The Rat Lumbosacral Spinal Cord Adapts to Robotic Loading Applied During Stance

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Received 26 December 2001; accepted in final form 12 August 2002

INTRODUCTION

Load-related sensory information plays an important role in shaping the muscle activity patterns during locomotion (Dietz and Duysens 2000; Duysens et al. 2000; Harkema et al. 1997; McCrea 2001; Pearson 1993). In both humans and cats, with and without intact cortical control, loading of the limb or whole body modulates locomotor activity in two ways. First, loading modulates the magnitude of extensor muscle activity during stance. For example, unloading a single hindlimb of a decerebrate cat during treadmill stepping by allowing the hindlimb to step into a hole reduces average ankle extensor amplitude during the 500-ms period after toe-down by 70% (Hiebert and Pearson 1999). Loading the ankle extensors with a cable-driven mechanism while the cats stepped into a hole restores the activity of those muscles to near normal levels. Mechanically unloading ankle extensors in humans with a cable-driven device also substantially reduces extensor muscle activity (Sinkjaer et al. 2000), while adding load at the center of mass during stance enhances extensor muscle activity (Stephens and Yang 1999). In spinal cord-injured humans, reducing body weight support while stepping at selected speeds on a treadmill can significantly increase the magnitude of ankle plantarflexor electromyographic (EMG) amplitudes, suggesting that the human lumbosacral spinal cord responds to the loading conditions by generating efferent output for stepping (Dietz et al. 1998; Harkema et al. 1997; Koijma et al. 1999). Consistent with these findings, electrical stimulation of load-related afferents in decerebrate cats during stance powerfully enhances ipsilateral extensor EMG activity (see review Pearson et al. 1998). Second, loading alters the timing of the transition between extensor and flexor muscle activity, with the onset of swing typically being delayed when the load is increased. For example, adding load to human infants during stepping prolongs stance (Pang and Yang 2000; Yang et al. 1998), although a similar prolongation was not seen in adults (Stephens and Yang 1999). Electrical stimulation of load-related afferents consistently prolongs extensor EMG activity during stance (Pearson et al. 1998). Such load-related responses have been hypothesized to play a key functional role in locomotor control in that they dynamically adjust the magnitude and timing of muscular activity to compensate for step-by-step variations in loading.

The presence of load-related locomotor responses in decerebrate animals, spinal-injured humans, and human infants suggests that the spinal cord itself plays a significant role in mediating the responses. The purpose of this study was to characterize the capacity of the lumbosacral spinal cord, iso-
Physiological Society Animal Care Guidelines.

University of California at Los Angeles and followed the American procedures used in this study were approved by the Animal Use Committee at surgery. The surgical procedures and maintenance of the spinal rats other rat as described previously (de Leon et al. 1994; Roy et al. 1991).

lateral from supraspinal control, to respond to load-related sensory input. Specifically, we applied loads to the lower shank of the hindlimbs of rats that received complete midthoracic spinal cord transections as neonates during bipedal stepping on a treadmill to determine if the spinal cord could adapt to a repetitively applied load on the hindlimbs. A unique feature of the experiment, in addition to defining load-related responses in animals spinally transected as neonates, was the use of small, lightweight robot arms to apply loads just above the ankle of the hindlimbs as well as to record the hindlimb trajectories during stepping (Reinkensmeyer et al. 2000; London et al. 2000; Edgerton et al. 2001; Timoszyk et al. 2002). This technique allowed us to quantify the effects of load-related sensory input on a broad array of features of stepping including step timing, hindlimb kinematics, and interlimb coordination across thousands of steps. Our findings indicate that the lumbosacral spinal cord responds to additional loading by modifying step timing and muscle activation patterns, while actively maintaining near-normal swing kinematics and interlimb coordination.

METHODS

Animals

The experiments were performed on nine rats that were completely transected at a midthoracic level 5 days after birth. The transected rat pups were returned to their mothers until they reached 21 days of age. At the time of the first and second series of experiments (see following text), the rats were 2 to 3 months old and could perform alternating, weight-bearing, bipedal stepping on the plantar surfaces of the paws on a treadmill. Six months after these experiments, two rats were implanted surgically with fine wire intramuscular electrodes bilaterally for a third series of experiments. The medial gastrocnemius (MG, a knee flexor and ankle plantarflexor) and tibialis anterior (TA, an ankle dorsiflexor) were implanted in one rat and the semitendinosus (ST, a hip extensor and knee flexor) and rectus femoris (RF, a hip flexor and knee extensor) in the other rat as described previously (de Leon et al. 1994; Roy et al. 1991). EMG recordings were made after 21 days of recovery from the implant surgery. The surgical procedures and maintenance of the spinal rats followed the protocols detailed for cats in Roy et al. (1992). All procedures used in this study were approved by the Animal Use Committee at University of California at Los Angeles and followed the American Physiological Society Animal Care Guidelines.

