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Variability in step training enhances locomotor recovery after a spinal cord injury

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Abstract

Performance of a motor task is improved by practicing a specific task with added 'challenges' to a training regimen. We tested the hypothesis that, in the absence of brain control, the performance of a motor task is enhanced by training using specific variations of that task. We utilized modifications of step performance training to improve the ability of spinal rats to forward step. After a complete thoracic spinal cord transection, 20 adult rats were divided randomly to bipedally step on a treadmill in the forward, sideward, or backward direction for 28 sessions (20 min, 5 days/week) and subsequently tested for their ability to step in the forward direction. Although the animals from all trained groups showed improvement, the rats in the sideward-trained and backward-trained groups had greater step consistency and coordination along with higher peak amplitudes and total integrated activity of the rectified electromyographic signals from selected hindlimb muscles per step during forward stepping than the rats in the forward-trained group. Our results demonstrate that, by retaining the fundamental features of a motor task (bipedal stepping), the ability to perform that motor task can be enhanced by the addition of specific contextual variations to the task (direction of stepping). Our data suggest that the forward stepping neuronal locomotor networks are partially complemented by synchronous activation of interneuronal/motoneuronal populations that are also a part of the sideward or backward stepping locomotor networks. Accordingly, the overlap and interaction of neuronal elements may play a critical role in positive task transference.

Introduction

Animals with a complete spinal cord transection (spinal) can learn to step (Barbeau *et al.*, 1993; Ichiyama *et al.*, 2005) and stand (de Leon *et al.*, 1998; de Leon *et al.*, 1999) with practice. A characteristic of such chronically learned motor behaviors, in general, seems to be that the greater the similarity of the trained task the greater the transfer of learning. Spinal cats that are trained to step perform better hindlimb stepping than cats that are trained to stand (hindlimb weight bearing) (de Leon *et al.*, 1998). Similarly, spinal cats that are trained to stand, learn to support their body weight for longer durations than cats that are trained to step or are not trained for either task (Hodgson *et al.*, 1994; de Leon *et al.*, 1998).

In light of the above examples, it seems reasonable to hypothesize that the more similar the muscles and the neural networks that execute a motor task, the more effective will be the transfer of the motor skill learned. Neuromuscular activation patterns differ during standing, walking, and swimming, suggesting that these behaviors may involve, at least in part, different neuronal but overlapping circuitries (Bigbee *et al.*, 2007; Magnuson *et al.*, 2009; Kuerzi *et al.*, 2010). In contrast to the idea of distinct circuitries for individual tasks, studies on multifunctional networks from invertebrates have established that, given the appropriate sensory modulation, many motor behaviors are driven by the coordinated activity of a common interneuronal pool (Briggman & Kristan, 2008; Berkowitz, 2010). These observations raise the issue as to whether the degree of commonality among the mammalian neural networks that are involved in different motor tasks could be a factor that could either facilitate or hinder the performance of one task when training for another task.

In task-specific neurorehabilitation training paradigms, attempts have been made to improve motor performance by introducing complexities to the specific motor task being trained (Behrman & Harkema, 2000). Musselman *et al.* (2009) report that training in a variety of relevant walking skills in varying situations and environments is more optimal than simply training forward walking on a treadmill for improving the over-ground locomotor capability of subjects with incomplete spinal cord injury (SCI). Moreover, we recently showed that spinal-transected mice and rats are able to bipedally step more effectively when the mode of forward bipedal step training allows for some critical level of variability during stepping (Cai *et al.*, 2006; Ziegler *et al.*, 2010). This strategy allows for

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variation in interneuron and motor unit activation patterns and also retains the common neuronal elements underlying bipedal stepping.

In the present study, we used qualitative variants of bipedal step training, i.e. sideward and backward stepping, to determine the effects of these 'challenges' on the recovery of forward stepping ability. Assuming that there are some common neural elements underlying different stepping behaviors (Courtine *et al.*, 2009), we hypothesized that the net ensemble of the neural networks that control forward stepping will be more extensive if spinal rats are trained with sideward or backward stepping than with forward stepping.

Materials and methods

Animals

Twenty adult female Sprague–Dawley rats (200–250 g body weight) underwent electromyographic (EMG) and epidural stimulating electrode implantations and complete spinal cord transection surgeries. The rats were assigned randomly to one of three groups – forward step trained (FT) (n = 6), sideward step trained (ST) (n = 7), or backward step trained (BT) (n = 7) for ∼5 weeks (28 sessions). In addition, five non-injured and non-trained control (Con) rats were used to obtain control data for EMG and kinematics analyses. All surgeries were performed under aseptic conditions with the animals deeply anesthetized with isoflurane gas (1.0–2.5% via facemask as needed). Surgery was performed with the animals on a water-circulating heating pad maintained at 37 °C to prevent hypothermia. All incisions were closed in layers using 4.0 Dexon for the muscle and fascia, and 4.0 Vicryl for the skin. After surgery, the rats were placed in an incubator maintained at 37 °C until fully recovered and administered antibiotics and analgesics once or twice per day as needed for 3–4 days. Thereafter, the rats were housed in a room maintained at 26 ± 1 °C and 40% humidity and on a 12 : 12 h light : dark cycle with access to food and water *ad libitum*. The cage floors were covered with CareFresh bedding. Pieces of fruit were given once daily. At the end of the experiment, animals were killed by intracardial perfusion with 4% paraformaldehyde. All experimental procedures were approved by the University of California Los Angeles Chancellor's Animal Research Committee and complied with the guidelines of the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals' (National Research Council, 2011).

