

Locomotor Capacity Attributable to Step Training Versus Spontaneous Recovery After Spinalization in Adult Cats

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de Leon, R. D., J. A. Hodgson, R. R. Roy, and V. R. Edgerton.

Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. *J. Neurophysiol.* 79: 1329–1340, 1998. Locomotor performance, hindlimb muscle activity and gait patterns during stepping were studied in step-trained and non-trained female, adult spinal cats. Changes in locomotor characteristics relative to prespinalization bipedal and quadrupedal stepping patterns were used to evaluate the effects of step training on the capacity to execute full weight-bearing stepping after spinalization. Step training consisted of full weight-bearing stepping of the hindlimbs at the greatest range of treadmill speeds possible at any given stage of locomotor recovery. In the initial stages of training the limbs were assisted as needed to execute successful steps. On the basis of two behavioral criteria, the maximum speed of treadmill stepping and the number of successful steps per unit time, the ability to step was at least 3 times greater in animals trained to step versus those allowed to recover spontaneously, i.e., the non-trained. The greater success in stepping was reflected in several physiological and kinematic properties. For example, the amplitude of electromyograph (EMG) bursts in the tibialis anterior (an ankle dorsiflexor), the amount of extension at the end of both the stance (E3) and swing (E1) phases of the step cycle, and the amount of lift of the hindlimb during swing were greater in step-trained than in non-trained spinal cats. The changes that occurred in response to training reflected functional adaptations at specific phases of the step cycle, e.g., enhanced flexor and extensor function. The improved stepping capacity attributable to step training is interpreted as a change in the probability of the appropriate neurons being activated in a temporally appropriate manner. This interpretation, in turn, suggests that step training facilitated or reinforced the function of extant sensorimotor pathways rather than promoting the generation of additional pathways. These results show that the capacity of the adult lumbar spinal cord to generate full weight-bearing stepping over a range of speeds is defined, in large part, by the functional experience of the spinal cord after supraspinal connectivity has been eliminated. These results have obvious implications with regards to 1) the possibility of motor learning occurring in the spinal cord; 2) the importance of considering “motor experience” in assessing the effect of any post-spinalization intervention; and 3) the utilization of use-dependent interventions in facilitating and enhancing motor recovery.

INTRODUCTION

There have been several detailed descriptions of the locomotor capacity of adult spinalized cats that have been trained to step (Barbeau et al. 1987, 1993; Barbeau and Rossignol 1987, 1991; Edgerton et al. 1991, 1997a,b; Hodgson et al. 1994; Lovely et al. 1986, 1990). These studies show that spinal cats can execute full weight-bearing hindlimb stepping on a treadmill while restrained in a harness with their forelimbs resting on a platform, within 1–3 wk of spinalization when some level of step training is imposed (Barbeau and

Rossignol 1987; Hodgson et al. 1994; Lovely et al. 1986, 1990) or when specific pharmacological agents, e.g., clonidine or strychnine, are administered to the trained spinal cats (Barbeau et al. 1993; Barbeau and Rossignol 1991; Edgerton et al. 1997a,b). As the postspinal time increases and the animals continue to be trained, hindlimb stepping ability progresses, i.e., increases in maximum treadmill speed and number of plantar surface steps performed, and usually reaches a plateau after 2–3 mo of training (Barbeau and Rossignol 1987; Edgerton et al. 1997a; Lovely et al. 1986). In addition, many features of the activation patterns (EMG) of the muscles and of the hindlimb kinematics of stepping are reestablished in trained adult spinal cats (Belanger et al. 1996; de Guzman et al. 1991; Forssberg et al. 1980; Lovely et al. 1990) and kittens (Grillner 1973, 1981; Smith et al. 1982).

Although these studies clearly demonstrate that an adult spinal animal can regain the ability to step when trained to step, only one study (Lovely et al. 1986) has used a non-trained group of spinal animals to differentiate the amount of recovery attributable to spontaneous, i.e., normally occurring recovery from spinal surgery, versus training interventions. Although Lovely et al. (1986) found that the maximum treadmill speed in step-trained cats was significantly greater than in non-trained cats 6 mo after spinalization (and 5 mo of training), there were no comparisons of the electromyograph (EMG) patterns or the kinematics of stepping between the groups.

In the present study, cats were trained to step beginning 1 wk after a complete low thoracic spinal cord transection and training continued for 3 mo. A greater improvement in locomotor performance and differences in the patterns of muscle activation and in the hindlimb kinematic characteristics were observed in these trained spinal cats compared with spinal cats that were treated similarly except for not being trained to step. In addition, the advantages of comparing bipedal hindlimb stepping before and after spinalization for differentiating the effects of training versus spontaneous recovery of stepping ability were addressed. The differentiation of training-induced effects versus the spontaneous recovery of stepping performance of spinal cats is important because it defines the degree to which the “experience” of the neuromotor system after spinal transection can shape its functional properties and potential for motor recovery. In addition, the implications of these results to rehabilitative strategies to postspinal interventions are overt.

METHODS

Experimental design

COMPARISON OF STEPPING IN TRAINED AND NON-TRAINED SPINAL CATS. Twelve adult (2–3 yr of age) female cats were

TABLE 1. Maximum treadmill speed* in step-trained and non-trained cats before and after spinalization

Group	Week Relative to Spinal Transection												
	-7 to 0	1	2	3	4	5	6	7	8	9	10	11	12
Step-trained cat													
1	1	0	0	0	0.7	1	1	-	-	0.6	1	1	1
2	1	-	0	0	0	0	0	1	0.8	-	0.8	-	0.8
3	1	0	0	0	0	0	0.6	1	0.6	0.8	-	0.4	0.4
4	1	0	-	-	0.5	1	1	0.6	0.8	0.8	0.6	0.8	0.6
5	1	-	0	0	0.4	0.8	1	1	1	1	1	0.8	1
6	1	0	0	0	0.4	0.7	0.6	0.6	0.5	0.8	1	0.7	0.8
Non-trained cat													
1	1	0	0	0.6	0.6	0.6	0.8	0.8	0.8	0.6	0.8	0.8	0.6
2	1	0	0	0	0	0	0	1	1	0.8	1	0.8	1
3	1	0	0	0	0	0	0	0	0	0	0	0.6	0
4	1	0	0	0	0	0	0	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0	1	0	0	0	0.2
6	1	0	0	0	0	0	0	0	0	0	0	0	0
7											0		
8											0		
9											0		

* Maximum speed is defined as the fastest speed at which 20 consecutive cycles of full weight-bearing, bipedal steps were performed on the plantar surfaces of the paws. Additionally, stepping must have been performed in the same manner at speeds below the fastest speed. Complete behavioral analyses could not be performed during some experimental weeks, denoted by a dash. Average maximum speeds from step-trained cats 1-6 and non-trained cats 1-9 are compared in Fig. 1 A.

trained to perform bipedal treadmill locomotion, i.e., only the hindlimbs stepped while the forelimbs rested on a platform. Age-related factors could not have explained the differences in recovery between the trained and non-trained spinal cats because all of the surgeries were performed in adult animals, i.e., after the period in which the "infant lesion effect" has been suggested to influence locomotor recovery (Robinson and Goldberger 1986a). EMG electrodes were implanted in selected hindlimb muscles and tests of bipedal stepping began 1 wk later. After sufficient EMG and kinematic data were collected, the spinal cords of the cats were completely transected (T₁₂-T₁₃). Beginning one week after spinalization, six of the spinal cats received bipedal step training (see Step-trained cats 1-6, Table 1) and six of the cats were not trained (see Non-trained cats 1-6, Table 1). Weekly tests of bipedal stepping on a treadmill were performed for 12 wk starting 1 wk after spinalization.

An additional three cats underwent spinal cord transection (no prespinal training or EMG electrode implants), received no training and were tested for bipedal stepping only once, at 10 wk after spinalization (see Non-trained cats 7-9, Table 1).

COMPARISON OF PRESPIAL QUADRUPEDAL STEPPING TO PRE- AND POSTSPIAL BIPEDAL STEPPING. Five cats (Step-trained cats 3-5 in Table 1 and 2 additional cats) were trained to perform quadrupedal and bipedal treadmill stepping before spinalization. After EMG and kinematic data were collected during normal stepping, the spinal cords were transected. All of the cats received bipedal step training, which began either 1 wk ($n = 3$) or 12 wk ($n = 2$) after spinalization. After training was initiated, tests of bipedal treadmill stepping were performed weekly for 12 wk.