Robotic apparatus

The rats stepped bipedally on a motorized, variable-speed treadmill with a robot arm attached to each of the lower shanks just above the ankle (Fig. 1) (Edgerton et al. 2001; Reinkensmeyer et al. 2000; Timoszyk et al. 2002). The robots were attached with padded neoprene cuffs and the rats stepped on a treadmill. Robot arms recorded the trajectories of the lower shank while applying a downward force proportional to the backward end-effector velocity.

FIG. 1. Rat stepper. Rats were placed in a harness and partially suspended and stabilized in a counterbalanced body weight support system. Toes position and orientation of the rat could be adjusted with a lockable ball joint. A robotic arm was attached to the lower shank of each hindlimb with a padded neoprene cuff and the rats stepped on a treadmill. Robot arms recorded the trajectories of the lower shank while applying a downward force proportional to the backward end-effector velocity.

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![Rat stepper](https://example.com/rat-stepper.png)

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Each rat was trained to step on the treadmill with the robots attached for approximately 3 min/day, for 3 days before each experiment. Because the rats received spinal cord transections as neonates, this training was sufficient for the rats to recover the ability to perform partial body weight–supported stepping. During the period between the second and third series of experiments, the rats were not trained. To facilitate the best stepping in each rat during the experiments, an experienced animal trainer adjusted the amount of body weight support and the orientation of the harness. The amount of support provided was 75 ± 8% (mean ± SD) of the body weight. The treadmill speed was set at 11 cm/s.

To assess the effects of the stance force field, the force field was applied and removed alternately while the rats stepped. The force field was activated or deactivated every 25 steps with a software switch until 10 bouts in the force-field-on condition and 10 bouts in the force-field-off condition were recorded (a total of 500 steps). For the experiments in the third series when EMG was recorded, five force-field-on and five force-field-off bouts were recorded. In all of the experiments, if the rat stopped stepping for more than 5 consecutive seconds, the experimenter briefly raised the rat using the weight-support lever (Fig. 1) and then lowered the rat to the treadmill. Typically, this would induce the rat to reinitiate stepping.

Data collection and analysis

The three-dimensional coordinates of the attachment point of the robot arms to the shanks and the force applied by each robot arm to the shank were sampled at 100 Hz. EMG recordings were sampled at 2 kHz. During all experiments, video recordings of the hindlimb movements were taken in the sagittal plane at 30 frames/s. Robotic, EMG, and video recordings were synchronized by sampling the video time code generator output. Details of the video and EMG data acquisition and analyses have been described previously (see de Leon et al. 1998).

Step detector

To identify individual steps from the robotic motion capture data, the following algorithm was used (Fig. 3). First, the horizontal trajectory of each hindlimb (Fig. 3, solid line) was low-pass filtered (forward and backward to prevent phase lag) with a cutoff frequency of 1.5 Hz (roughly 1.5 times the mean step frequency) (Fig. 3A, dashed line). This filtering procedure removed high-frequency variations in the horizontal trajectory, typically leaving a single minimum point for each step. These local minima were identified (Fig. 3A, squares), and the minimum of the unfiltered, horizontal trajectory within a 500-ms interval centered about each filtered minimum was defined as the beginning of a step cycle (Fig. 3, A–D, circles). The change from positive to negative horizontal velocity was defined as the beginning of stance (Fig. 3, A–D, ×). Thus the swing and stance durations of each identified step were calculated as the duration of time during which the horizontal velocity was positive or negative, respectively. The length of each step was calculated as the difference between the maximum and minimum of the horizontal trajectory of the robot tip within a step (Fig. 3D). Note that, following the step identification procedure, the unfiltered position data were used to calculate the step parameters, as well as for all other analyses and presentations.

