RetentionPolicy of Hindlimb Stepping Ability in Adult Spinal Cats After the Cessation of Step Training

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Retention of hindlimb stepping ability in adult spinal cats after the cessation of step training. J. Neurophysiol. 81: 85–94, 1999. Adult spinal cats were trained to perform bipedal hindlimb locomotion on a treadmill for 6–12 wk. After each animal achieved the ability to step, locomotor training was withheld, and stepping was reexamined 6 and 12 wk after training ended. The performance characteristics, hindlimb muscle electromyographic activity patterns, and kinematic characteristics of the step cycle that were acquired with training were largely maintained when training was withheld for 6 wk. However, after 12 wk without training, locomotor performance declined, i.e., stumbling was more frequent, and the ability to consistently execute full weight-bearing steps at any treadmill speed decreased. In addition, the height that the paw was lifted during the swing phase decreased, and a smaller range of extension in the hindlimbs occurred during the E3 phase of stance. When three of the spinal cats underwent 1 wk of retraining, stepping ability was regained more rapidly than when trained initially. The finding that stepping ability in trained adult spinal cats can persist for 6 wk without training provides further evidence that training-induced enhancement of stepping is learned in the spinal cats and that a memory of the enhanced stepping is stored in the spinal networks. However, it appears that the spinal cord can forget how to consistently execute stepping if that task is not practiced for 12 wk. The more rapid learning that occurred with retraining is also consistent with a learning phenomenon. These results in conjunction with our earlier findings suggest that the efficacy of the neural pathways that execute a motor task is highly dependent on the periodic activation of those pathways in a sequence compatible with that motor task.

INTRODUCTION

We previously have shown that the locomotor ability of adult low-thoracic spinal cats can be improved considerably above the level that can be expected due to spontaneous recovery, if the hindlimbs receive step training (de Leon et al. 1998a; Lovely et al. 1986). On the basis of locomotor tests that were performed weekly for 12 wk after spinalization, step-trained spinal cats executed more full weight-bearing steps at a greater range of treadmill speeds compared with spinal cats that were not trained. An enhanced flexion during swing and a greater range of extension during stance were acquired with training based on the electromyogram (EMG) and kinematic characteristics of stepping (de Leon et al. 1998a). Similar locomotor performances after spinalization and training in cats were reported elsewhere (Barbeau and Rossignol 1987; Belanger et al. 1996), although the recovery characteristics in nontrained spinal control animals were not examined. In addition to the effects of step training on locomotor ability, we have shown that spinal cats trained to stand on their hindlimbs could perform full weight-bearing standing for longer durations than nontrained spinal cats (de Leon et al. 1998b). The longer periods of standing in the trained cats were associated with an acquired ability to sufficiently activate hindlimb extensor muscles and thereby maintain weight-bearing extension at the knee and ankle joints. Furthermore, it appears that adaptations in the mass or force-generating potential of the hindlimb musculature cannot account for the acquisition of stepping or standing by the trained spinal cats (Roy and Acosta 1986; Roy et al. 1991, 1998). Together these findings suggest that the spinal networks that controlled stepping and standing could be modified by the training experience and further provide evidence that the lumbar spinal cord could learn how to improve the execution of these hindlimb tasks after a complete spinal cord transection (de Leon et al. 1998a,b; Edgerton et al. 1997a,b; Hodgson et al. 1994).

To further test whether training-enhanced stepping in spinal cats is learned and whether a memory of the enhanced stepping is formed, the following question was asked: “Are the behavioral and physiological characteristics of stepping that are acquired in response to step training maintained when the task is no longer practiced?” On the basis of reflex conditioning studies in spinal animals, changes in hindlimb withdrawal responses can be acquired and maintained for hours after conditioning ceased (Beggs et al. 1983; Durkovic 1985). In addition, Wolpaw and colleagues demonstrated that operantly conditioned changes in the amplitude of the H-reflex in intact monkeys can be maintained for ≈3 days after the spinal cord is completely transected (Wolpaw and Lee 1989). Thus it appears that the lumbar spinal cord is capable of storing memories of simple motor responses that are acquired through conditioning. Whether the improved execution of a complex motor task, e.g., stepping, that is acquired by spinal circuits persists or is forgotten in the absence of training has not been adequately examined. Stepping ability in spinal cats has been shown to regress when daily training is not maintained (Barbeau and Rossignol 1987); however, there were no studies that systematically examined the effects of withholding step training on locomotor characteristics.