Surgical procedures

During all surgical procedures sodium pentobarbital (35 mg/kg ip) was administered to each cat after pretreatment with atropine (ip) and acepromazine (im). Supplemental doses of anesthesia were administered as needed during surgery to maintain a low level of arousal.

Before spinalization, intramuscular recording electrodes were

chronically implanted in selected hindlimb muscles in the right hindlimb [deep region of the distal compartment of the semitendinosus (St), lateral deep portion of the vastus lateralis (VL), distal portion of the iliopsoas (IP), midbelly of the soleus (Sol), midbelly deep portion of the tibialis anterior (TA), medial deep portion of the medial gastrocnemius (MG)] as previously described (de Leon et al. 1994; Pierotti et al. 1989). After each muscle was back-stimulated to ensure proper electrode placement, each wire was secured in the muscle with a suture at its entry and exit from the muscle.

The spinal cords were transected at the T₁₂-T₁₃ junction as described in detail previously (Roy et al. 1992). Briefly, a skin incision was made on the back to expose the vertebral processes between ~T₁₀ to L₁. A partial laminectomy was performed to expose the spinal cord at the T₁₂-T₁₃ junction. Fine scissors and forceps were used to cut the dura and to perform the transection beginning on the dorsal surface of the cord between T₁₂-T₁₃. After the transection, no spinal cord matter was visible between the two cut ends of the cord. The ends of the cord retracted leaving a clear space between the two cut ends. This procedure allowed for the preservation of the large ventral artery of the spinal cord. Gelfoam was inserted in the space and the muscle and skin above the lesion site were closed with sutures.

Animal care procedures

Postspinalization management of the spinal cats has been detailed elsewhere (Roy et al. 1992). Cats were housed in spacious cages, 2-4 cats per cage, with the cage floors covered with shredded newspaper. The bladders and colons of the cats were expressed twice daily for the duration of the experiment. Dry kibble and water were given ad libitum and wet food was given once daily. All procedures were performed in accordance with the American Physiological Society Animal Care Guidelines and were approved by the Animal Use Committee at the University of California, Los Angeles.

Hindlimb training and testing procedures

PRESPIALIZATION. Before spinalization, bipedal hindlimb step training on a motorized treadmill was performed for 15-30 min/

day, 5 days/week for 4–8 wk. The forelimbs of the cats rested on a platform raised ~2.5 cm above the treadmill belt. A cloth harness was fitted over the shoulders, between the forelimbs and around the upper trunk of all trained cats. To accustom the cats to stepping while supported in the harness, a food reward (Gerbers Baby Food) was provided. After ~2 wk of training, the cats performed consistent bipedal locomotion while in the harness at treadmill speeds of 0.2–1.0 m/s. In some animals, quadrupedal step training also was performed on the same treadmill as part of their daily training regimen. During quadrupedal stepping the animals were not restrained and food rewards were used to encourage the animals to maintain a steady gait at treadmill speeds of 0.2–1.0 m/s. Prespinalization stepping was tested at speeds up to 1.0 m/s, usually in increments of 0.2 m/s. Stepping at slower speeds was tested first and a minimum of 10 consecutive steps per speed was required before continuing testing at the next higher speed.

POSTSPINALIZATION. After spinalization, training of bipedal hindlimb stepping was performed for 30 min/day, 5 days/week on the same treadmill that was used to train normal stepping. As during training of prespinal bipedal stepping, the forelimbs of the spinal cats rested on a platform above the treadmill belt and a cloth harness was used to support the upper trunk. In addition, a thin sheet of plexiglass, ~7 cm in height, was placed longitudinally to separate the hindpaws during stepping. The same platform, harness and divider were used during locomotor testing.

Initially, step training in the spinal cats consisted of manually assisting the hindlimbs to produce plantar surface stepping on a slowly moving treadmill belt. The amount of load during stance in one hindlimb was facilitated by lifting the tail. The hindpaw was placed forward or a single pinch was applied to the skin around the ankle to facilitate the initiation of swing. As stepping ability progressed the hindlimbs were able to bear more weight and initiate flexion, thus less assistance was necessary. After 5–8 wk of training, paw contact with the moving treadmill belt was sufficient to trigger stepping episodes at speeds between 0.2–0.6 m/s. At this time, the trainers held the tail only to provide lateral support during stepping.

To evaluate bipedal locomotion after spinalization, a standard testing procedure was used for both the trained and non-trained cats. On the days when the trained cats received testing in addition to training, stepping was tested several hours before the start of the training session. To minimize unintended effects of testing on step recovery, particularly in the case of non-trained spinal cats, only 45 s of testing was performed at a given speed. Paw placing or perianal stimulation was used for no longer than 15 s to attempt to trigger stepping at a given speed. Thereafter, the ability of the hindlimbs to generate stepping without such assistance was observed for the remainder of the 45 s trial. Testing began at the slower treadmill speeds (0.2–0.4 m/s) and if 10 or more full weight-bearing steps were performed, testing proceeded at progressively faster speeds, usually in increments of 0.2 m/s, up to 1.0 m/s. However, if difficulties in initiating stepping persisted at the slow speeds, the locomotor test was ended. The minimum and maximum amounts of time spent on the treadmill during a single test were 90 and 225 s, respectively. The results presented here are based on locomotor tests performed without the use of trainer assistance, e.g., mechanical stimulation of the tail, to maintain stepping.

Assessment of locomotor ability

Stepping ability during a locomotor test was measured by the maximum speed of stepping and by the number of steps performed at a given speed. The maximum speed was defined as the fastest speed at which full weight-bearing steps were performed on the plantar surfaces of the paw in one hindlimb. To ensure that the

animals could step reliably at the maximum speed, it was required that the cats execute at least 20 consecutive steps at the peak speed and at all speeds tested (in increments of 0.2 m/s) below the fastest speed. Thus, a maximum speed of 0.6 m/s indicates that the cat exhibited consistent stepping at 0.2, 0.4, and 0.6 m/s. In cases of inconsistent stepping performance, i.e., the inability to execute 20 consecutive steps at any speed, a maximum speed of 0 m/s was recorded. In these instances, the number of steps performed at slow speeds (0.2–0.4 m/s) provided a more useful indicator of stepping ability (e.g., compare maximum speed and step number for Non-trained *cat 3*, Tables 1 and 2). Step number was determined by counting the number of full weight-bearing plantar surface steps performed by the two hindlimbs at a given speed during a 45-s trial.

Data recording and analysis

EMG and kinematic data during stepping were recorded as described in detail previously (de Leon et al. 1994). Briefly, raw EMG signals were amplified and recorded on an FM tape recorder (TEAC Model XR-510, TEAC Corporation, Montebello, CA), while a camera and video cassette recorder (Panasonic System Camera, WV D5100 and Panasonic AG1280P; Cypress, CA) were used to record the video signals. An SMPTE time code generator (Model F30, Fast Forward Video; Irvine, CA) was used to synchronize video frames with the EMG signals recorded on FM tape.

A range of 8–50 EMG bursts corresponding to full weight-bearing, plantar-surface stepping were analyzed by computer as described previously (de Leon et al. 1994). Briefly, the EMG signals from each muscle during 10–20 s bouts of stepping were sampled into an AMIGA computer at 2 kHz and calibrated. The EMG signals were rectified and smoothed by using a moving average (9-point moving average, i.e., 110 Hz low-pass filter). Computer software designed in-house was used to detect and display the start and end of each burst based on a given threshold level above the baseline noise for a channel. The starting and ending points of bursts were used to determine the relative timing of EMG activity recorded from different muscles. Cycle periods were calculated as the time between the starting points of successive bursts of EMG activity. Burst durations were calculated as the time between the start and the end of each burst. Mean EMG amplitude was calculated by dividing the integrated area of each unsmoothed burst by the burst duration. Averaged EMG waveforms were generated from unsmoothed bursts triggered to the start of TA activity in each recording channel. EMG activity corresponding to poorly executed steps, i.e., dorsal-surface steps or steps that were not full weight-bearing, were excluded from analyses (with the exception of Non-trained *cat 6* in Table 2, which failed to exhibit any weight-bearing thus, rhythmic EMG activity during unsuccessful attempts at treadmill stepping was analyzed).