The identified step durations and step lengths were approximately normally distributed. Because the step detection algorithm did not distinguish actual steps from short flexion movements, large withdrawal movements, or step resetting by the experimenter, very short and very long steps, as well as steps with very small step lengths, were discarded from further analysis. The step duration (mean ± SD) was 980 ± 270 ms and the results presented below excluded steps whose durations fell above or below two SDs from the mean. Step length was 38.0 ± 17.6 mm with step lengths shorter than two SDs from the mean excluded from the analyses. Steps that did not contain either a swing or a stance phase with at least a duration of 90 ms were also discarded. Based on the above criteria, 7.6 ± 4.1% (mean ± SD) and 8.3 ± 1.7% of steps were omitted from each rat in the force-field-on and force-field-off conditions from bilateral force field experiments, and 5.6 ± 1.2 and 5.3 ± 0.8% were omitted from unilateral force field experiments. An analysis was performed to check the sensitivity of the results to the percentage of discarded steps based on step cycle duration only. No changes in statistical significance were observed.

FIG. 3. Step detection algorithm. A: horizontal trajectory of the robot attachment point (solid line) was low-pass filtered with a cutoff frequency of 1.5 Hz (dashed line) to remove high-frequency variations in the trajectory, typically leaving a single minimum point for each step. The local minima of the filtered trajectory then were identified (squares). The minima of the unfiltered horizontal trajectory within a 500-ms interval centered about each filtered minimum were defined as the beginning of a step cycle (circles). Swing and stance were defined when the horizontal velocity was positive or negative, respectively. Circles and crosses indicate stance-to-swing and swing-to-stance phase transitions, respectively. Vertical trajectory (B), horizontal velocity (C), and position trajectory (D) in parasagittal plane (markers as in A).

J Neurophysiol • VOL 88 • December 2002 • www.jn.org
when between 0 and 60% of the shortest and longest steps were discarded.

Mean trajectory/velocity profiles

To calculate mean step trajectory profiles, each identified step was normalized with respect to time, and the normalized trajectory was resampled with 100 points (cubic spline interpolation). The mean step trajectory then was calculated as the ensemble average of the resampled steps. This resampling procedure allowed the individual step trajectories to be averaged even though the trajectories contained a varying number of points. This procedure yielded trajectories that accurately represented the spatial path of the hindlimbs. Mean step trajectories were calculated for the force-field-on and force-field-off conditions for each rat and averaged for each condition across rats.

The velocity of the shank attachment point was calculated by taking the finite difference of the raw position trajectories. Mean step velocity profiles then were calculated with the same resampling method used to calculate the mean step trajectories. Note that the velocity profiles were resampled after finite differentiation of the position trajectories to preserve the measured velocity information despite the time normalization.

EMG analysis

EMG activity that corresponded to weight bearing, plantar surface stepping was analyzed in two rats. For these rats, an observer blinded to the robotic motion analysis reviewed video data to identify stepping bouts during which alternating plantar stepping occurred. Data depicting poor stepping were discarded. For the rat with hip/knee muscle (RF and ST) electrodes implanted, 20% of the data were eliminated and for the rat with knee/ankle muscle (MG and TA) electrodes 12% of the data were eliminated.

Onsets of EMG bursts were defined as the times at which the rectified and filtered (cutoff 400 Hz) EMG signal exceeded the threshold voltage (mean of the baseline + 3 SD) for 5 ms (cf. Harkema et al. 1997), and ends of bursts were the times at which the signal remained below the threshold for 60 ms (Fig. 4). If three or more burst