Electromyography implantation procedures

A small incision was made at the mid-line of the skull. The muscles and fascia were retracted laterally, small grooves were made in the skull with a scalpel, and the skull was dried thoroughly. Two Omnetics connectors with Teflon-coated stainless steel wires (AS632; Cooner Wire, Chatsworth, CA, USA) were attached securely to the skull with screws and dental cement as previously described (Roy *et al.*, 1991; Ichiyama *et al.*, 2005). A skin incision was made in the mid-dorsal region of the back and 24 wires from the connector were routed subcutaneously to the most distal end of the opening. Two wires were coiled subcutaneously for later implantation on the spinal cord (see below). Skin and fascial incisions were made to expose the belly of the adductor brevis, vastus lateralis (VL), semitendinosus (St), medial gastrocnemius (MG), and tibialis anterior (TA) muscles bilaterally. All EMG data reported are from the muscles in the right limb, which was the lead leg for ST rats. Two wires were routed subcutaneously from the back incision to each muscle site. Bipolar intramuscular EMG electrodes were formed and secured into the mid-belly of each muscle as described previously (Roy *et al.*, 1991). The EMG wires were coiled near each implant site to provide stress relief. Stimulation through the connector implanted on the skull was used to verify the proper placement of the electrodes in each muscle. In addition, proper placement of the electrodes was verified post-mortem via dissection.

Spinal cord transection procedures

Spinal cord transections were performed at 14 days after the implantation of the EMG electrodes. A dorsal mid-line skin incision was made from ∼T6 to T10 and the paravertebral muscles and fascia from ∼T7 to T9 were reflected laterally to expose the vertebrae. To expose the spinal cord, a partial laminectomy was performed via removal of the spinous processes and a portion of the lateral bodies of the T7 and T8 vertebrae. The dura was picked up using fine forceps and microscissors were used to completely transect the spinal cord (including the entire extent of the dura). Small cotton balls were used to separate the cut ends of the spinal cord and to clean the transection site. Two surgeons independently verified a complete transection by gently passing a fine glass probe through the transection site and then lifting the cut ends of the spinal cord. Gelfoam was inserted in the transection site to minimize bleeding and to separate (∼2–3 mm) the cut ends of the spinal cord. Post-surgery, the bladders of all animals were expressed manually three times daily for the first 2 weeks and twice thereafter throughout the study.

Epidural stimulation electrode implant procedures

Epidural electrodes were implanted at the same time as the spinal cord transection surgery. A partial laminectomy was performed over spinal cord segments L2 (between vertebral levels T12 and T13) and S1 (vertebral level L2). Two Teflon-coated stainless-steel wires were inserted through an opening made between the T10 and T11 vertebrae and one wire was passed epidurally to each partial laminectomy site. A small region (∼1 mm notch) of the Teflon coating was removed from each wire to form the stimulating electrodes; the exposed surface was positioned towards the spinal cord and the wire was secured to the dura at the mid-line of the spinal cord at each site with 9.0 silk sutures.

Locomotor training procedures

Rats that are spinalized as adults do not recover any stepping ability spontaneously (Ichiyama *et al.*, 2008b; Kubasak *et al.*, 2008). To facilitate stepping, all rats received an injection of the non-selective serotonergic agonist quipazine (0.3 mg/kg, i.p.) at 10–15 min prior to each training session as described previously (Gerasimenko *et al.*, 2007; Ichiyama *et al.*, 2008a). A treadmill was mounted over and secured with clamps to a rotating turntable such that the direction of the treadmill could be changed easily to enable stepping in one of three directions – forward, sideward, or backward. Evidence from our laboratory highlights the importance of using a combination of pharmacological facilitation and epidural stimulation to evoke a good bipedal stepping response in spinal rats (Ichiyama *et al.*, 2005, 2008a; Gerasimenko *et al.*, 2007). Accordingly, epidural electrical stimulation at L2 and S1 was delivered continuously during the training and testing sessions at 40 Hz with 200 μs rectangular pulses as described previously (Ichiyama *et al.*, 2005). An upper body harness was used to position the rats over a treadmill belt and to partially support their body weight during bipedal locomotion. Rats were trained 5 days/week, 20 min/session for ∼5 weeks (28 training sessions) starting at 7 days after the spinal cord transection surgery. The treadmill belt speed was increased progressively from 6 to 13.5 cm/s. By the eighth training session, all FT and ST rats were able to step forward and

sideward, respectively, at 13.5 cm/s for 20 min. BT rats, however, stepped only at slower treadmill speeds (9 cm/s or slower) up to the 18th session and were dependent on tail pinching to step backward. For the last 10 sessions (18th session onwards) of backward step training, the treadmill speed was increased to 13.5 cm/s, but most rats continued to require tail pinching to step backward. Tail pinching was not used during any testing session.