The videotaped stepping sequences were reviewed on video monitors and the number of successful steps at a given speed was counted. Kinematic analyses were performed on 8–10 step cycles from which EMG activity was analyzed (for details, see de Leon et al. 1994). Briefly, the bony landmarks on the hindlimb were digitized and calibrated and the stick figure representations were plotted. The knee position was triangulated as described previously (Goslow et al. 1973). Hip, knee, and ankle joint angles were generated by triangulating the *x* and *y* coordinates. Specifically, the greatest amount of flexion during the F and E2 phases of the step cycle (Philippson 1905) and the greatest amount of extension during the E1 and E3 phases were measured. The maximum amount of forward and backward hindlimb movement was measured by the displacement of the *x* coordinate of the paw marker (head of the 5th metatarsal) relative to the *x* coordinate of the hip marker (greater trochanter). The amount of paw lift during swing was measured by the displacement of the *y* coordinate of the ankle

TABLE 2. Number of successful steps* in step-trained and non-trained cats before and after spinalization

Group	Week Relative to Spinal Transection												
	-7 to 0	1	2	3	4	5	6	7	8	9	10	11	12
Step-trained cat													
1	90 ^{†‡}	0	0	0	74 ^{†‡}	50	78	–	95	83	92	105	92 ^{†‡}
2	113 ^{†‡}	–	0	0	0	0	41 [‡]	37 [†]	77	117	84	65	116 ^{†‡}
3	96 ^{†‡}	0	0	0	0	43 ^{†‡}	110	74	18	71	–	96 ^{†‡}	58
4	106 ^{†‡}	7	–	–	31 ^{†‡}	77	106	76	100	85	88	80	83 ^{†‡}
5	90 [†]	0	0	0	–	35	–	110 [†]	–	–	–	–	103 [†]
6	90 [†]	–	–	–	99 [†]	–	–	–	–	–	–	–	82 [†]
Non-trained cat													
1	85 ^{†‡}	0	54 ^{†‡}	124	120	108	104	94	99	94	63	105	101 ^{†‡}
2	108 ^{†‡}	0	0	0	0	3	3	89 ^{†‡}	69	83	82	86	82 ^{†‡}
3	94 ^{†‡}	0	0	29 ^{†‡}	22	35	9	47	0	29	36	70	26 ^{†‡}
4	102 ^{†‡}	0	22 ^{†‡}	16	41	0	0	0	10 ^{†‡}	0	2 ^{†‡}	9 ^{†‡}	4 ^{†‡}
5	82 ^{†‡}	0	0	0	0	15 ^{†‡}	14 [†]	3	72	55	2	2	33 [‡]
6	95 ^{†‡}	0	0	0	0	0	0	0	0	0	0	0	0
7											3		
8											20		
9											25		

* Full weight-bearing bipedal steps performed on the plantar surfaces of the paws during 45 s of locomotor testing at a speed of 0.4 m/s (data shown for step-trained cat 6 was obtained from stepping at 0.3 m/s). [†] Electromyograph (EMG) activity recorded from muscles in the right hindlimb during 8–50 steps were analyzed (these data are shown in Figs. 3 and 4). [‡] Kinematic analyses were performed on 8–10 steps to measure toe and ankle displacement (these data are discussed in text) and joint angles at different phases of the step cycle (shown in Fig. 6). Average step number from step-trained cats 1–6 and non-trained cats 1–9 are compared in Fig. 1B. Complete behavioral analyses could not be performed for some tests, denoted by a dash.

marker (lateral maleolus). Swing was defined as the period starting with forward movement of the paw (toe off) and ending with, but not including, paw contact (touchdown). Stance was defined as the period beginning with paw contact and ending before forward paw movement. These definitions were based on the reported characteristics of the swing phase of stepping in spinal cats, i.e., the paw drags before it is lifted (Belanger et al. 1996).

All comparisons of the EMG and kinematic characteristics of stepping between step-trained and non-trained cats were based on analyses performed on the number of cats shown in Table 2 (see [†] and [‡]). Most EMG and kinematic data for spinal cats were from single tests performed between 4–8 wk after spinalization, i.e., when full weight-bearing stepping was initially observed, and/or 12 wk after spinalization, i.e., when a plateau in locomotor performance was attained. When a sufficient number of step cycles from one cat was not available from a single test (<8 cycles), data from multiple tests were pooled for analysis (see Non-trained cats 4, 5, Table 2). Comparisons of the EMG characteristics of quadrupedal and bipedal locomotion were based on analyses of stepping in 5 step-trained cats. Data from 3 of the 5 cats (Step-trained cats 3, 4, 5, Table 2) were from tests performed before and 12 wk after spinalization. Data from the remaining 2 cats (not shown in Table 2) were from tests performed before and 24 wk after spinalization. EMG activity during prespinal quadrupedal and bipedal stepping and postspinal bipedal stepping after 12 wk of step training was analyzed in these cats.

Statistics

Comparisons of group mean values (trained versus non-trained; pre- versus postspinal) were statistically analyzed using computer-based resampling (“bootstrap”) software (Resampling Stats 4.0.2, Arlington, VA), which is better suited for analyzing nonnormally distributed data than the more traditional population techniques (Efron and Tibshirani 1991). In the resampling approach, significance is judged by comparing statistics calculated from actual data to analogous statistics calculated from computer simulations that

realize the null hypothesis of the experiment. In this case, the actual difference between group means was compared with the differences between means of groups generated from the original data by randomizing group membership. If the actual difference was >95 or 99 of 100 of the differences in the randomized data, the actual result was considered significant at the $P < 0.05$ or $P < 0.01$ level, respectively. Paired *t*-tests were used to compare mean cycle period before and 12 wk after spinalization in individual cats.

RESULTS

Locomotor performance after spinalization

On the basis of two behavioral criteria, the ability to step bipedally on a treadmill after spinalization improved more in cats that were trained to step than in those that were not trained. By week five, the step-trained cats could step at speeds that were on average ~6 times faster than the speeds attained by the non-trained cats (0.6 ± 0.2 vs. 0.1 ± 0.1 m/s; Fig. 1A). A second behavioral measure of performance, the number of successful steps occurring in a 45 s period at a speed of 0.4 m/s, also demonstrated the beneficial effects of step training. The step-trained cats performed ~30–50 more steps than the non-trained cats (Fig. 1B, weeks 6–12).

Marked individual differences in the level of locomotor performance were evident among the non-trained cats (Tables 1 and 2). For instance, in two of six non-trained cats, maximum speeds and step number recovered to prespinal levels; whereas, three other non-trained cats could not maintain weight-bearing stepping for 20 consecutive cycles at any speed. In the latter non-trained cats, occasional short bouts of full weight-bearing stepping occurred but were interrupted frequently by stumbling or collapsing of the hindlimbs to a nonweight-bearing position resulting in a low step

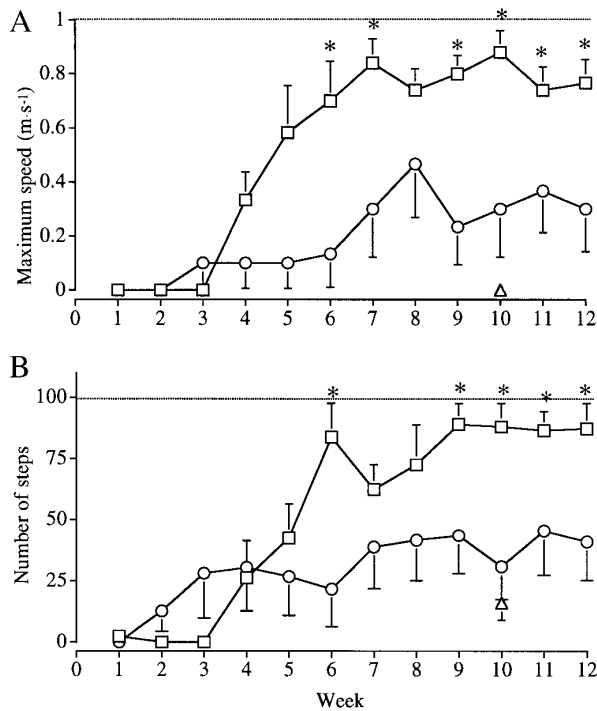


FIG. 1. Performance of bipedal hindlimb stepping on a treadmill after spinalization in step-trained and non-trained cats. Average maximum speeds (A) and number of steps performed during 45 s of testing (0.4 m/s) (B) from 6 step-trained (□) and 6 non-trained (○) cats are shown 1–12 wk after spinalization and 3 non-trained cats 10 wk after spinalization (△) (see text for definition of maximum speed). The horizontal line indicates average maximum speed or number of steps observed during prespinalization tests of stepping. Data from individual cats are shown in Tables 1 and 2. Bars, standard errors of mean. * Significantly different from non-trained ($P < 0.05$).

number. One non-trained cat failed to exhibit any weight-bearing stepping throughout the study. Poor locomotor performance was observed in each of three additional non-trained cats that underwent a single locomotor test at 10 wk after spinalization rather than the weekly testing that occurred in the six other non-trained cats (see Fig. 1 and Tables 1 and 2). In contrast to the non-trained cats, all step-trained cats performed hindlimb stepping similarly.