**FIG. 4.** Examples of EMG recordings (two top traces, 4th-order, low-pass Butterworth filtered, 400 Hz cutoff frequency) for the medial gastrocnemius (MG), tibialis anterior (TA), rectus femoris (RF), and semitendinosus (ST) muscles with and without application of the bilateral force field during stance and vertical trajectories of the robot end effector. C: expanded view of data in A indicated by thick horizontal bar. TA (A and C) and ST (B) recordings are inverted. Thin, horizontal bars indicate the force-field-on condition. Upward arrows indicate burst onset and downward arrows indicate burst end as detected by the EMG analysis algorithm. The last bursts before and during a change of force field condition were not analyzed. Circles indicate end of stance (A–C) on horizontal, end-effector trajectories (bottom). In one rat during application of the force field, the magnitude of the MG bursts increased and their durations remained constant, while the magnitude and duration of TA bursts remained relatively constant in the right hindlimb (A). In a second rat, the left hindlimb seldom exhibited ST bursts without application of the force field, but, with application of the force field, the ST more frequently exhibited bursts. Statistical analysis for burst amplitude and duration is shown in Fig. 9. Note that, during application of the force field, small oscillations sometimes were seen during stance due to an interaction of the force field with the hindlimb dynamics (see position trajectory in B). Power spectrum analysis of individual hindlimb trajectories confirmed increased power between 4 and 12 Hz during stance in 6 of 8 rats, but the amount of increase was not significantly correlated with the reduction in stance time across rats. The step cycle duration and stance duration in A decreased 40% (P < 0.01) and 35% (P < 0.05), respectively, with application of the force field, while the burst durations of the MG and TA did not decrease significantly. Step cycle durations, stance durations, or burst durations did not change significantly in B.
onsets were detected within a single step segment, the bursts were combined by decrementing the minimum time the EMG signal needed to remain below threshold to result in a burst end (the resulting mean minimum time across all analyzed bursts was 57.2 ± 10.0 ms SD), until two or less bursts remained. Sometimes small, short, apparently spurious bursts were detected by this algorithm. To eliminate these bursts, the integral of the EMG signal with respect to time of each burst was calculated, and bursts whose integral was below 0.002 v/s were discarded. This threshold value was determined by examining a histogram of the burst integrals, which had a bimodal distribution with the lower values corresponding to the small, spurious bursts. The duration of each burst was defined as the time from the burst onset to the first burst end. The magnitude of each burst was defined as the magnitude of the EMG signal at the burst peak. Figure 4 provides a detailed example of the results of this burst detection procedure.

As described previously (Nicolopoulos-Stournaras and Iles 1984), two bursts were sometimes seen for the biarticular RF and ST muscles during a single step cycle. One burst occurred near toe-off in association with knee flexion, whereas the other burst occurred near toe-down in association with hip extension. If the peak of a burst occurred closer to the end of stance it was classified as a “toe-off” burst, and if it occurred closer to the beginning of stance it was classified as a “toe-down” burst. Toe-off and toe-down bursts were analyzed separately.

**Statistics**

To determine the effects of the robotic loading, the mean values of the timing and kinematic parameters were first determined across all steps within each exposure (sample size = 25 steps per exposure). The means of those values were then calculated across exposures to the force-field-on and force-field-off conditions (sample size = 10 exposures for each condition). This calculation yielded two numbers for each rat hindlimb: a parameter value for the force-field-on condition and a value for the force-field-off condition. Paired, two-tailed t-tests were performed on the force-field-on and force-field-off values (sample size = number of rats = 8 for each hindlimb) to test the hypothesis that their mean difference was equal to zero. For the EMG analysis, a paired t-test across field exposures (sample size = 5) was used to compare burst parameters for each of the two rats implanted with EMG electrodes.

**Results**

The mean force applied bilaterally to the shank during stance (averaged across rats) was 21 ± 5 gF and about 6% of body weight. When this force was applied, the position of the ankle was lowered during stance (Figs. 5A and 6A). All rats continued to step consistently when the perturbation was imposed to one or both hindlimbs. The total number of steps analyzed for the eight rats for the bilateral and unilateral fields was 7,682 and 8,455, respectively.

**Imposing an additional load at the lower shank during stance decreased stance duration while maintaining a normal swing trajectory and duration**

In response to the bilateral perturbation, step cycle duration decreased significantly in both hindlimbs (Fig. 7). The decrease in step duration was primarily due to a shorter stance duration since swing duration was unaffected. Correspondingly, step length, measured from the toe-down to the toe-off position on the treadmill by video analysis, decreased significantly (Fig. 6D). The step length measured by the robots at the shank (as opposed to the toes) was relatively unchanged (Fig. 5A) because application of the force field caused the ankle to dorsiflex (Fig. 6B) and the lower shank to move downward as well as backward (Fig. 6A). Consequently, the lower shank moved farther backward along the treadmill during stance compared with the distance traveled backward by the toes (Fig. 6D). The backward speed of the lower shank increased during stance (Fig. 5C) due to this rotation (Fig. 6A), even though the treadmill speed was constant.

During swing, no force was applied to the hindlimb, yet modifications were apparent in the spatial trajectory and velocity of the hindlimb as it moved forward. Specifically, the arc-length traveled by the lower shank during swing increased.