Behavioral testing procedures

All kinematics data were collected after 28 sessions of locomotor training. Throughout the testing session, both EMG and kinematics data were collected from each rat as the rats (irrespective of training status) stepped in the forward direction. The connector implanted on the rat's head was connected to a Grass S88 Stimulator (Grass Technologies, Astro-med Inc., Rockland, MA, USA) through a stimulus isolation unit (Grass SIU5). EMG signals (2 kHz) were amplified and filtered (10–1000 Hz bandpass). Rats in all groups stepped bipedally on a moving treadmill (13.5 cm/s) and three-dimensional video recordings (Basler Vision Technologies) were made using four cameras (two cameras on each side at 100 fps) oriented at 45° and 135° bilaterally with respect to the forward direction of locomotion. Reflective markers were attached bilaterally to the shaved skin overlying the anterior superior iliac spine of the iliac crest, greater trochanter, lateral condyle, lateral malleolus, distal end of the fifth metatarsal, and lateral surface of the fourth metatarsal. Motion-capture software (SIMI Reality Motion Systems, Unterschleissheim, Germany) was used to obtain three-dimensional coordinates of the markers. All rats were tested in the presence of quipazine (0.3 mg/kg, i.p., administered at ~15 min prior to the testing) as well as epidural electrical stimulation (at spinal segments L2 and S1, a frequency of 40 Hz, and an intensity of 2.5–3.5 V) (Ichiyama *et al.*, 2008b; Courtine *et al.*, 2009).

Data analysis

The body was modeled as an interconnected chain of rigid segments, and joint trajectories and angles were generated accordingly. A range of kinematics gait parameters including cycle period, stance phase, swing phase, drag (defined as the time that the foot lags behind the ankle just before toe-off during the initial swing phase in a step cycle) normalized to the step cycle, step trajectories including step length, step height, and joint angle measurements were computed during forward stepping behavior in all rats. Six to ten step cycles when the rats were stepping consistently were analysed for each rat. The quality of forward stepping was compared among the three groups by quantifying the following. (i) Percentage of the average number of plantar steps taken, where a plantar step is defined as foot placement on the treadmill with extended as opposed to curled toes; (ii) Variability in step length between adjacent steps (step length consistency), as measured by the first order principal component analysis (PCA); (iii) Horizontal mid-swing stepping velocity, as defined as the mean horizontal velocity of the foot during the period representing 30–80% of the completion of the swing phase of a normalized step cycle. This portion of the swing phase was chosen because we observed an obvious slower foot velocity in the mid-swing phase of the step cycle in some rats; (iv) Gait timing variability, as assessed by the coefficient of variation of the lag time between the onset of the right and left leg stance phases within a step cycle.

Activation patterns (muscle waveforms and timing) for each muscle were obtained by taking an average of 6–10 filtered, rectified, and normalized (to the step cycle) EMG bursts from each muscle of each rat during consistent stepping. The threshold for inducing locomotor activity with epidural stimulation (40 Hz) was assessed for all rats after 10 days of training and at the end of the last testing session. These data were used to determine any change in threshold voltage required to induce locomotion (see below) at the end of 28 training sessions. Mean peak amplitudes, durations, and integrals of identified EMG bursts from each muscle were computed for each rat during the testing at the end of the 28 training sessions (Ichiyama *et al.*, 2008b; Courtine *et al.*, 2009).

Statistical analyses

All data are reported as mean ± SEM or median values. Six to ten consecutive weight-bearing steps from each rat in each group were included in the analyses of all measures. Overall significant differences in trajectory characteristics (trajectory length, step length, and step height), kinematics measures (joint angles, velocity characteristics) and stepping quality (step length consistency, lag time between the onset of the right and left hindlimbs, percentage of plantar steps) between the Con, FT, ST, and BT rats were obtained using univariate ANOVA measures. For all ANOVA measures, animal groups were treated as the independent variable and the levels (sub categories) of outcome variables (e.g. trajectory characteristics) as the dependent variable. Two-way ANOVA was run to test the difference in EMG burst characteristics (cycle period, burst durations, amplitudes and integrals) between animal groups for all muscles tested (with animal groups and muscle groups defined as the two factors). Change in stimulation threshold over time was calculated using a repeated-measures one-way ANOVA. There was homogeneity of variance between groups as assessed by Levene's test for equality of error variances. Normality of distribution was assessed by the Shapiro Wilk test. For data sets that were not distributed normally and that had differences in variances, step cycle parameters (stance, swing, and drag durations) and consistency in the interlimb phase differences (coefficient of variation of the lag time), the non-parametric Kruskal–Wallis rank test was utilized. Bonferroni *post-hoc* adjusted tests (and Dunn's test for non-parametric measures) were used to identify significant differences among individual groups and to reduce Type I errors. Differences between groups were considered statistically significant at $P < 0.05$. All statistical analyses were performed using MATLAB (Mathworks) and GRAPHPAD (GraphPad Software, Inc.) for Windows Software.