EMG burst characteristics

Several changes in the activation patterns of selected muscles reflect greater improvements in locomotion in the trained than in the non-trained cats. Valid comparisons of EMG patterns from prespinal through the 12 wk of training could be made by using the chronically implanted intramuscular electrodes. Examples of the EMG patterns during bipedal stepping before and after spinal transection are shown for one step-trained and one non-trained cat in Fig. 2. Before spinalization, clear consistent bursts of activity were recorded from each hindlimb muscle. When the cat was positioned over the treadmill during the first week after spinalization, little EMG activity was evident in either the step-trained or non-trained cats; none could execute weight-supported stepping. Four to six weeks after spinalization, rhythmic EMG activity was recorded from the hindlimb muscles in all step-trained and some non-trained cats, but the episodes

of rhythmic activity were shorter in the non-trained cats. A typical non-trained cat could execute a few weight-supported steps on the dorsal or plantar surfaces of the paws before the hindlimbs stumbled (Fig. 2, d, c), usually followed by a series of unsuccessful attempts at stepping. In most muscles the EMG amplitudes after 4–6 wk approximated those recorded before spinalization in both trained and non-trained cats. The EMG patterns 12 wk after spinalization generally did not change in the trained and non-trained cats from that observed at 4 wk, although changes in the waveforms of flexor EMG bursts were apparent, e.g., TA EMG amplitudes in the trained but not in the non-trained animals were higher after than before spinalization.

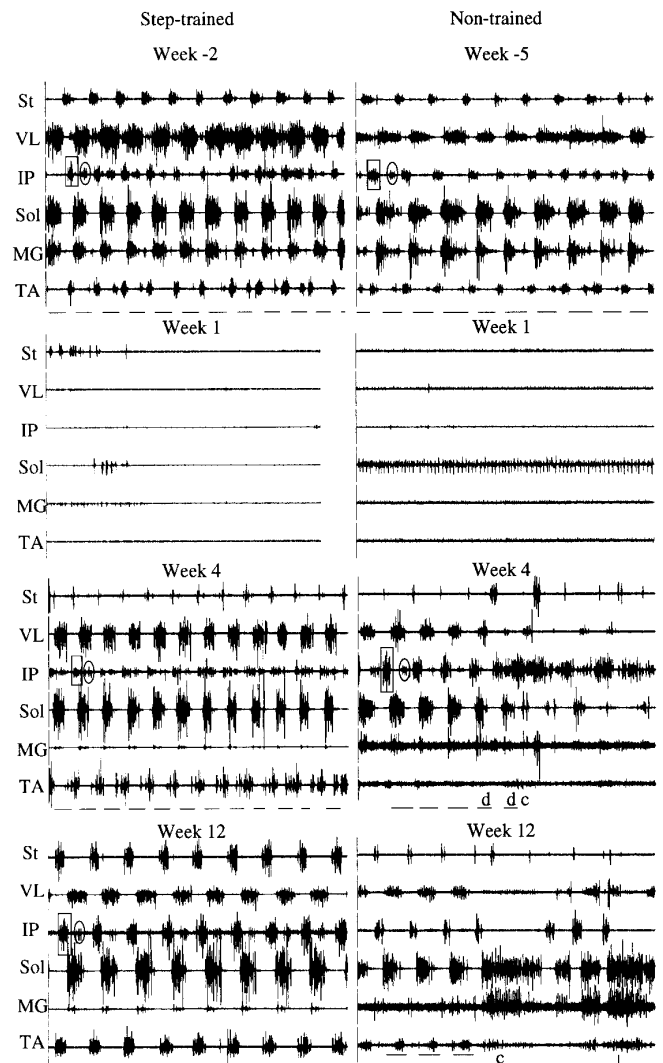


FIG. 2. Electromyograph (EMG) activity during bipedal hindlimb stepping in a step-trained and a non-trained cat before (–2 or –5 wk) and 1, 4, and 12 wk after spinalization. Raw EMG was recorded from selected hindlimb muscles (St, semitendinosus; VL, vastus lateralis; IP, iliopsoas; Sol, soleus; MG, medial gastrocnemius; TA, tibialis anterior) during stepping at a treadmill speed of 0.4 m/s. Stance phase during full weight-bearing step cycles is indicated by horizontal lines below EMG records. c, a step failure (collapse); d, contact of dorsal surface of paw on treadmill belt (toes curled). □, IP bursts during swing (IP_{sw}) ○, stance (IP_{st}) in one step cycle. Horizontal calibration, 1 s, and vertical calibration, 1.0 mV, for all muscles except for Sol (2.0 mV). Data are from Step-trained cat 4 and Non-trained cat 3 (see Tables 1 and 2).

TABLE 3. Cycle period during bipedal hindlimb locomotion before and after spinalization

Group	Week				
	-7 to 0	4-8		12	
	(ms)	(ms)	(%)	(ms)	(%)
Step-trained cat					
1	969 ± 25	873 ± 22*	90	919 ± 20	95
2	779 ± 15	634 ± 7*	81	702 ± 10*†	90
3	912 ± 18	717 ± 6*	79	920 ± 11†	100
4	891 ± 17	719 ± 8*	81	1,000 ± 14*†	112
5	1,023 ± 13	844 ± 21*	83	934 ± 13*†	91
6	1,058 ± 66	829 ± 22*	78	1,115 ± 12†	105
Mean			82 ± 6		99 ± 3‡
Non-trained cat					
1	1,168 ± 41	649 ± 11*	55	769 ± 17*†	66
2	1,015 ± 16	954 ± 16*	94	966 ± 9*	95
3	1,168 ± 97	966 ± 17*	83	1,294 ± 19†	111
4	834 ± 18	708 ± 26*	85	630 ± 40*	75
5	1,037 ± 24	848 ± 29*	82	-	-
6	1,059 ± 14	548 ± 15*	52	621 ± 24*	59
Mean			80 ± 7		86 ± 9

Values shown are means ± SE. Cycle period was measured from 8–50 consecutive EMG bursts during bipedal stepping at 0.4 m/s (data from step-trained *cat 6* was obtained from stepping at 0.3 m/s) before and after spinalization. Data shown are from the soleus (Sol) or vastus lateralis (VL) muscle during full weight-bearing stepping (except the data from Non-trained *cat 6*, which are from nonweight-bearing attempts at stepping and were not included in the group mean). The data collected from weeks 4–8 and 12 are shown as a percentage of the prespinal values on weeks -7 to 0 (%). Complete analyses could not be performed during some weeks, denoted by a dash. See Tables 1 and 2 for corresponding behavioral data. *, †, and ‡, significantly different than weeks -7 to 0 at $P < 0.01$, weeks 4–8 at $P < 0.01$, and non-trained during the same time period at $P < 0.01$.

The EMG patterns of step-trained and non-trained cats during full weight-bearing cycles of bipedal locomotion were quantified with the exception of Non-trained *cat 6*, which never achieved any weight bearing. For this animal, EMG activity during nonweight-bearing cyclic hindlimb movements on the treadmill was analyzed but not included in group means. EMG activity from only a moderate speed of stepping (0.4 m/s) was compared because of the lack of weight-bearing steps in the non-trained cats at the faster speeds. It is important to note that all comparisons of EMG represent the most successful stepping that was observed in each cat. Further, the EMG bursts analyzed in non-trained cats often were not consecutive step cycles because of the inconsistency in the stepping performance of these cats.

