to 25% of the body weight supported by the hind limbs.
and acepromazine maleate (5 mg/kg body weight) administered intraperitoneally. A laminectomy was performed at a mid-thoracic level and the right side of the spinal cord was isolated using a probe and then transected using microscissors. Data on bipedal hind limb stepping performance before and 32 days after hemisection for Exp1 and 3 and 28 days after hemisection for Exp2 are reported. The surgical procedures and maintenance of the animals followed the protocols detailed in Roy et al. [18].

A robotic device was used for testing hind limb locomotor function as previously described [2, 3, 20, 21]. Briefly, robotic arms were attached to the ankles of the hind limbs to record horizontal and vertical movements during stepping (for 30-60 sec) on a treadmill. The amount of load on the hind limbs was controlled via a computer controlled body weight support device that was set to provide 25 and 50% of the weight of the body. Weight-bearing steps were analyzed using computer algorithms that detect toe off and touch down events. The determination of the step kinematics, e.g., toe off and touch down, was based on a combination of velocity magnitudes in the x (horizontal) and y (vertical) directions. In addition to the robotic

![Figure 2. Hind limb locomotor performance of a representative rat that was hemisected 32 days earlier (Exp1). Scatterplots in (A) show the step cycle trajectories in the left and right hind limbs as measured by a robotic arm attached, but functioning in a passive mode, to the ankle of each limb. Corresponding horizontal (thick lines) and vertical (thin lines) position and velocity data over time are shown (B and C). Dots above the velocity traces indicate robotically-detected step cycles. Plots of the number of steps performed by the combination of the two hind limbs during 30 sec of testing for all rats (n = 7) are shown (D). Note that during most of the 30-sec test the movements of the right (impaired) hind limb were erratic. However, one trajectory cycle of the impaired hind limb (A, right panel) was similar to most of the cycles shown in Figure 5 (A, left panel). * significantly different from intact group, (p< 0.05); # significantly different from left hind limb (p< 0.05).]
data, the locomotor capacity of the rats was assessed from video recordings of the hind limbs during the tests.

Results

Experiment 1

Asymmetry in robotic-detected steps

The locomotor performance of rats measured during the robotic tests was characterized by consistent step cycle trajectories and periodic displacement and velocity traces in both hind limbs (Fig. 1). The number of steps that was detected by the robotic device was the same in the left and right hind limbs for the rats prior to the hemisection. In Exp1, bipedal stepping on a treadmill was examined 32 days following hemisection of the right half of the spinal cord. Stepping ability was tested using the robotic arms to measure ankle trajectories during locomotion. An asymmetry in stepping was apparent, i.e., the right (impaired) hind limb performed significantly fewer steps than the left hind limb (Fig. 2). The left hind limb (unimpaired) performed a normal amount of steps for the speed and time tested, whereas the right (impaired) hind limb produced significantly fewer steps relative to intact rats and to the contralateral hind limb in hemisected rats. In effect, the right hind limb of the hemisected rats executed some detectable steps, but failed to maintain a consistent stepping pattern.

To determine if the attachment of the robotic arms to the hind limbs contributed to the observed asymmetry in stepping, we compared hind limb stepping with and without the robotic arms attached to the ankles in a subset (n = 4) of rats and counted the number of steps in each limb using video-based analyses (Fig. 3). The right (impaired) hind limb performed a significantly greater number of steps without than with the robotic arm attached. In short, the locomotor deficit in the impaired hind limb was detected only when the robotic device was used in the assessment of stepping.

To compare the hind limb coordination patterns before and after the spinal hemisection, horizontal movements in the left and right hind limbs were analyzed (Fig. 4). All of the data recorded during the 30-sec robotic tests for each of the rats were included in the analyses. Before the spinal hemisection, the hind limbs moved either in the forward or backward direction throughout the robotic tests (Fig. 4A, C). In addition, left and right hind limbs were coordinated to move in either opposite directions or in the same direction for an equal proportion of time during stepping (Fig. 4E).