**FIG. 5.** Average step trajectories (A) and velocities (B–D) of one hindlimb across all rats with (solid lines) and without (dashed lines) bilateral application of the stance force field. Trajectories were averaged across steps (n ~ 500) for a given rat, and then the mean trajectories were averaged across rats (n = 8). Ellipses indicate ± SD in the horizontal and vertical directions. Markers indicate positions and velocities at the beginning of swing (O), maximum step height (+), and the beginning of stance (X). In C and D, the difference between the mean velocities for the entire stance or swing phase during the force-field-off (dashed lines) and force-field-on (solid lines) conditions are labeled (Δ). Markers indicate the significance of the difference (****P < 0.001, ***P < 0.01, **P < 0.05, *P < 0.1, paired t-test) for each phase. During application of the force field, the lower shank was perturbed downward during stance but quickly returned to its unperturbed trajectory during swing (A), while its vertical velocity increased during both swing and stance (B and D). Application of the force field resulted in an increase in backward speed during stance (C). The horizontal distance traveled by the lower shank did not change significantly with application of the force field (A), but the distance traveled by the toes did (see Fig. 6A).
when the force field was applied because the hindlimb initiated swing from a lower position (Fig. 5A). Despite this increase in distance traveled during swing, the duration (Fig. 7) and spatial trajectory (Fig. 5A) of the swing were relatively unchanged. In other words, after the beginning of swing, the hindlimb quickly returned to the unperturbed swing path. To accomplish this, the vertical velocity of the hindlimbs was increased during early swing (Fig. 5, B and D).

An analysis was performed to determine how quickly the spinal control of stepping responded to the application and removal of the force field. When the force field was applied, step cycle duration decreased significantly on the first step to approximately its mean force-field-on value (Fig. 8A). In contrast, when the force field was turned off, step cycle duration did not increase significantly until the third step (Fig. 8B). Thus the spinal cord responded immediately to application of the force but responded over the course of a few steps to its removal. An analysis also was performed to determine whether the response to the force field varied with repeated exposures. Using a linear regression approach, no significant relationships were found for step cycle or stance duration as a function of the number of exposures to the force field (data not shown).

Hindlimb muscle activation patterns were modified by the force field application

Figure 4 shows ankle, knee, and hip muscle EMG recordings (filtered and rectified) from two rats with and without bilateral application of the force field. For the rat implanted with MG/TA electrodes, the peak MG burst amplitude increased by 102% in the right hindlimb and 23% in the left hindlimb in the force-field-on compared with the force-field-off condition (Fig. 9A). No significant differences in burst amplitude were observed for the TA (data not shown). Neither MG nor TA burst duration changed significantly with application of the force field.

With application of the bilateral stance field, the peak EMG amplitude of the ST at toe-down increased significantly (33%) in the right hindlimb (Fig. 9B) but was unchanged in the left hindlimb. The magnitude of the right hindlimb RF bursts also increased significantly (348%) with application of the force field. The ST in both hindlimbs seldom exhibited EMG bursts when the force field was off (about one burst per 25 steps at toe-down and none at toe-off), but, when the bilateral force field was on, the frequency of occurrence of toe-down bursts increased to about five per 25 steps and the occurrence of toe-off bursts increased to about one burst per 20 steps (Fig. 9D). The duration of the toe-off bursts of the RF in the right hindlimb increased significantly (from 322 to 914 ms) with application of the bilateral force field. No significant changes in burst duration for the ST muscle were observed.

Both the ipsilateral and contralateral hindlimbs adapted to the unilateral force field

When the force field was applied to only one hindlimb, the downward perturbation during stance was experienced only by the hindlimb to which the force field was applied (Fig. 10, A and B). However, the perturbation resulted in a small but significant decrease in step cycle duration (Fig. 11). Similar to the response observed with the application of the bilateral force field, stance duration decreased in the perturbed hindlimb with
application of the unilateral force field (Fig. 11A). This adaptation was accompanied by a decrease in step cycle duration in the contralateral, nonperturbed hindlimb, primarily due to a decrease in swing duration (Fig. 11B). The combination of these changes was sufficient to maintain the interlimb coordination during stepping.