Results

Our results demonstrate that, irrespective of the training regimen, all spinal rats were able to step in a forward direction in the presence of quipazine treatment and epidural stimulation. Detailed analyses, however, revealed distinct differences in the kinematics and EMG characteristics of the forward-stepping behavior among the rats in the three groups. Overall, our results demonstrate that the rats that were trained to step sideward or backward showed greater stepping consistency, a higher percentage of plantar steps, faster horizontal velocities during the swing phase, and more highly coordinated interlimb coordination during forward stepping than rats that were trained to step forward. These behavioral distinctions between groups were reflected in the hindlimb kinematics and EMG activation patterns.

Step kinematics

Qualitatively, the stepping consistency between the right and left hindlimbs (interlimb coordination) was more similar to Con rats in most ST and BT rats than in FT rats (Fig. 1). In addition, more ST and

BT rats than FT rats displayed a relatively normal alternating pattern, i.e. similar to Con rats, between the right and left hindlimbs across step cycles. This more consistent interlimb coordination in BT and ST rats is reflected in the L-shaped pattern in the joint probability plots of vertical step heights between the two hindlimbs, whereas a more variable pattern is observed in the FT rats.

The kinematics of a typical forward step cycle in ST and BT rats more closely resembled the step cycle of Con rats than did the step cycle of FT rats (Fig. 2). The percentage of the swing phase duration in a step cycle was longer (Kruskal–Wallis statistic = 13.17, $P = 0.0043$) and the stance phase duration was shorter (Kruskal–Wallis statistic = 14.43, $P = 0.0024$) in all SCI groups than in Con rats (Fig. 2A). FT rats had a longer period of foot drag during a step cycle than Con, ST, and BT rats (Kruskal–Wallis statistic = 13.10, $P = 0.004$).

The mean step length ($F_{3,21} = 6.602$, $P = 0.002$) and trajectory lengths ($F_{3,21} = 8.424$, $P = 0.0007$) were shorter in FT rats than in Con, ST, and BT rats (Fig. 2B). The mean step height was similar among all four groups. The mean maximum hip joint angle was smaller in all SCI groups ($F_{3,21} = 4.196$, $P = 0.017$) than Con rats and the mean minimum hip joint angle was smaller in FT ($t = 3.358$, $P = 0.026$) than Con (Fig. 2C) rats. The excursion (range) of the hip joint was shorter in FT rats than in Con, ST and BT rats ($F_{3,21} = 7.803$, $P = 0.001$). The mean maximum knee joint angle was smaller in FT rats than in Con and BT rats ($F_{3,21} = 8.448$, $P = 0.0006$), whereas the minimum knee joint angle was larger ($F_{3,21} = 4.218$, $P = 0.021$) in ST and Con rats than in both FT and BT rats. The knee excursion (range) was larger in Con and BT rats than in FT rats ($F_{3,21} = 5.604$, $P = 0.005$).

Stepping ability

The percentage of plantar steps was higher in Con rats than in FT and BT rats ($F_{3,21} = 9.230$, $P = 0.0004$) and was higher in ST (~60%) than FT (~10%) rats ($t = 2.472$) (Fig. 3A). Based on principal component analysis (PCA), mean step length consistency was greater in Con and ST rats than in FT and BT rats ($F_{3,21} = 13.78$; $P = 0.002$, Fig. 3B). The mean horizontal velocity of the foot during the mid-swing phase was slower in all SCI groups than in Con rats ($F_{3,21} = 36.19$; $P < 0.0001$) and in FT rats than in ST and BT rats ($F_{2,17} = 6.174$; $P = 0.020$) (Fig. 3C). The coefficient of variation of the lag time between the onset of the right and left hindlimbs for consecutive steps (reflecting consistency in the interlimb phase differences for each step cycle) was lower in Con and ST rats than in BT and FT rats (Kruskal–Wallis statistic = 13.90; $P = 0.003$, Fig. 3D).