CYCLE PERIOD. The mean cycle periods between weeks four to eight after spinalization were 82 and 80% of the mean prespinalization cycle periods in step-trained and non-trained cats, respectively (Table 3). By week 12, the cycle periods in each step-trained cat had recovered to at least 90% of prespinalization values (Table 3). In contrast, the non-trained cats (except Non-trained *cat 3*) showed no further recovery in cycle period after week eight. Further, the mean cycle periods were significantly less in non-trained than step-trained cats at week 12 (Table 3).

MEAN EMG AMPLITUDE AND BURST DURATION. During bipedal stepping, one EMG burst per step cycle was observed consistently in all muscles. The IP had two distinct EMG bursts per cycle, i.e., one burst during swing (IP_{sw}) and one during stance (IP_{st}) (Fig. 2). In some cats two bursts of activity also occurred per step cycle in the TA, both before and after spinalization. All TA EMG activity, however, was analyzed as a single burst for all cats.

A significantly higher EMG amplitude was observed in the TA of step-trained than non-trained cats (Fig. 3A). Compared with prespinal means, EMG amplitude increased in the TA and IP_{sw} (burst during swing phase) and decreased in the VL and MG in the trained cats. Only a significant increase in the IP_{sw} and decrease in the VL occurred in non-trained cats (Fig. 3A). The magnitude and direction of change observed in each of the step-trained and non-trained cats for each muscle are illustrated in Fig. 4. These data indicate that flexor EMG activity tended to be elevated, whereas extensor EMG activity tended to be reduced after spinalization.

Mean burst durations for step-trained and non-trained cats were not significantly different (Fig. 3B). After spinalization, mean burst durations were shorter than before for the St and VL and longer for the TA in both groups (Fig. 3B). The pre- and postspinal comparisons between the two groups, however, differed for the IP_{st} and Sol. Compared with prespinal, the IP_{st} EMG burst was longer in the non-trained but not the step-trained cats and the duration of the Sol was shorter in the step-trained but not the non-trained cats (Fig. 3B).

Hindlimb excursion during the step cycle

Excursions of the hip, knee, ankle, and toes during bipedal stepping before spinalization and 12 wk afterward for one step-trained and one non-trained cat are shown in Fig. 5. Although there were some similarities in the steps performed by step-trained cats and the successful steps of non-trained cats, e.g., dragging of the toes at the initiation of the flexion phase followed by lifting the paw, three principal differences were evident. First, the ankle was lifted higher during swing

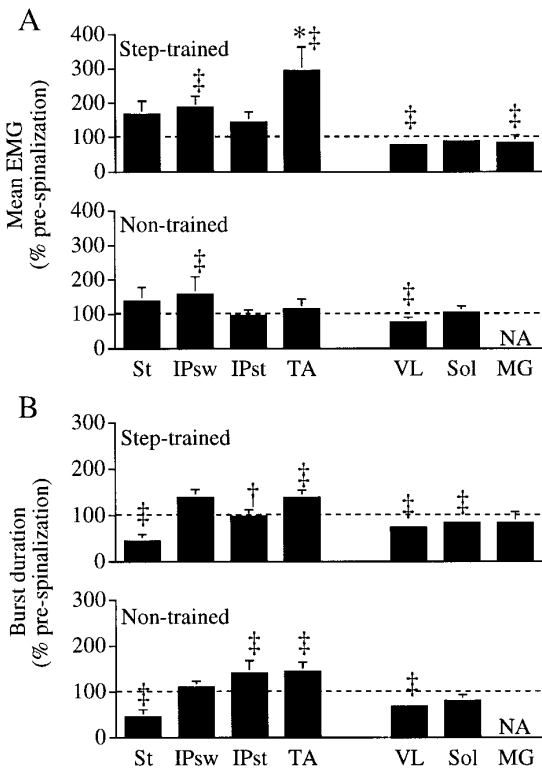


FIG. 3. Amplitude (A) and duration (B) of EMG activity during bipedal locomotion in 6 step-trained and 6 non-trained spinal cats. Mean EMG amplitude and burst duration of 8–50 EMG bursts from selected hindlimb muscles (see Fig. 2 for muscle abbreviations) during full weight-bearing bipedal steps (0.4 m/s) before and 12 wk after spinalization were measured. Postspinal values were expressed as a percentage of prespinalization values in each cat. Data shown are mean \pm SE of normalized values. Complete analyses were not performed for every cat (see Fig. 4 for number of cats). *, †, and ‡, significantly different from non-trained at $P < 0.01$, non-trained at $P < 0.05$, and prespinalization (100%) at $P < 0.01$.

of a successful step in step-trained compared with non-trained cats. Thus the vertical displacement of the ankle was significantly higher after spinalization in the step-trained cats (240% of prespinal values) than in the non-trained cats (114% of prespinal values). Second, the hindlimbs extended further at the end of stance (E3) in step-trained than in non-trained cats. For example, when the amount of backward movement was measured by the displacement of the toe at the beginning of swing (toe off) relative to the position of the hip, a significantly shorter distance was found in non-trained cats (79% of prespinal values) than was measured in the step-trained cats (108% of prespinal values; compare TO in Fig. 5, B and D). Third, the swing phase tended to project further forward at placement (beginning of stance) in step-trained than in non-trained cats. For example, relative to the hip position at touchdown, the forward placement of the toe was significantly greater after spinalization in step-trained cats (124% of prespinal values) than in the non-trained cats (110% of prespinal values; compare TD in Fig. 5, B and D).

Greater ankle angles at F, E1, and E3 occurred in step-trained compared with non-trained cats when tested 12 wk after spinalization (Fig. 6). In step-trained cats, the hip and ankle were more extended at F and E3, respectively, after spinalization. No differences in pre- versus postspinal

joint excursions were observed in non-trained cats. There were no differences in the incidence of yield in trained (knee, 82%; ankle, 39%) and non-trained (knee, 79%; ankle, 51%) cats after spinalization. These results are similar to that reported previously in adult spinal cats trained for 6 mo and tested at the same treadmill speed (Lovely et al. 1990).

Comparison of quadrupedal and bipedal stepping

Differences in mean EMG amplitudes and burst durations for the St, TA, VL, Sol, and MG were found when prespinal, quadrupedal stepping was compared with bipedal stepping after spinalization (Fig. 7). Furthermore, these differences were similar to those found when bipedal stepping before and after spinalization were compared (compare dark and

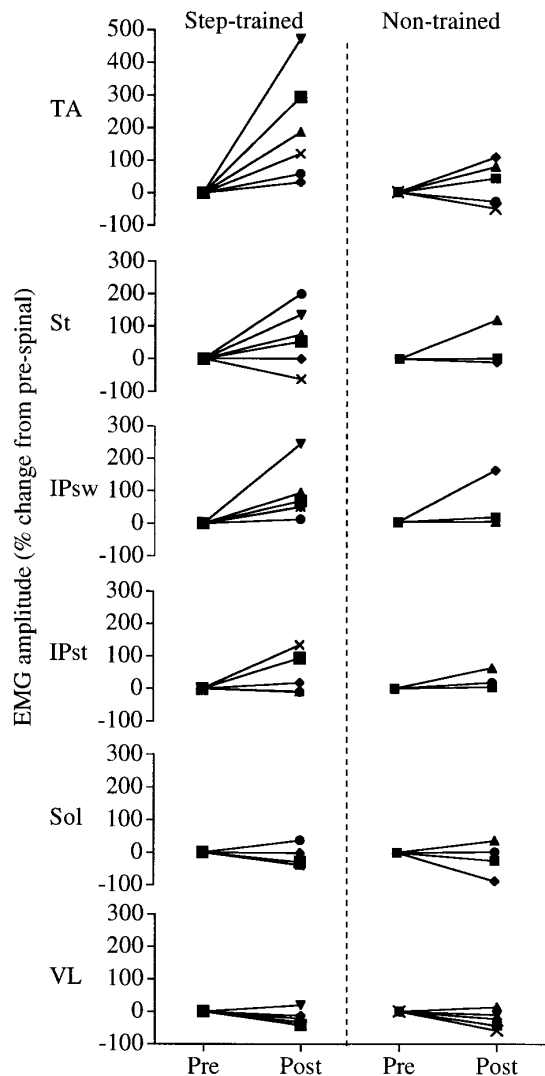


FIG. 4. Changes in EMG amplitude in step-trained and non-trained cats after spinalization. Mean EMG amplitude of 8–50 EMG bursts from selected hindlimb muscles (see Fig. 2 for muscle abbreviations) during full weight-bearing bipedal steps (0.4 m/s) before (Pre) and 12 wk after (Post) spinalization were measured. Data shown are changes from prespinal values (100%) for individual cats (average values of mean EMG amplitude across cats are shown in Fig. 3). Each symbol represents a different cat in each group. Note, scale of vertical axes for each plot is the same.