**Discussion**

In this study loads that were applied just above the ankle during stance reduced stance duration and step length while enhancing the level of extensor muscle activity in spinal transected rats. In addition, the normal swing trajectory was preserved even though the loading substantially altered the hindlimb configuration from which swing was initiated. When loading was applied unilaterally the stance phase was shortened on the perturbed side, but interlimb coordination was preserved by reducing the swing duration of the unperturbed hindlimb. These results indicate that the spinal cord coordinated multiple joints and both limbs depending on the sensed state of the limbs, thereby sustaining locomotion in the varying dynamic environment.

**Elevated loading during stance increased EMG activity but shortened the step cycle duration**

The observation that robotic loading enhanced the magnitude of MG EMG bursts during stance is consistent with many previous findings. For example, imposed loading enhances extensor muscle activation in cats and humans (Guertin et al. 1995; Harkema et al. 1997; Hiebert and Pearson 1999; Sinkjaer et al. 2000; Stein et al. 2000). These load-related responses apparently function to sustain stepping in the presence of variations in hindlimb loading.

The downward load applied to the lower shank by the robots decreased both stance and step cycle durations, demonstrating, as has been shown previously (Duysens et al. 2000), that load-related afferent input alters the control of step timing by the spinal cord. However, some previous studies have found that stance is prolonged if stance forces are sustained during the end of stance (Duysens and Pearson 1980; Hiebert et al.
Similar effects have been observed if load-related afferents from the ankle plantarflexors are electrically stimulated at selected points in the step cycle of decerebrate rats (Fouad and Pearson 1997) and cats (Pearson et al. 1998). Furthermore, step cycle and stance durations were longer when some loading of the limbs was allowed in human infants (Yang et al. 1998). Other studies have found different effects on step timing when a load was applied. For example, pulling downward on the hips of adults (Stephens and Yang 1999) or adding mass to the ankles of running humans (Cavanagh and Kram 1989) did not change stride length or step cycle duration. Adding weights to the feet of walking humans did not change stride length or step cycle duration (Bonnard and Pailhous 1991), although it shortened bilateral stance duration and lengthened swing duration. When the mass was added to the waist, however, bilateral stance duration was lengthened and swing duration was shortened.

The differences in the results between the present and some of the previous studies of loading may be due to different stimulation methods, experimental models, and species studied (i.e., decerebrated, acute spinal, chronic spinal, intact, neonatal, adult, rat, cat, or human). Alternately, it may be that the robotic loading technique used in this study generated a novel sensory input pattern to the spinal cord. This latter suggestion implies...
that the differences observed in the duration of stance with load reflect differences in the sensory inputs derived from the varied experimental designs used to study this issue.

For example, the loading technique used in the present study produced local pressure at the attachment point to the lower shank, which could have induced flexor reflexes. Thus the step timing changes might be explained by an increased occurrence of flexor reflexes that abbreviated stance. However, it is unlikely that stimulation of a gross flexor reflex would create nearly the same swing trajectory and interlimb coordination as when the reflexes did not occur. For example, in designing the attachment interface, we found that if the robot attachment cuff were placed around the paw rather than the shank, then the rat would frequently initiate a flexion reflex, hold the limb in the flexed posture, and stop stepping (Timoszyk et al. 2002). In the present study, the rats shortened stance but continued stepping, moving the hindlimb along approximately the same swing trajectory as when the force was not applied and preserving interlimb coordination.

Another unique feature of the loading technique was that elevated loads were applied at the lower shank throughout stance, thus imposing a prolonged perturbation but only to the paw and ankle, rather than to the entire hindlimb. Thus the loading technique applied force to a unique subset of load sensors in the hindlimb. In addition, the loading technique changed the configuration of the hindlimb during stance in a way different from what would be expected for a load applied through the torso (i.e., the ankle was dorsiflexed, while the knee and hip were not flexed). Thus information from muscle length or joint angle sensors was also uniquely altered and may have contributed to the observed reduction in stance duration.

Based on the results of the present and other experiments noted above, we suggest that the spinal circuitry does not interpret isolated parameters such as joint position, muscle length, muscle force, or cutaneous input but rather an instantaneous “state” that is defined by the patterns of sensory input from all mechanoreceptors in both hindlimbs, i.e., as an ensemble. If such is the case, then a modulation of a single parameter such as muscle force or joint angle for example will be interpreted in the context of all other input and may evoke unique responses. A systems-level response is also consistent with the encoding of global responses by more than half of the spino-cerebellar neurons responding to limb position in the cat (Bosco et al. 2000). From this perspective it seems feasible that loading of the plantarflexors could trigger swing in some experimental paradigms, but not in others. Based on the present experiments, it appears that the strategy of the control circuitry of the hindquarters during stepping when the paw (not the whole hindquarters) is loaded only during stance is to shorten the stance phase and cycle duration.