Hindlimb muscle electromyography burst characteristics

The threshold of epidural stimulation required to produce reliable locomotion in the forward direction was lower after 28 than 10 days of training in the ST rats (2.2 ± 0.1 vs. 1.7 ± 0.1 V, $t = 3.771$, $P = 0.0093$), but not in FT (2.4 ± 0.3 vs. 2.2 ± 0.2 V) or BT (1.7 ± 0.1 vs. 1.4 ± 0.2 V) rats. There was a significant interaction between the effects of animal groups and muscle peak amplitude values ($F_{2,4,8} = 5.071$, $P < 0.0001$). Simple main effects analysis showed that the EMG burst peak amplitudes were different between groups for all muscles tested ($F_{2,4,8} = 23.51$, $P < 0.0001$). *Post-hoc* analysis showed significantly higher EMG burst peak amplitudes in the ST and BT rats compared with the FT rats for all muscles ($P < 0.001$) except for the adductor brevis and MG in BT rats (Fig. 4A). In addition, the maximum amplitudes of the adductor ($t = 3.858$, $P < 0.001$) and extensor muscles ($t = 2.947$, $P < 0.01$; MG, $t = 5.211$, $P < 0.001$) were higher in ST than BT rats. There was a significant interaction between the effects of animal groups and burst durations ($F_{2,4,8} = 7.21$, $P < 0.0001$). Simple main effects analysis showed that the EMG burst durations were different between groups ($F_{2,4,8} = 2.28$, $P < 0.0001$) with *post-hoc* analysis demonstrating

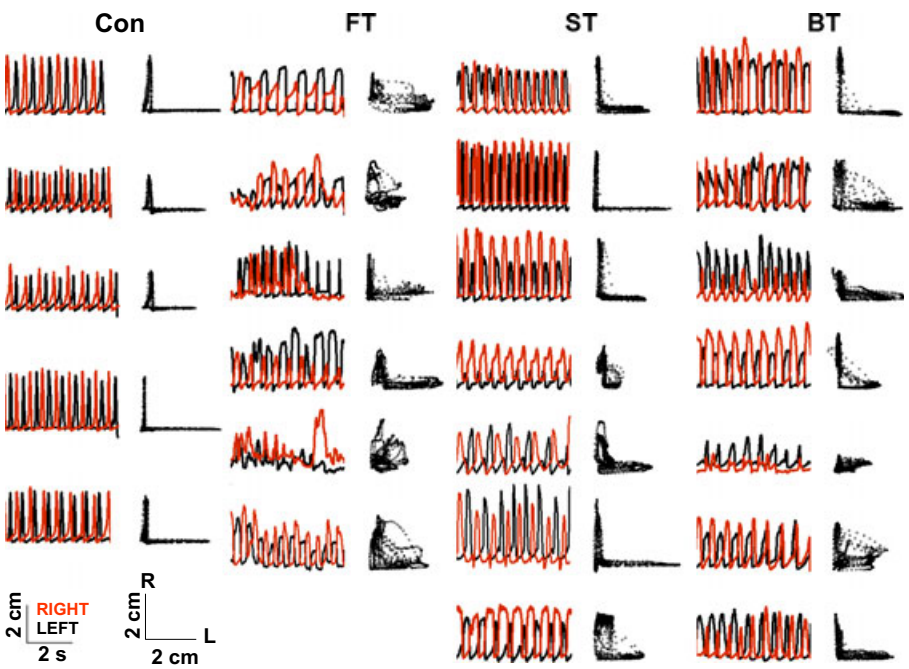

FIG. 1. Interlimb coordination during forward stepping. Vertical step height trajectories of the metatarsophalangeal joint during consecutive left (black) and right (red) hindlimb forward stepping in individual rats in the Con ($n = 5$), FT ($n = 6$), ST ($n = 7$), and BT ($n = 7$) groups. Adjacent traces to the right (black) are joint probability distributions of the left and right metatarsophalangeal (MTP) joint movements; an L-shaped pattern indicates an alternating motion of the two hindlimbs, whereas a D-shaped pattern indicates less alternation between the two hindlimbs.