The findings from this study demonstrate that the recovery...
of stepping in trained spinal cats is long lasting, i.e., maintained for 6 wk after the cessation of step training. However, stepping ability decays when training is withheld for 12 wk. These results provide further evidence that the spinal cord acquires an improved ability to step by practicing that task, but, if stepping is no longer practiced, the spinal cord can forget how to successfully execute full weight-bearing stepping. These findings have important implications regarding the impact of motor training on the long-term recovery of stepping after spinal cord injury and its persistence, once it was acquired.

METHODS

Experimental procedures

EMG electrodes were implanted in selected hindlimb muscles of seven adult female cats. EMG and kinematic data were collected from the hindlimbs, while each cat performed bipedal treadmill locomotion, i.e., only the hindlimbs stepped while the forelimbs rested on a platform. After data from ≥20 consecutive step cycles were collected from treadmill speeds ranging between 0.2 and 0.8 m·s⁻¹, the spinal cords of the cats were completely transected (T12–T13).

The spinal cats received daily hindlimb locomotor training on a treadmill, which began either 1 or 12 wk after spinalization. Each animal was trained (6–12 wk) until 20 or more consecutive full weight-bearing steps at each speed between 0.2 and 0.8 m·s⁻¹ could be performed successfully. Thus all animals acquired a similar level of stepping ability before training was suspended and the retention tests were initiated. The amount of recovery and the rates of retention were not affected by differences in the time at which training began after spinalization.

Stepping ability was tested after the step training period (Week 0) and the retention of stepping in the absence of training was examined during locomotor tests performed 6 and 12 wk after training ended (Weeks 6 and 12). During all tests, EMG activity from selected hindlimb muscles and hindlimb kinematic data was recorded.

After the last retention test, three of the seven spinal cats were retrained to perform stepping for 1 wk, and thereafter locomotor performance was retested. The three spinal cats that exhibited the fastest recovery rates during the initial training period were selected to facilitate retraining. The rates of recovery during the initial training and the retraining periods were compared in these three cats.

Surgical procedures

During all surgical procedures, sodium pentobarbital (35 mg/kg ip) was administered 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 (Roy et al. 1992).

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 ilio-psoas (IP), midbelly of the soleus (Sol), and midbelly deep portion of the tibialis anterior (TA)] 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 completely 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 is detailed elsewhere (Roy et al. 1992). Cats were housed in spacious cages, two to four cats per cage, with the cage floors covered with shredded newspaper. The bladders and colons of the cats were expressed manually 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

During all training and testing procedures, a cloth harness was fitted over the shoulders, between the forelimbs, and around the weight-bearing steps at each speed between 0.2 and 0.8 m·s⁻¹, the spinal cords of the cats were completely transected (T12–T13).

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Hindlimb training and testing procedures

During all training and testing procedures, a cloth harness was fitted over the shoulders, between the forelimbs, and around the upper trunk, and the forelimbs of the cats rested on a platform raised ~2.5 cm above the training surface. Food rewards (Gerbers Baby Food) were used to encourage the animals to maintain a steady posture while in the harness.

Bipedal stepping before spinalization. Bipedal hindlimb step training on a motorized treadmill was performed for 15–30 min/day, 5 days/wk (for details see de Leon et al. 1998a). Four to 8 wk of prespinal training were necessary before the cats could perform long episodes of stable hindlimb stepping (range of 0.2–0.8 m·s⁻¹) while they were positioned in the harness.