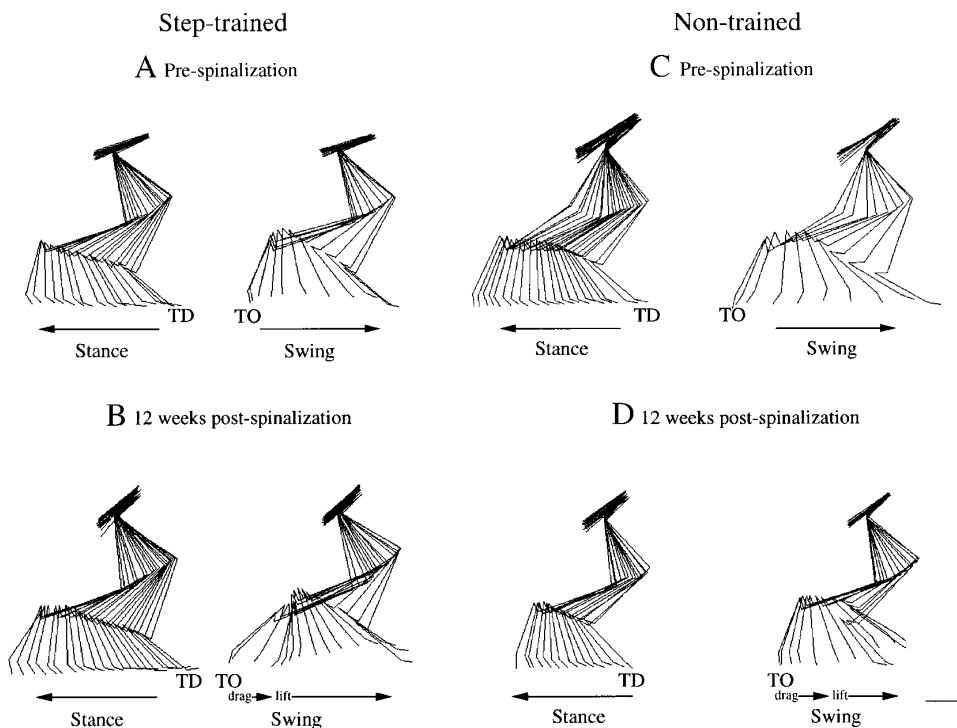


FIG. 5. Stick figure representations of hindlimb from swing and stance phases of one step cycle (0.4 m/s) in a step-trained cat before (A) and after spinalization (B) and from a non-trained before (C) and after spinalization (D). \rightarrow , progression of hindlimb movement. After spinalization, swing was composed of a drag and lift component (see text) indicated by arrows. Touchdown at beginning of stance and toe off at beginning of swing are denoted by TD and TO, respectively. Horizontal position of each hindlimb representation was normalized to position of hip (greater trochanter). Horizontal and vertical calibration, 5 cm. Data are from Non-trained *cat 1* and Step-trained *cat 4* (see Tables 1 and 2).

light bars in Fig. 7). Therefore, based on burst amplitude and duration, similar conclusions could be drawn when either quadrupedal or bipedal stepping was used to evaluate the changes in EMG activity after spinalization. There were, however, some clear distinctions in the EMG waveforms between bipedal and quadrupedal stepping. For example, during quadrupedal stepping, IP activity consisted of a single burst that occurred during swing (Fig. 8, IP_{sw}). In contrast, two bursts of activity, IP_{sw} and IP_{st} (Figs. 2 and 8), were observed in 85% (117/137) of the step cycles analyzed from the spinal cats. A similar incidence of double bursts in the IP, 92% (143/156 cycles), were observed during prespinal bipedal stepping. The IP_{st} burst, on the other hand, was absent during quadrupedal stepping (0/83 cycles). Thus IP_{st} burst data obtained after spinalization could not be compared with normal IP activity recorded during prespinal quadrupedal stepping (Fig. 7).

The timing of the TA activity differed between quadrupedal and bipedal stepping (Fig. 9). During quadrupedal stepping, the onset of TA activity occurred 22 ± 19 (SE) ms before the initiation of swing and preceded IP_{sw} and Sol activity by an average of 2 ± 18 and 252 ± 17 ms, respectively (Fig. 9). In contrast, when the cats stepped bipedally, TA activity began 200 ± 32 ms before swing was initiated (see Fig. 9). During pre- and postspinal bipedal stepping, TA activity preceded IP_{sw} by an average of 250 ± 42 ms and preceded Sol activity by 425 ± 44 ms (Fig. 9). No significant differences in the timing of TA activity between pre- and postspinal bipedal stepping were found. Thus on the basis of comparisons with prespinal quadrupedal stepping, the TA muscle was recruited earlier in the step cycles of spinal cats. However, an earlier onset of TA activity was not apparent when bipedal conditions of stepping were compared before and after spinalization.

DISCUSSION

Spontaneous versus training-induced recovery of stepping after spinalization

The present data differentiate the magnitude of the recovery of stepping after complete low thoracic spinal cord transection that can be attributed to routine daily care, i.e., spontaneous recovery, versus that which can be associated with full weight-bearing stepping for 30 min/day, 5 days/week. Multiple measures of stepping performance reflect a clear difference between the stepping capability that is attributable to spontaneous recovery versus that which occurs as a result of the practice of the motor task that is being tested, i.e., stepping. On the basis of the criteria of the maximum speed of full weight-bearing stepping, about four times more recovery occurred in the spinal cats that were trained to step. On the basis of the number of successful steps there was a threefold greater improvement if trained. In a previous study, we found an approximate threefold increase in the maximum speed that adult spinal cats could step (Lovely et al. 1986). This similarity in results between studies was observed despite two notable differences between the experiments of Lovely et al. (1986) and the present study. First, in the earlier study the cats were tested 6 mo after spinalization and step training was initiated one month after spinalization compared with the present study where 3 mo of training were performed beginning 1 wk after spinalization. Secondly, the criteria for reaching the maximum speed were based on five consecutive steps in the earlier study and 20 consecutive steps in the present study.

One may be surprised by the clear discriminatory results from the behavioral measures with respect to the effects of training versus spontaneous recovery. However, the ability to step at faster speeds requires a high level of accuracy and

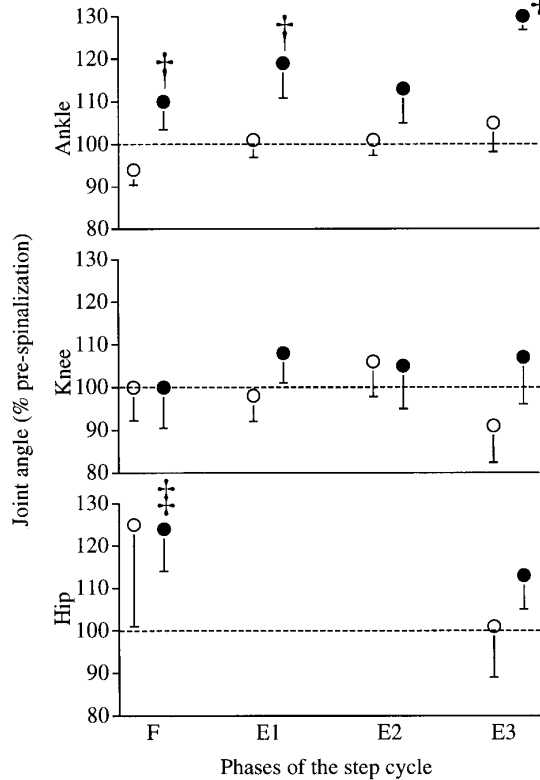


FIG. 6. Ankle, knee, and hip angles in step-trained (●) and non-trained (○) spinal cats during phases of step cycle. Maximum amount of joint flexion, i.e., smallest joint angles, at F and E2 and maximum amount of joint extension, i.e., greatest joint angles, at E1 and E3 were measured from 8–10 cycles of full weight-bearing bipedal hindlimb stepping (0.4 m/s) in 4 step-trained and 5 non-trained cats before and 12 wk after spinalization. Postspinal values were expressed as a percentage of prespinal values (---). Mean ± SE of normalized values are shown. E1 and E2 (yield) phases are absent at hip. *, †, and ‡, significantly different from non-trained at $P < 0.01$, non-trained at $P < 0.05$, and prespinalization (100%) at $P < 0.01$.

consistency in the fidelity of the neural control. Essentially, stepping at the faster speeds imposes a stricter performance demand than at the slower speeds and thus differentiates the levels of stepping performance among the spinal animals.