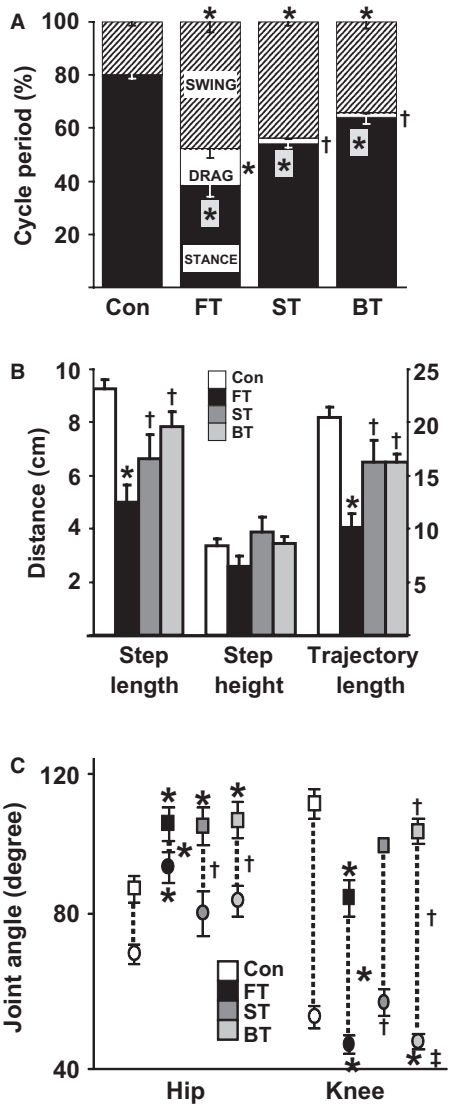

FIG. 2. Mean (± SEM) kinematics measures for 6–10 step cycles for all rats in the Con, FT, ST, and BT groups during forward stepping after 5 weeks of training in a specific direction. (A) Percentage of stance duration (black), swing duration (hatched), and foot drag at the beginning of the swing phase (white) normalized to the step cycle duration. (B) Step lengths, step heights, and trajectory lengths of the step cycles for each group. (C) Minimum (circles), maximum (squares), and range of hip and knee joint angles during forward stepping for each group. Numbers of animals are the same as in Fig. 1. *, †, and ‡Significantly different from the Con, FT, and ST groups, respectively, at $P < 0.05$.

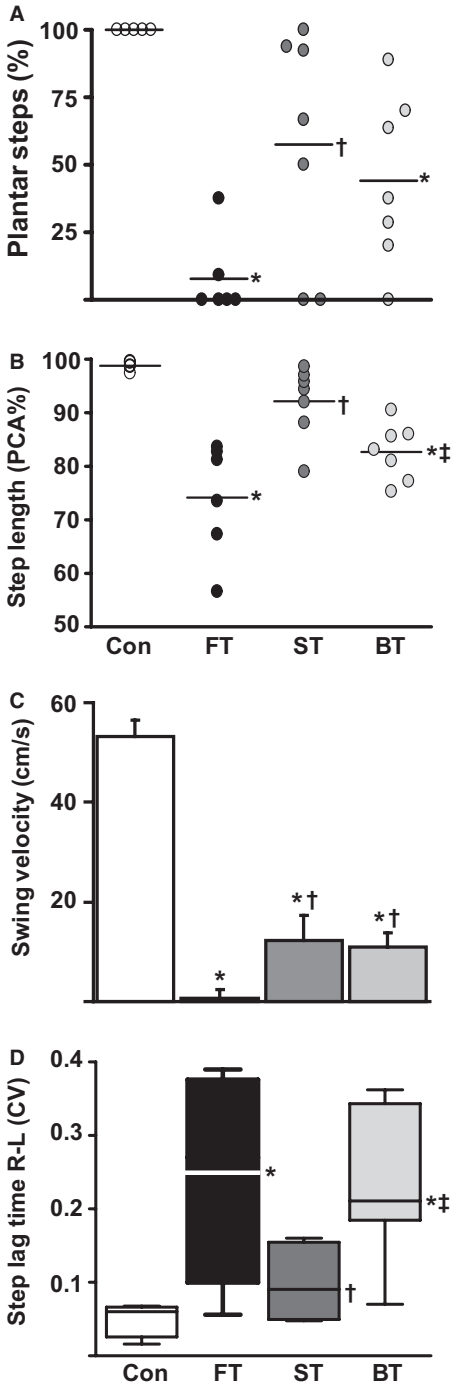

FIG. 3. Phase-dependent measures of stepping. (A) Percentage of plantar steps performed by the rats in each group during forward stepping after 5 weeks of forward, sideward, or backward training. (B) Consistency of the hindlimb horizontal trajectory as measured by the first principal component; higher principal component analysis (PCA) values indicate greater consistency in stepping. Values for individual rats (circles) and the mean values (horizontal line) are shown for each group in both A and B. (C) Mean (+ SEM) horizontal velocity during the mid-swing phase for each group. (D) Coefficient of variation (CV) of the lag time between the onset of the right and left leg movements within a step cycle. Lower values indicate less variation between the right and left hindlimbs during forward stepping. Horizontal lines on the box plot demonstrate quartile and median values in each group. Number of animals and steps analysed in each group are the same as in Figs. 1 and 2. *, †, and ‡Significantly different from the Con, FT, and ST groups, respectively, at $P < 0.05$.

shorter mean EMG burst durations for the TA and St in BT rats than in FT and ST rats, and shorter mean EMG burst durations for the TA in ST than FT rats ($P < 0.001$, Fig. 4B). There was a significant interaction between the effects of animal groups and burst integrals ($F_{2,4,8} = 4.29$, $P < 0.0001$). Simple main effects analysis showed that the EMG burst integrals were different between groups ($F_{2,4,8} = 3.98$, $P < 0.0001$). *Post-hoc* analysis demonstrated that the mean EMG burst integrals for both extensors (VL and MG) were smaller ($P < 0.01$) in FT rats than in ST and BT rats, and smaller in all muscles ($P < 0.01$) in BT rats than in ST rats (Fig. 4C). In addition, the mean burst integral of the St ($t = 9.970$, $P < 0.001$) was smaller in FT rats than in ST rats.