After the cats could perform stable locomotion, data were collected during tests of bipedal stepping on the treadmill. Stepping at slower speeds was tested first, and after ~20 consecutive steps were executed testing continued at the next higher speed, usually in increments of 0.2 m·s⁻¹. Three or four tests were sufficient to collect data from ≥20 consecutive step cycles at treadmill speeds of 0.2, 0.4, 0.6, and 0.8 m·s⁻¹.

Bipedal stepping after spinalization. After spinalization, training of the bipedal hindlimb stepping was performed on the motorized treadmill for 30 min/day, 5 days/wk, as described previously (de Leon et al. 1998a). A thin sheet of Plexiglas, ~7 cm in height, was placed longitudinally to separate the hindpaws during stepping. Initially, step training in the spinal cats consisted of manually assisting the hindlimbs to produce plantar surface stepping on a slowly moving treadmill belt while the tail was held to provide vertical and lateral support for the hindquarters. As stepping ability progressed 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 and 0.6 m·s⁻¹. At this time, the trainers held the tail only to provide lateral support during stepping.

A standard procedure was used to test bipedal hindlimb stepping after spinalization and is described in detail elsewhere (de Leon et al. 1998a). Paw placing or perianal stimulation was used for to ensure proper electrode placement, each wire was secured in the muscle with a suture at its entry and exit from the muscle.

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ceed at progressively faster speeds, usually in increments of 0.2 m·s⁻¹ up to 1.0 m·s⁻¹. However, if difficulties in stepping persisted at the slow speeds, e.g., full weight-bearing stepping did not occur, the locomotor test was terminated. Tests were performed after the training period, i.e., when the spinal cats acquired the ability to consistently execute stepping at a range of treadmill speeds and after training was withhold for 6 and 12 wk. In addition, locomotor tests were performed 1, 4, and 6 wk after training was initiated to examine the rate of recovery during training.

Assessment of locomotor ability

Stepping ability during a locomotor test was measured by the maximum speed of stepping and by the number of plantar steps performed at a given speed (de Leon et al. 1998a). No differences in stepping performance were observed between the two hindlimbs; therefore, the analysis of a single hindlimb (right) was sufficient for evaluating the maximum speed and step number. The maximum speed was defined as the fastest speed at which ≥20 consecutive full weight-bearing step cycles were executed on the plantar surface of one paw. To ensure that the maximum speed was an accurate measurement of stepping ability over a range of speeds, it also was required that ≥20 consecutive steps were executed at all speeds below the fastest speed. 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⁻¹ was recorded. Plantar step number was determined by counting the number of full weight-bearing steps executed on the soles of the paws in the two hindlimbs at a speed of 0.4 m·s⁻¹ during a 45-s trial. Full weight-bearing steps occurring on the dorsum of the paw were not included in plantar step number. However, the number of dorsal steps was counted and the percentage of dorsal steps relative to the total number of steps was calculated [(dorsal/planar + dorsal)·100].

Data recording and analysis

EMG and kinematic data during stepping were recorded as described by 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), and a camera and videocassette recorder (Panasonic System Camera, WV D5100; Panasonic AG1280P Panasonic, Cypress, CA) were used to record the video signals. A 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 was analyzed by computer (de Leon et al. 1994). The EMG signals from each muscle during 10- to 45-s bouts of stepping were sampled into an Amiga computer at 2 kHz and calibrated. The EMG signals were rectified and smoothed with 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. 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. EMG activity corresponding to poorly executed steps, i.e., dorsal steps or steps that were not full weight-bearing, were excluded from the analyses.

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, bony landmarks on the hindlimb were digitized and calibrated, and stick figure representations were plotted. The knee position was triangulated as described previously (Goslow et al. 1973). Swing was defined as the period starting with forward movement of the paw (toe-off) and ending with, but not including, paw contact (touch-down). 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). The lengths of E2 and E3 (Philippson 1905) were measured by the horizontal displacement of the x-coordinate of the paw marker (head of the 5th metatarsal) during each phase. The amount of paw lift during swing was measured by the vertical displacement of the y coordinate of the paw marker.