It is important to recognize, however, that these behavioral measures of step performance must be used under well-controlled conditions. For example, the animal's upper trunk was stabilized with a thoracic vest that helped to minimize movement of the upper body. This procedure, in turn, helped maintain a constant balance of the upper body and head, thus avoiding disrupting the movement of the center of gravity of the lower body laterally or vertically. When these variables were controlled carefully, the locomotor capability of the hindlimbs of complete low thoracic spinal cats while fully bearing the weight of the hindlimbs could be assessed validly and reliably. Even as effective as these behavioral criteria were in comparing groups, detailed analyses of EMG and joint kinematics demonstrated further differences that were not detected by the behavioral measures, i.e., speed and number of successful steps.

In assessing the recovery after spinal transection in the cat, a common practice has been to rely on data obtained during unrestrained quadrupedal locomotion for an intact

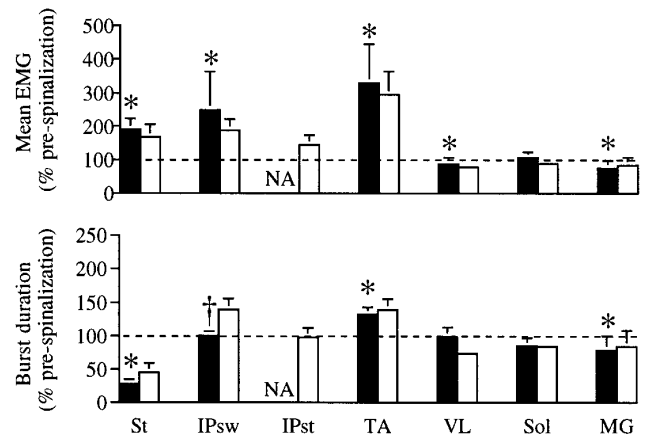


FIG. 7. Amplitude and duration of EMG activity during bipedal hindlimb stepping in step-trained spinal cats relative to prespinal quadrupedal stepping. Mean EMG amplitude and burst duration of 8–50 EMG bursts from selected hindlimb muscles (see Fig. 2 for muscle abbreviations) during prespinal quadrupedal stepping and postspinal bipedal stepping were measured in 5 cats. Data shown are mean ± SE of normalized values. Postspinal values were expressed as percentage of prespinalization values for individual cats (■). For comparison, postspinal data normalized to bipedal stepping before spinalization are also shown (□; see Fig. 3). NA, no IPst burst observed. * and †, significantly different from prespinalization (100%) and data normalized to prespinal bipedal values at $P < 0.01$, respectively.

control comparison (Belanger et al. 1996; Forssberg et al. 1980; Lovely et al. 1990). In the present study, similar conclusions could be made for some, but not all, measures

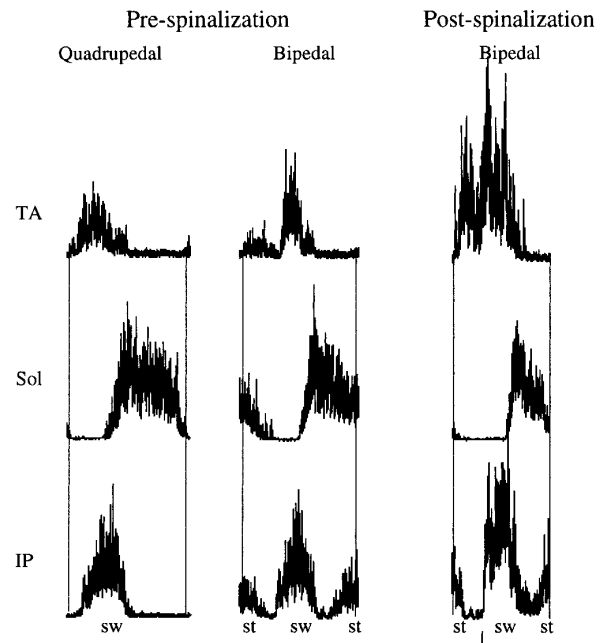


FIG. 8. Average EMG waveforms during quadrupedal and bipedal stepping before spinalization and bipedal stepping after spinalization. Ten to 14 cycles of rectified EMG activity from Sol and IP muscles triggered from start of TA activity were averaged for each locomotor condition. IP bursts during swing and stance are denoted by sw and st, respectively. Note, average waveforms are shown relative to one cycle of TA activity (vertical lines denote onset of TA activity); thus, Sol and IP_{st} activity from 2 cycles (bipedal stepping) are depicted. Horizontal calibration, 1 s, and vertical calibration, 0.1 mV for TA and IP and 0.2 mV for Sol. Data are from 1 step-trained cat (see METHODS).

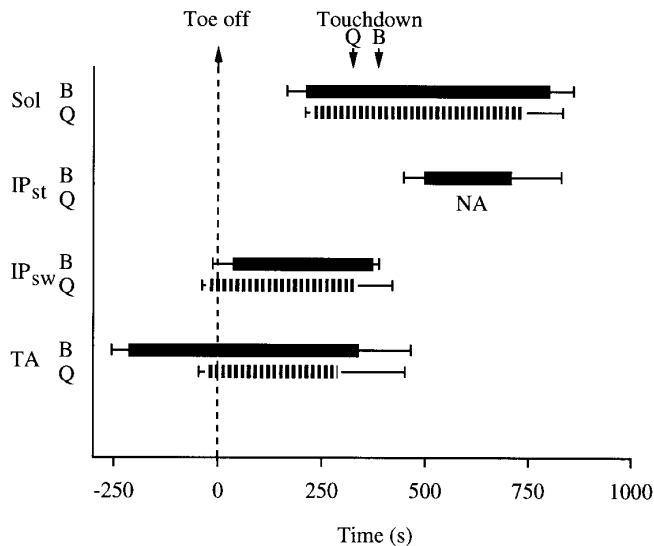


FIG. 9. Comparison of timing of EMG activity from selected hindlimb muscles during a single cycle of bipedal (dark bars; B) and quadrupedal (hatched bars; Q) stepping. Onset and offset for TA, IP_{sw} , IP_{st} , and Sol EMG bursts were normalized to onset of toe off at beginning of swing (see up arrow and vertical, dashed line) for bipedal and quadrupedal stepping. Eight to 50 cycles (0.4 m/s) of prespinal quadrupedal and pre- and postspinal bipedal stepping in 5 step-trained cats were analyzed to generate average onset and offset values shown. Average onsets of touchdown for 2 locomotor conditions are denoted by down arrows. IP_{st} burst was absent during quadrupedal stepping, denoted by NA. Bars, SE of mean onset and offset.

of locomotor performance whether bipedal or quadrupedal stepping was used as a criteria. For example, the appearance of double bursting in the IP and the earlier onset of TA activity relative to IP activity occurred during bipedal stepping before and after spinalization, but not during quadrupedal stepping. Other differences between quadrupedal and bipedal stepping were found by de Guzman et al. (1991) who reported a decrease in MG activity after spinalization when compared with prespinal quadrupedal, but not to prespinal bipedal, stepping. These data illustrate that, at least in some cases, the more appropriate prespinal control is bipedal rather than quadrupedal stepping. The explanation for the differences in the EMG patterns during bipedal and quadrupedal stepping cannot be determined from the present study. It is possible, for instance, that propriospinal and/or mechanical coupling between the forelimbs and hindlimbs might be the bases for these differences. Differences in the forelimb-hindlimb coordination during different locomotor gaits in cats have been associated with unique temporal patterns of muscle activity, i.e., the relative timing of forelimb flexor and hindlimb extensor EMG activity differs during pacing versus trotting on a treadmill (Miller et al. 1975). Furthermore there is evidence that these recruitment strategies are programmed at the spinal level (Miller and Van Der Meche 1976).