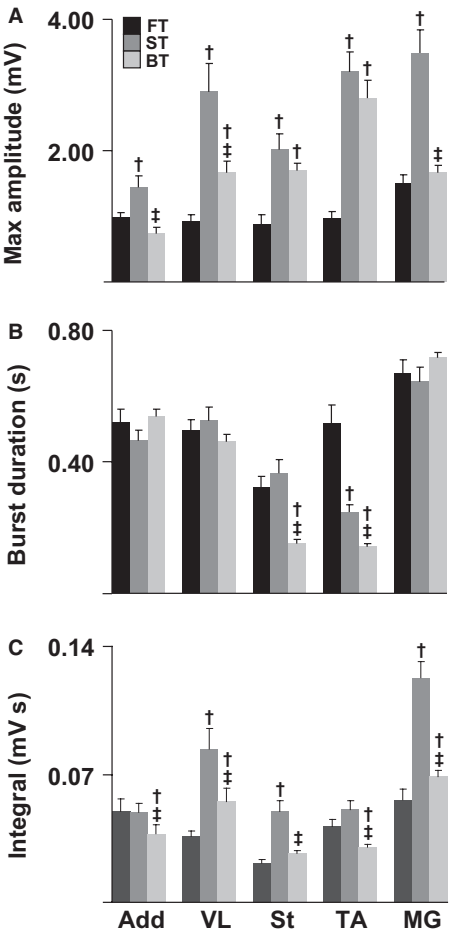

FIG. 4. EMG characteristics from select hindlimb muscles during forward stepping averaged for all rats in each group while forward stepping (n = 10 steps). (A) Mean (+ SEM) maximum amplitudes, (B) burst durations, and (C) burst integrals normalized to the step cycle period for each muscle in each group during forward stepping. Add, adductor. Number of rats and number of steps analysed in each group are the same as in Fig. 1. † and ‡Significantly different from the FT and ST groups, respectively, at $P < 0.05$.

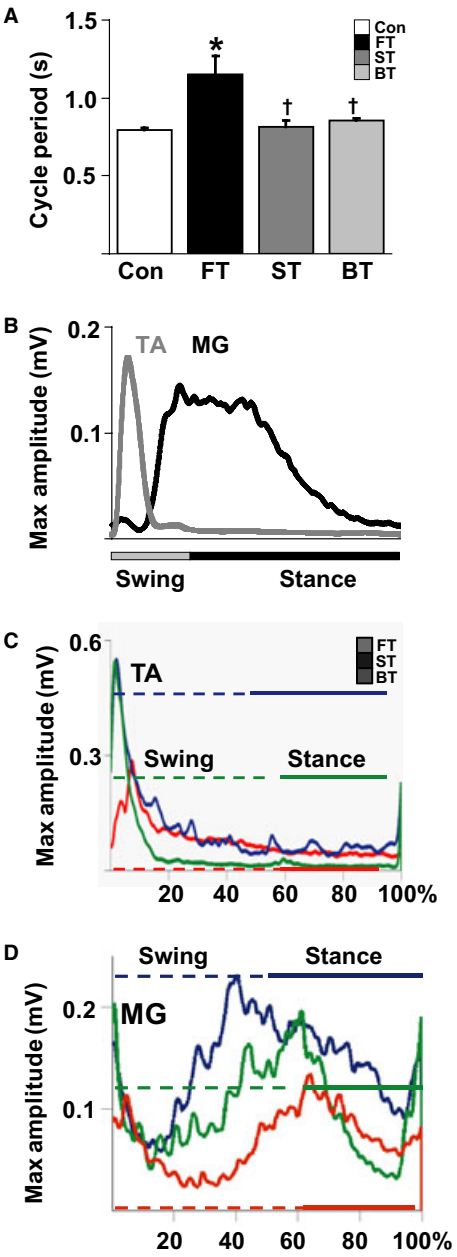

FIG. 5. Phase-dependent EMG characteristics. (A) Mean (+ SEM) cycle period obtained from the MG EMG burst activity during forward stepping in each group. *, and †Significantly different from the Con and FT group at $P < 0.05$. (B) Mean integrated EMG activity of the TA and MG muscles from non-injured Con rats (n = 5) showing a typical flexor–extensor activation pattern for a normalized step cycle during forward stepping. Mean rectified EMG activity of the (C) TA and (D) MG normalized to a step cycle for all rats in each group. The horizontal lines in the TA and MG muscle trace depict the swing (dashed) and stance (solid) phase durations for each group. Number of rats and number of steps analysed in each group are the same as in Fig. 1.

Muscle activation patterns

The cycle period measured using the TA EMG burst was longer in FT rats than in Con, ST, and BT rats ($F_{3,21}$ = 8.233, $P < 0.0001$, Fig. 5A). The relatively long cycle period in FT rats appears to reflect the slow horizontal velocities (and consequent longer time) in the mid-swing phase of the step cycle (Fig. 3C) and not other step characteristics such as trajectory length and step height (Fig. 3B). Flexor–extensor muscle synergies were similar in all three groups such that the flexors (TA, St) were active primarily during the swing phase and the extensors (MG, VL) were active primarily during the stance phase of each normalized step cycle (Fig. 5C and D, only TA and MG shown). This alternating antagonistic muscle activity pattern is similar to that seen in Con rats (Fig. 5B). The extensor, but not the flexor or adductor, muscles showed different temporal patterns across groups (data not shown for adductor brevis, VL, and St). Qualitatively, MG activity was initiated at ~40% of the normalized step cycle in FT rats, but much earlier, i.e. at ~17% of the step cycle, in ST and BT rats (Fig. 5D). Similarly, the VL activity was initiated at ~50% of the normalized step cycle in FT rats, but much earlier, i.e. at ~30% of the step cycle, in ST and BT rats (data not shown).