Statistics

Comparisons of group mean values were statistically analyzed as described previously (de Leon et al. 1998a) by using computer-
RESULTS

Locomotor performance in spinal cats: enhancement with training and decline after withdrawal of training

The trained spinal cats acquired the ability to perform hindlimb stepping at a prespinal level of performance based on two criteria, the maximum speed of treadmill stepping and the number of full weight-bearing plantar steps that were executed (Fig. 1, A and B, see Week 0 and dashed lines). Consistent, full weight-bearing stepping at a range of treadmill speeds (0.2−0.8 m·s⁻¹) was observed in four of the spinal cats after 6 wk of training, whereas three other spinal cats required an additional 3–6 wk of training to reach the same level of performance. In addition, most steps were executed on the plantar surfaces of the paws, whereas only a small percentage of steps was performed on the dorsal surfaces (Fig. 1C). Before spinalization, only plantar surface stepping was observed (percentage of dorsal steps = 0%, see Fig. 1C, dashed line). These results are consistent with our previous findings that prespinal levels of stepping were acquired after spinalization in cats that received step training but not in cats that were untrained (de Leon et al. 1998a).

In the absence of training for 6 weeks, six of seven spinal cats maintained the ability to execute ≥20 consecutive steps at a range of treadmill speeds (0.2−0.8 m·s⁻¹), and thus the maximum speed and the number of steps that were executed did not change significantly (Fig. 1, A and B). Only one spinal cat lost the ability to step after just 6 wk of no training. During the training period, this animal was slow to recover stepping and required several more weeks of training than the other cats before it regained the ability to step.

After 12 wk without locomotor training, none of the spinal cats executed more than seven steps without stumbling at any speed, and a significantly lower maximum speed (0 m·s⁻¹) was found relative to Weeks 0 and 6 (Fig. 1A). Although bouts of full weight-bearing stepping were observed, there was greater difficulty in initiating stepping sequences and the majority of steps were executed on the dorsal surface of the paw, i.e., the toes were curled (percentage of dorsal steps = ~60%, Fig. 1C; see also Week 12, Fig. 2).

Characteristics of the hindlimb step cycle

Figure 2 shows stick figures of the hindlimb during the swing and stance phases of the step cycle in one representa-
Relative to prespinal stepping, a different pattern of hindlimb movement was observed after spinalization and 12 wk of step training; specifically, the execution of swing was altered (compare Swing in Week 0 and in Prespinal). Before spinalization, the paw was lifted after toe-off as it was brought forward. However, after spinalization, the paw dragged on the treadmill belt immediately after toe-off. After the initial dragging, a sufficient amount of flexion occurred to lift the paw and complete the swing phase. These features of swing, i.e., drag followed by lift, have been reported in several studies of spinal locomotion in trained (Barbeau and Rossignol 1987; Belanger et al. 1996; de Leon et al. 1998a; Lovely et al. 1990) and nontrained spinal cats (de Leon et al. 1998a; Lovely et al. 1986). Generally, the hindlimb movement during stance was similar before and after spinalization, although a greater hip extension was observed throughout the step cycle after compared with before spinalization as was reported previously (Belanger et al. 1996; de Leon et al. 1998a).

After training was withheld for 6 wk, the general appearance of the step cycle did not change. A drag and lift of the paw remained.

FIG. 4. Raw electromyogram (EMG) recorded from selected hindlimb muscles of 1 spinal cat during tests of stepping at a treadmill speed of 0.4 m·s⁻¹ after step training (Week 0) and after the cessation of step training (Weeks 6 and 12). St, semitendinosus; VL, vastus lateralis; IP, iliopsoas; Sol, soleus; TA, tibialis anterior. Lines drawn under the EMG records indicate the stance phases in which full weight-bearing occurred on the plantar surface of the paw. d, dragging of the paw on the treadmill belt occurred throughout forward swing. Horizontal calibration, 1 s; vertical calibration 1 mV for all muscles except for the Sol, 2 mV.