Does training reinforce existing functional pathways or generate new pathways?

The acquisition of stepping reflects a change in the probability of the execution of a stepping event rather than the induction or absence of a pathway, which enables a new event (stepping) to occur. For example, training a spinal

cord transected animal to stand, but not step, results in the inability to step (Edgerton et al. 1997b). Although these stand-trained spinal animals had not executed successful stepping for ~3 mo (none since spinal transection), they could execute full weight-bearing stepping within minutes after administration of strychnine, i.e., blocking glycine receptors. This finding suggests that the neural pathways needed to execute stepping were present even in those animals that could not step, but that these pathways were non-functional. It appears that strychnine modulated the balance of excitatory and inhibitory inputs to the interneurons and motor pools such that the probability of the appropriate sequence of synaptic events in the appropriate pathways increased dramatically and rapidly. Similar observations were observed when bicuculline was administered to non-trained spinal cats (Robinson and Goldberger 1986b).

In the present study, the acquisition of the ability to step after complete spinal cord transection also appears to be a manifestation of a change in the probability of a given combination of neural pathways being activated in a predictable and functionally appropriate temporal sequence. Another possibility, however, is that the acquisition of the ability to step was attributable to the formation of new pathways that could generate stepping. It appears, on the other hand, that the neural networks that generated stepping were also present in the animals that could not step consistently, but were not in a similar mode physiologically in that they could not function with enough consistency to support sustained stepping. Therefore, the training effect seems to have been, in large part, a manifestation of an increase in the probability of executing successful steps. By increasing this probability, the animals were more likely to execute more consecutive steps at faster speeds. This change in the probability of occurrence is similar to measures of phenomena related to learning, i.e., indices of learning based on the probability of an event occurring (Tully and Quinn 1985).

The changes in EMG waveforms seen in the present study, in effect, also reflect changes in the probability of activation of selected neural pathways. For example, the higher EMG amplitudes observed in the TA after step training indicate that more motoneurons were recruited and/or there was a higher frequency of excitation of the motoneurons recruited in the TA motor pool. Therefore, the training increased the probability of more motoneurons being active at a higher frequency. On the other hand, the level of activation of the fast extensor muscles seemed to be mildly reduced relative to that observed during bipedal stepping prespinalization. Thus step training in spinal cats appeared to reduce the probability of excitation of the extensor motor pools. As noted above, although the probability of execution of a successful sequence of activations was lower in non-trained compared with trained animals, the non-trained cats also were able to execute some full weight-bearing steps. Further, it is important to recognize that when successful steps were taken, the EMG amplitudes and burst durations were similar in many respects to those observed in trained cats.

Role of muscle adaptations in improving stepping in trained spinal animals

Another factor that could have contributed to the greater postspinal acquisition of stepping in trained compared with

non-trained cats could have been the recovery of some of the muscle mass that was lost after spinal injury. We have examined the potential role of muscle plasticity in explaining the differences in stepping performance of spinal animals in a wide variety of experiments (Roy and Acosta 1986; Roy et al. 1991, 1998). The overall conclusion is that the recovery in muscle mass that can occur as a result of motor training cannot account for the recovery in motor performance. Neither the magnitude nor the time course of these changes adequately paralleled the changes in stepping ability. Thus the larger changes in motor capacity observed in the trained cats were attributable primarily to changes in the probability of a given combination of neural pathways being activated in a temporally appropriate manner.

Specificity of training

These present data alone do not provide evidence that the enhanced recovery in step-trained animals resulted specifically from the enhancement of only those neural pathways that contribute to stepping. For example, it is possible that the step training had a generalized or systemic effect on the recovery of ancillary neural functions as well as those that generate stepping.

There is some evidence, however, that those afferent and efferent pathways used repetitively are highly specific to the acquisition of a motor task. For example, preliminary evidence suggests that a cat can learn to stand if it is trained to stand, whereas stand training has a minimal effect on the ability to step (Edgerton et al. 1997b; Hodgson et al. 1994). There is also evidence that the specific source of afferent information used to initiate and maintain stepping during training becomes essential for the animal to execute the trained task. For example, if the tail is "crimped" to increase afferent stimulation during step training, then the ability to step becomes highly dependent on the presence of this source of stimulation (Edgerton et al. 1997a).

It is not clear why some of the non-trained animals in the present study recovered a significant level of stepping ability without any training on the treadmill. However, similar levels of animal to animal variability have been reported previously (Eidelberg et al. 1980; Lovely et al. 1986; Robinson and Goldberger 1986a). One possibility is that the animals that regained some stepping ability spontaneously executed weight-supporting stepping during routine movements in their cages. Brief, incidental experiences of weight-supporting stepping could have occurred each day and this could have had an effect. This raises an issue regarding how much training or experience is needed to trigger an improvement in the probability of successfully generating stepping. Long-term continuous activity recordings are needed to determine the degree to which the motor tasks that are practiced after spinal transection are the motor tasks that are acquired, either coincidentally (spontaneously) or purposely as part of a training regimen.

Significance of different plateau levels for the trained and non-trained spinal animals

Both the maximum speed of stepping and the number of consecutive successful steps suggest that a maximum level

of performance for each group was reached within seven weeks after surgery. Thus, the rate of recovery, as well as the plateau level reached, was markedly enhanced by step training. These experiments, however, cannot determine whether or not the higher plateau reached in the training group was due to the fact that the training occurred during the first seven weeks after surgery. There are several suggestions that the plateau level could be affected by subsequent modification of the postsurgical treatment. If animals are trained to stand or not trained at all for several months and then are trained to step, there is significant improvement in their locomotor capability (Edgerton et al. 1997b; Hodgson et al. 1994). In addition, if an animal that is trained to step is subsequently placed on a regimen of stand training, that animal loses its ability to step effectively. Therefore, on the basis of the present results and the observations that the behavioral potential of spinal cats can be modified by repetitive training for periods of up to two years, there appears to be a long-term persistence of spinal plasticity after spinalization. Further experiments are needed, however, to determine whether or not early training can result in greater improvements when imposed immediately after spinal transection.

Conclusions and perspective

The recovery of stepping after spinal transection could have resulted from changes in the efficacy of the existing neural networks that normally generate stepping. On the other hand it seems unlikely that the neural pathways in the spinal cord that generated or contributed to stepping while the CNS was intact were the same when supraspinal control was lost. Although the original lumbar intraspinal pathways probably remain intact after spinalization, they would be expected to function differently in a widely different intercellular milieu, because of the marked changes in synaptic densities and the type of synapses that are left intact. For example, the relative effect of any given intraspinally derived synaptic input will be higher given the absence of supraspinally derived synapses. The net effect is that the postspinal reacquisition of stepping occurred with a different spinal cord, because of the combination of neural pathways and synaptic efficacies that had generated stepping before spinal transection were unlikely to be the same combination as when stepping was executed after spinalization.

Although the specific mechanisms to which the changes in stepping performance can be attributed is unknown, the present data demonstrate that some use-dependent phenomena can significantly modulate the level of functional recovery. These data raise new issues about which use-dependent factors modulate locomotor recovery, how specific the trained pathways are to the pathways that adapt, and how dependent this adaptive potential is on the elapsed period after spinalization. Further, the present data demonstrate the importance of considering experience-dependent factors in evaluating the effectiveness of any postspinal intervention, such as tissue implants and the use of growth-promoting and growth-inhibiting factors.

The authors thank S. Lauret for excellent care of the animals and M. Recktenwald for technical assistance.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-16333.

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Received 14 August 1997; accepted in final form 4 November 1997.

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