A limitation of the present study is that EMG recordings were taken from only two animals and from only two muscles in each leg of each animal. These recordings may have been atypical given the increased variability of EMG within and between spinalized rats. The MG and TA burst patterns observed in the present study were similar in timing and amplitude to our previous studies of EMG patterns during normal rat locomotion (de Leon et al. 1994; Roy et al. 1991). Moreover, there appears to be a similar response to loading during normal rat locomotion, i.e., increasing loading on the hindlimbs of intact rats by raising the incline level of the treadmill significantly increased EMG amplitudes in extensor (MG) but not flexor (TA) muscles.

**Persistence of a loading effect for several steps after the elevated loading was stopped**

Whereas the spinal cord adjusted its step duration at the onset of additional loading, it normalized the step cycle timing over the course of several steps following the removal of the loading perturbation. Similar “aftereffects” have been observed when an obstacle is placed in the path of the leg trajectory during the swing phase of stepping of spinal transected cats (Nakada et al. 1994). On evoking a stumbling response, there was an increase in flexion in the same and the next step. When the obstacle subsequently was removed, the spinal cord still “anticipated” the obstacle for several steps, continuing to produce an enhanced flexion during the swing phase. The swing kinematics did not return to the prestumble features until several steps after removal of the obstacle. These results underscore the possibility that load-related control of stepping in spinal animals can accommodate over the course of several steps reflecting a type of “short-term memory” of a complex, dynamic motor task in the lumbosacral spinal cord.

**A normal kinematic pattern was maintained during the swing phase when greater than normal loading was applied to the shank during stance**

The stance field perturbed the hindlimb trajectory so that the position of the ankle at the beginning of swing was shifted downward. During swing, however, the hindlimb quickly returned to the unperturbed swing path. The early increase in swing velocity may have been due, in part, to a mechanical spring-like effect arising in the ankle musculature. In particular, ankle plantarflexors may have stored energy during stance as they were stretched by the robot-applied load and then released this energy during early swing. An alternative explanation may be that the ankle plantarflexors generated greater force at toe-off because they were stretched to a more advantageous operating point on their force-length curve. Arguing against a purely mechanical effect, however, is the observed increase in hip flexor (RF) EMG at swing initiation, suggesting that the spinal cord modifies upcoming flexor activity based on load-related input obtained during stance. Also the accommodation of the timing of the swing phase of the contralateral leg during unilateral application of the stance force field suggests a systems-level response that cannot be attributable to a mechanical recoil phenomenon of the ankle extensors. In particular, the swing duration of the nonperturbed hindlimb was adjusted during exposure to the unilateral force field, thereby reducing step duration in the contralateral leg. Generation of coordinated bilateral motor responses in response to unilateral perturbations also has been observed during stepping in decerebrate cats (Hiebert et al. 1994) and human infants (Pang and Yang 2001; Yang et al. 1998).

**Robotic devices can communicate with and thus potentially assess and teach the injured spinal cord through modulation of afferent input to the spinal cord**

There has recently been a surge of interest in developing robotic devices for assessing and retraining upper and lower
extremity movement following neurologic injury (Colombo et al. 2000; Hesse and Uhlenbrock 2000; Lum et al. 2002; Matjasic et al. 2000; Reinkensmeyer et al. 2002a,b; Volpe et al. 2001). The present results demonstrate the feasibility of modulating afferent input patterns during locomotion with robotic technology. This control includes the possibility of enhancing load-related afferent information during stance to enhance muscle output during both stance and swing. Identification of the patterns of sensory inputs that optimize locomotor recovery and a clearer understanding of how these sensory signals are processed to generate a systems-level response in a given physiological state e.g., following spinal cord injury and/or regeneration, will be necessary to decipher the neural strategies used in in vivo systems to control locomotion.

The authors thank H. Zhong and M. Herrera for assistance in the surgeries. This work was supported by Christopher Reeve Paralysis Foundation Grant DA1–9902–2 and by National Institute of Neurological Disorders and Stroke Grants NS–16333 and NS–42951.

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