FIG. 3. Height of the paw during swing (A) and the lengths of E3 (B) and E2 (C) during prespinal stepping (dashed lines) and in spinal cats after step training (Week 0) and the cessation of training (Weeks 6 and 12). Data are from full weight-bearing step cycles at a treadmill speed of 0.4 m·s⁻¹ in which steps were executed on the plantar surfaces of the paws (n = 7 cats). * ‡ Significantly different from Week 0 or prespinal values, respectively (P ≤ 0.05).
were still observed during swing, and when the hindlimb was weight bearing the plantar surface of the paw remained in contact with the treadmill belt throughout stance (Fig. 2, Weeks 0 and 6). However, after 12 wk without training, the amount of lift of the paw during swing decreased, and the toes often dragged on the treadmill belt throughout forward hindlimb movement (Fig. 2, Week 12). Contact of the dorsum of the paw during the stance phase was prevalent, and weight bearing often occurred on the tips of the toes at Week 12.

Selected characteristics of the swing and stance phases, i.e., the trajectory of the paw during swing and the length of the two phases of stance, E2 and E3 (see Fig. 2), were measured at each time point. Only full weight-bearing cycles in which stepping was executed on the plantar surface of the paw were included in these kinematic analyses. The height of the paw during swing and the E2 phase of stance were similar to prespinal levels after the training period (Fig. 3, A and C, compare Week 0 with dashed line) whereas the length of E3 was significantly shorter after spinalization (Fig. 3B, compare Week 0 with dashed line).

Compared with Week 0, there were no significant changes in the amount of paw lift during swing or in the length of E2 or E3 after 6 wk of no training (Fig. 3). However, after the cats were not trained for 12 wk, paw height during swing and the length of E3 were decreased significantly relative to prespinal and Week 0 levels (Fig. 3, A and B, compare Week 12 with Week 0 and dashed line). Greater variability in the length of E2 was measured when training was withheld (Fig. 3C).

**EMG burst characteristics**

Raw EMG recorded from selected hindlimb muscles in one spinal cat is shown in Fig. 4. A consistent pattern of
EMG burst activity was recorded in all channels after stepping was acquired (Week 0) and after training was withheld for 6 wk (Week 6). In addition, the general shapes of the EMG bursts appeared similar for each hindlimb muscle. For example, the EMG bursts recorded from the Sol resembled short spikes of activity, whereas the VL, Sol, and TA EMG bursts were longer in duration (see Weeks 0 and 6). IP EMG activity was composed of two bursts, one main burst that occurred mostly during swing and a second, smaller burst that was present mainly during stance (see Weeks 0 and 6). The second burst was not present in every step cycle but occurred at a frequency similar to that previously reported in spinal cats stepping bipedally (de Leon et al. 1998a). Less consistent EMG burst patterns and shapes were recorded after the cats were not trained for 12 wk (see Week 12). This inconsistency was reflected in the increased occurrence of sporadic EMG bursts corresponding to non–full-weight-bearing steps and/or dorsal steps.

The EMG burst activity during full weight-bearing steps performed on the plantar surface of the paw was analyzed. After the training period, prespinal levels of EMG activity were recovered in the Sol, VL, and TA, whereas St activity was elevated (compare Week 0 to dashed lines, Fig. 5). The duration of burst activity was lower after spinalization in the Sol, VL, and St, whereas TA activity was unchanged (compare Week 0 to dashed lines, Fig. 6).

Compared with Week 0, no significant changes in EMG amplitude or duration occurred when training was withheld for 6 wk (compare Weeks 0 and 6, Figs. 5 and 6). However, both mean EMG activities and burst durations tended to decrease in the extensor muscles after 12 wk of no training: VL burst duration and mean EMG and Sol burst duration were significantly less at Week 12 relative to Weeks 0 and 6 (Figs. 5 and 6). In contrast, the flexor mean EMG amplitudes, i.e., TA and St, increased from Week 0 to Week 12 (Fig. 5). High-amplitude EMG spikes were observed in the step cycles in which paw dragging occurred throughout forward swing (see d in Fig. 4).