After the hemisection, deficits in hind limb movement patterns were evident (Fig. 4 B, D, F). There were periods during the robotic tests in which the hind limbs did not move at all (Fig. 4 B, D, F). When hind limb movements occurred, less time was spent moving backward and more time was spent in forward movement relative to the stepping observed before the hemisection (Fig. 4 B, D). In addition, left-right coordination was affected by the spinal hemisection. The two hind limbs of the hemisected rats spent less time moving in opposite directions, i.e., in an alternating gait pattern, relative to pre-hemisection stepping patterns (Fig. 4 E, F).

Experiment 2

Comparison of hind limb coordination patterns in Experiment 1 and 2

Unlike the observations for the hemisected rats in Exp1, in Exp2 there was no significant asymmetry when comparing the percentage of time within the step cycle occupied by the stance or the swing phase for either the left (unimpaired) or the right (impaired) limb (Fig. 5). This symmetry was evident as early as 3 days following surgery and persisted for at least 28 days (Fig. A-D). Symmetry also was examined by comparing the direction of movement of the right vs. the left limb. Although symmetry persisted using this analysis, it was apparent that some disruption of the stepping pattern was imposed by the hemisection. For example, normally there is little or no time during the step cycle when no movement is occurring in one or both limbs. Three days after surgery, one or both limbs were not moving for about 8 percent of the step cycle (Fig. 5E). After 28 days this time had decreased to about 1% of the step cycle (Fig. 5F).
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Discussion

The issue being addressed in the present paper is whether the symmetry of hind limb stepping is dependent on symmetrical descending neural control systems. Our results suggest that the ability to perform a relatively complex motor task following a thoracic hemisection is related to the difficulty of the task. An alternative explanation could be the differences in body weight and age of the rats between the two experiments. For example, the rats in Exp1 had a mean body weight one month after hemisection of ~500 g while the rats in Exp2 had a mean body weight of ~350 g. We think that this could be an important difference between the two experiments because previous experiments have demonstrated the critical nature of the level of body weight support in determining the quality of stepping [20, 21].

Webb and Muir [22] reported that a hemisection of one side of the rat spinal cord at a thoracic level affected the hind limbs more severely than a cervical hemisection. However, a cervical hemisection resulted in the same BBB [1] score as a cervical sham (same surgery except no damage to the spinal cord). When a quantitative and more stringent analysis was used, significant alterations in the gait of rats with either a cervical or a thoracic hemisection were observed for up to 6 weeks post-surgery. Saruhashi et al. [19] also reported that the motor performance was normal 4 weeks after a thoracic hemisection in young rats based on the recovery of movement.

![Figure 4](image_url)

Figure 4. Data related to the coordination of the two hind limbs (Exp1) are illustrated. The percentage of the time during the robotic tests that the hind limbs (Exp1, n=7) moved forward and backward (horizontally) or did not move at all is shown in A-D. A and C represent data obtained before the hemisection surgery. B and D illustrate the proportion of time distributed within the step cycle 32 days after hemisection. Note that the biggest pre-post hemisection difference is in the greater amount of time that the limb is not moving in either a horizontal or vertical direction post-hemisection. The amount of time that the two limbs were moving in an opposite horizontal direction, the same horizontal direction, or not moving in a horizontal direction in one or both limbs are illustrated before hemisection (E) and 32 days after hemisection (F) when supporting 25% of their body weight. Note that the biggest difference between the before and after hemisection conditions is that there was a greater proportion of time in which one or both limbs remained stationary.
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on a rating scale of an open field test. These authors also noted that the recovery of motor function was correlated with the amount of 5-HT terminals that were present below the lesion, with 20% of the normal level of 5-HT terminals seeming to be the threshold needed to achieve full recovery. Thus, the behavioral results reported by Webb and Muir [22] and Saruhashi et al. [19] suggest that when a hemisection is performed in rats at an adult stage and a relatively non-challenging motor task is used to assess the impact of the spinal injury, the level of motor performance can appear to be normal.