Performance of stepping after training was reinstated

To further examine the importance of training to locomotor ability, three of seven spinal cats were retrained to perform hindlimb stepping after training was withheld for 12 wk. Stepping ability improved significantly within 1 wk of retraining, i.e., the average maximum speed of stepping increased from 0 to 0.7 m·s⁻¹ (Fig. 7), and no recovery occurred in the four cats in which training was not reinstated. Relative to the rate of acquisition during the initial training period, a more rapid recovery of stepping ability was observed when the three spinal cats were retrained (Fig. 7).

DISCUSSION

This study demonstrates that the performance characteristics, hindlimb muscle EMG activity patterns, and kinematic characteristics of the step cycle acquired with training in spinal cats are largely maintained when training is withheld for 6 wk. However, after 12 wk without training, locomotor performance declines, and the acquired EMG and kinematic characteristics are lost. Furthermore, the locomotor performance recovers more rapidly when the spinal cats are retrained to step than during the initial acquisition of stepping after spinalization.

Is memory formed in the lumbar spinal cord?

Memory in the spinal cord can be defined as a persistence of changes within spinal pathways that are acquired by activity induced in the spinal circuits from descending and/or sensory pathways. Wolpaw and Lee (1989) provided evidence for memory traces in the spinal cords of monkeys that were conditioned to modulate the spinal H-reflex, i.e., the animals were rewarded for appropriately enhancing or decreasing the H-reflex. When a complete spinal cord transection was performed, the specific effect of conditioning on the reflex response persisted, thus indicating that the associated spinal circuitry was modified. A similar activity-dependent phenomenon, “spinal fixation,” reportedly occurred in intact rats when descending or sensory input to the spinal cord was used to induce an asymmetric posture in the hindlimbs (Steinmetz et al. 1981). Evidence for long-term neural changes was that the postural asymmetry persisted for ≤2 h after a complete spinal transection was performed 45 min after the initial acquisition of the postural response.

In other studies that examined retention phenomena medi-
ated by spinal circuits, hindlimb reflexes were conditioned in animals after a complete spinal cord transection. Durkovic (1985) and others (Beggs et al. 1983; Shurrager and Culler 1940) used classical conditioning techniques to enhance the withdrawal reflex in spinal animals and found that the amplitude of the reflex remained elevated for hours after acquisition. These findings suggest that the neural changes in the spinal circuits were driven by specific patterns of afferent activity in the absence of supraspinal input. In other words, memories were formed in the lumbar spinal cord by “sensory” experiences.

It is likely that sensory stimuli such as load and/or proprioceptive information generated during hindlimb locomotor training produced long-term changes in the spinal pathways, which controlled stepping after supraspinal input was eliminated. Thus a memory was formed in the spinal cord so that the enhanced ability to step was maintained even after stepping was not practiced for several weeks. It appears that, in addition to the retention of discrete hindlimb motor responses, improvements in movements that were executed at multiple joints in the two hindlimbs were mediated by long-lasting changes within the underlying spinal circuits.

Significance of retention phenomena to learning in the spinal cord

The retention of an acquired motor response mediated by spinal circuits provides additional evidence that a motor response can be learned by the spinal cord. For a motor response to be learned, the effect of conditioning must have some long-lasting features to rule out factors that could transiently alter performance. For instance, in studies of spinal reflex conditioning, the acquired responses persisted for hours (Beggs et al. 1983; Durkovic 1985) and even days (Dykman and Shurrager 1956). Similarly, the finding that stepping was maintained even without practice for 6 wk indicated that manipulating the hindlimbs during daily training did not transiently enhance spinal reflexes or “warm-up” the hindlimb musculature in a manner that facilitated stepping.

Other evidence for learning related to retention is “savings,” i.e., the facilitation of the acquisition of a motor response by an initial acquisition experience. Previous studies provided evidence that the rate of re-acquisition of a flexion response in spinal animals was faster after the initial conditioning of the reflex (Sherman et al. 1982; Shurrager and Culler 1940). In this study, when step training was reinstalled in three spinal cats after their performance decayed (12 wk without training), the recovery of stepping was more rapid than the initial recovery after spinalization (see Fig. 7). This also occurred more rapidly than the recovery in cats that were trained to step immediately (de Leon et al. 1998a) or 1 mo (Lovely et al. 1986) after spinalization. Thus it appears that an initial training period benefited subsequent acquisition of stepping. Further studies in which behavioral and physiological characteristics are examined in a larger number of animals, however, are needed to establish the role that this savings phenomenon may have in learning motor tasks.

In summary, we demonstrated that the effects of locomotor training on hindlimb stepping after spinalization are long lasting, i.e., there is retention and savings, although, in the prolonged absence of training, stepping ability decayed. Furthermore, we have previously shown that spinal cats that received training for 12–28 wk after spinalization maintained a superior ability to step relative to nontrained spinal cats during the same time period (de Leon et al. 1998a; Lovely et al. 1986). On the basis of these long-term studies of recovery, the changes in locomotor performance after spinalization appear to be highly dependent on the presence or absence of training and that other factors, e.g., length of time after spinalization, could not explain these recovery characteristics. In addition to these behavioral and physiological findings, there is also evidence that changes in the spinal cord mediated the acquisition of stepping after spinalization. Preliminary biochemical studies of the γ-aminobutyric acid synthetic enzyme (glutamate decarboxylase, GAD67) in the lumbar spinal cord showed that the GAD67 mRNA levels were enhanced throughout the ventral and dorsal horn regions after spinalization but that normal levels were observed in the spinal cords of cats that received step training after spinalization (Edgerton et al. 1997a; Tillakaratne et al. 1995). There is also evidence that glycinergic neurotransmission in the spinal cord of rats is modulated by

Spinal cord “forgets” if stepping is not practiced

The finding that stepping ability decayed when training was withheld for 12 wk indicated that the memory of enhanced stepping formed in the spinal cord was not permanent. Reflex responses that were acquired via classical conditioning also have been shown to decrease when extinction procedures were used after acquisition, i.e., the conditioned stimuli were not consistently presented with reinforcement (Beggs et al. 1983). However, during the retention period in this study, the animals were not exposed to any known training-related stimuli, and thus no attempts were made to extinguish the stepping behavior, i.e., the decrease in stepping ability was a passive phenomenon. Thus the decay in stepping ability over time indicated that the spinal cord lost the improved ability to step through processes resembling “forgetting.”

The decline in stepping, however, also could have been related to hindlimb muscle atrophy or the development of joint stiffness when the cats were no longer exercised for long periods. We previously examined the physiological properties of several hindlimb muscles after spinalization in cats, and it is clear that the adaptations in the musculature cannot account for the characteristics of locomotor recovery that occur in the presence or absence of training (Roy and Acosta 1986; Roy et al. 1991, 1998). The amount of force that can be generated in the hindlimb muscles of step-trained and nontrained spinal cats is similar, based on muscle mass and twitch and tetanic tension characteristics (Roy and Acosta 1986; Roy et al. 1991, 1998). In addition, the fatigability of the soleus muscle, the primary slow extensor of the ankle, did not change after spinalization in the trained or nontrained cats (Roy et al. 1991, 1998). Each of the animals was maintained in good health throughout this study, i.e., there were no joint deformities, and body weight was maintained at a normal level. Thus loss of locomotor performance after the cessation of training was not due to the inability to produce sufficient levels of force in the muscles nor to execute an adequate range of motion in the hindlimb joints.
spinalization and hindlimb step training. The expression of glycine receptors was closer to normal levels in spinal rats that received step training and acquired the ability to step, whereas greater than normal levels were found in nontrained spinal rats that exhibited poor stepping (Talmadge et al. 1996). Adaptations in the hindlimb musculature can be ruled out as possible mechanisms for locomotor recovery based on the similarity in the force- and fatigue-related properties of hindlimb muscles in trained versus nontrained spinal cats (Roy and Acosta 1986; Roy et al. 1991, 1998). Together these findings suggest that the recovery of stepping after spinalization in step-trained spinal cats is an experience-dependent process that is mediated by spinal plasticity.