In this respect, our data derived from the hemisected rats in Exp1 are consistent with others [8, 12, 13, 15, 16] suggesting that more stringent motor tasks would reveal limitations imposed by a thoracic hemisection. For example, Prendergast et al. [17] reported that the level of motor performance in adult rats following a thoracic hemisection was not as great as when the hemisection occurred at birth. Differential effects during reflex and locomotor testing also were observed between neonatal and adult hemisected rats by Kunkel-Bagden et al. [14]. It is important to note that the hemisected rats in Exp2 of the present study were young adults, at a stage when the sensory motor system is mature.

Although hind limb bilateral coordination can be clearly preserved following a complete spinal cord transection [4-6] and even after transection plus curare administration [7], interlimb coordination obviously can be modulated by supraspinal centers. To address the

Figure 5. Data related to the coordination of the two hind limbs in rats after hemisection of the right side of the spinal cord (Exp2) are illustrated. Comparisons of the relative durations of the stance and swing phases and when no horizontal movement was occurring are shown for the unimpaired (A and B) and the impaired (C and D) limbs 3 (A and C) or 28 days (B and D) after hemisection. The relationship between the limb from the side receiving the hemisection vs. the limb on the intact side is shown in E and F. In comparing the 3 and 28 day post-hemisection coordination of the two limbs, the amount of time in which the limbs are moving in phase and out of phase as well as when there is no movement in either limb is shown. Note that at both 3 and 28 days after hemisection the properties of the two limbs were essentially the same. However, there was some apparent improvement from 3 to 28 days in both limbs in that there was almost no period within the step cycle in which the limbs were stationary at 28 days. A similar interpretation was evident when comparing the relationship between the two limbs.

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issue of whether intraspinal coordination is essential for interlimb coordination, Kato [10] examined the locomotor performance of cats after a longitudinal myelotomy in which the two sides of the spinal cord of adult cats were separated surgically from L2-L3 to L7-S1. Interlimb coordination was well preserved after the myelotomy, demonstrating that ipsilateral-contralateral communication at the level of the lumbar segments was not essential to coordinate the two limbs. Kato [11] further observed that when a hemisection and longitudinal myelotomy were combined to isolate one leg of the cat from descending and contralateral neural control, the two hind limbs regained a significant level of coordination within 42 days after surgery. These data emphasize the importance of the mechanical linkage between the isolated limb and the remainder of the body in improving some level of interlimb coordination in the absence of neural interconnections. The present results demonstrate that symmetrical descending control of the sensory and motor systems of the spinal cord is not necessary to sustain bilateral symmetry in locomotion. We observed that asymmetric descending signals can support locomotion when the spinal cord is not injured directly. Thus, it appears that whatever descending signals are important in modulating posture and locomotion, these signals readily become functional bilaterally. This observation raises a variety of questions regarding why many individuals that are impaired by a bilateral spinal cord injury are able to use one leg or the other. These studies do not demonstrate an absence of control of descending tracts in the control of locomotion, but the existence of alternative means of coordinating the motor output from both sides of the spinal cord. It also seems likely that some motor tasks will be more dependent on supraspinal control than others. For example, the locomotor pattern in a non-human primate following a thoracic hemisection is remarkably similar to the pattern observed before the lesion. One apparent important difference following a thoracic corticospinal lesion in the Rhesus is the prevalence of a modest deficit in the initial portion of the swing phase, often referred to as footdrop. Another alteration in the motor control capability is particularly evident when the animal is attempting to perform fine motor skills requiring precise control of the more distal muscles of the lower limb [9]. Whether a difference in the control of the more proximal joints during locomotion compared to the distal joints during a fine motor task exists in the rat remains to be determined. Although there are considerable data on the anatomical supraspinal-spinal connections in a number of species, there is very limited understanding of the functional significance of specific supraspinal projections to specific sites, e.g., motor pools, within the spinal cord in any species.

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