Relationship of locomotor characteristics that are acquired and lost

This study identifies which features of the locomotor task are acquired and lost that account for the successful execution of locomotion. On the basis of the recovery of stepping 4–12 wk after the spinalization, step-trained spinal cats acquired the ability to execute full weight-bearing plantar steps at a range of treadmill speeds (0.2–1.0 m s\(^{-1}\)) with few step failures (Barbeau and Rossignol 1987; Belanger et al. 1996; de Leon et al. 1998a; Lovely et al. 1986). One specific adaptation to locomotor training that contributed to stepping ability was the enhancement of hindlimb flexion during swing. Spinal cats that received step training lifted their paws after initially dragging them on the treadmill belt after toe-off (Belanger et al. 1996; de Leon et al. 1998a). In contrast, an appropriate pattern of hindlimb flexion was generated infrequently in spinal cats that were not trained (de Leon et al. 1998a).

The importance of flexion in the success of the step cycle was further demonstrated when training was withheld. After 12 wk without training, step cycles in which the dorsum of the paw contacted the treadmill during stance and swing occurred frequently relative to well-executed step cycles. Thus the performance of stepping deteriorated to nontrained levels of locomotor performance, i.e., a maximum speed of 0.2 m s\(^{-1}\) in nontrained spinal cats (de Leon et al. 1998a; Lovely et al. 1986). These findings suggested that the activity in the spinal pathways that controlled hindlimb flexion were particularly dependent on the training status of the animal. The probability of the flexor pathways being properly activated was markedly reduced in the absence of training compared with that in successfully trained cats.

Some of the changes that occurred in the absence of training, however, were not expected. For example, mean EMG activity levels in the hindlimb flexor muscles increased when training was withheld for 12 wk. This seemed inconsistent with our previous finding that flexor muscle activity levels were enhanced by step training after spinalization (de Leon et al. 1998a). However, EMG bursts during step cycles in which the paw was dragged forward were analyzed in this but not the previous study. These types of steps resulted in overall high activity spikes in flexor muscles and could account for the apparent discrepancy (see d in Fig. 4).

Implications for rehabilitation after spinal cord injury

The implications of these findings are that not only is the potential for locomotor recovery after a spinal cord injury enhanced by undergoing training but that these beneficial effects persist for relatively long periods of time even when daily practice of the stepping task is not maintained. Furthermore, if stepping ability is lost because of a prolonged period of inactivity, the subsequent recovery of locomotor function can be expected to be more rapid than during the initial training period.

How the level of locomotor recovery acquired with training impacts the characteristics of retention cannot be determined from these results. Each spinal cat reached a criterion level of performance before the retention tests were initiated and the performance of stepping decayed at a uniform rate when training was withheld. Thus it remains to be determined whether there is a faster rate of decay in stepping ability when acquisition is not complete or conversely whether retention of stepping can be improved if the maximal recovery potential is reached. Another question raised by this study is “after acquisition, what is the amount of practice needed to sustain the acquired levels of locomotor recovery for long periods of time, e.g., over the lifetime of a spinal-injured individual?” A sustained level of locomotor ability for 6 mo was reported after rehabilitative training in spinal-injured humans, but it is unclear how often one must train to maintain the improvements in stepping (Wernig et al. 1995). It seems prudent to continue to examine use-dependent effects of step and stand training after spinal cord injury as well as to determine the effects of other types of motor training. Such studies should provide valuable insight into designing rehabilitation strategies for humans and may provide unique perspectives on the mechanisms of neuromotor plasticity after brain and spinal cord injury.

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REFERENCES