Discussion

We compared three qualitatively different task-specific bipedal step training strategies, i.e. forward, sideward, and backward step training, for their ability to improve forward bipedal stepping in rats receiving a complete spinal cord transection as adults. We found that the best forward stepping, as described by similarity to non-injured Con rats, was observed in spinal rats that were trained to step sideward. The better performance in the forward stepping behavior of the ST group

was associated with higher peak EMG amplitudes and muscle activation patterns that were more similar to that observed in intact rats than either the FT or BT groups. In addition, the horizontal velocity during swing, foot trajectories, and the quality and coordination of stepping were more similar to stepping in Con rats in ST and BT rats than in FT rats.

Imposing variations to task-specific training improves the performance of that task

Given the many examples that task-specific training enhances motor performance after an SCI (Hodgson *et al.*, 1994; Behrman *et al.*, 2005; Bigbee *et al.*, 2007; Harkema, 2008; Magnuson *et al.*, 2009), the present results were unexpected. For instance, spinal cats that were trained to step can regain more effective hindlimb stepping than cats that were trained to stand (de Leon *et al.*, 1998). Similarly, spinal cats that were trained to stand, learn to support their body weight for longer durations than cats that were trained to step or not trained for either task (Hodgson *et al.*, 1994; de Leon *et al.*, 1998). Rats that were trained to reach and grasp perform better in this task, but worse in skilled running across a ladder, and vice versa, rats that were trained to run across a ladder performed better in this task but worse in reaching and grasping (Girgis *et al.*, 2007; Garcia-Alias *et al.*, 2009). An essential difference among the different modes of training in these studies compared with the present study is that the fundamental feature of the different training modes in the present study involved bipedal stepping in different directions. In contrast, standing vs. stepping entails qualitatively different patterns – a static postural task as opposed to a dynamic repetitive task. Likewise, reaching is a fundamentally different task to stepping on a ladder.

Physiologically, the implication from the combination of all of these studies is that there must be some critical level of qualitative similarity of the spinal circuits engaged within the trained tasks and the task that is being tested. As a consequence, if the neuronal circuits being trained and those being tested partially overlap, then this could reinforce the synaptic efficacy of a more expansive neural circuit. This, in turn, could result in engagement of a larger circuitry that can become more responsive to the intrinsic variability that appears to be important to the spinal locomotor circuitry (Cai *et al.*, 2006; Ziegler *et al.*, 2010). Our results are consistent with studies showing that practicing variations of a task along with the specific task can be much more beneficial than training in a single task (Shea & Kohl, 1990). After SCI, rats that were trained to stair climb (Singh *et al.*, 2010) or injured mice that were trained on a running wheel (Engesser-Cesar *et al.*, 2005) showed improvement in open-field locomotion. Similarly, Starkey *et al.* (2011) observed enhanced performance in a novel untrained staircase-grasping test after training incomplete SCI rats in a specific single pellet-reaching task. In the clinical setting, imposing a more 'challenging' training task, such as negotiating obstacles during step training, walking on different terrains, and walking while reaching/carrying objects, can improve the walking behavior of patients with an SCI (Behrman *et al.*, 2005; Musselman *et al.*, 2009; Manella *et al.*, 2010). Some of the ways to 'challenge' a walking task are to increase the speed and/or incline of the treadmill belt and/or add weights to the ankle during locomotion – each of these parameters alters the magnitude of the same behavior in a graded, continuous manner. In the present work, we selected the direction of stepping as a specific contextual variation to the task of forward stepping, and define sideward and backward stepping as 'challenging' tasks for the forward stepping task.

Based on the present results, we propose that by adding variations of a qualitatively similar task to a specific motor task a wider ensemble of afferent feedback could enhance the performance of the specific motor task. Consequently, because the cutaneous receptor fields are densely distributed on the foot pad of a rat (Leem *et al.*, 1993), the inherent nature of sideward step training (sideward brushing of the foot against the treadmill) may have activated a relatively larger ensemble of afferent feedback from cutaneous receptors as well as mechanoreceptors of the lower limb. As a result, sideward step training might have engaged and thereby trained a broader spinal neural circuitry than forward step training, leading to greater neuronal activation (and hence the resultant elevated EMG activity) in ST rats as compared with FT rats during forward locomotion. Our observation is supported by the evidence that the threshold for stimulation that evoked forward locomotion was significantly lower in the ST rats than the other two SCI groups, perhaps due to a compensatory facilitating input from peripheral afferents and a corresponding greater excitability of specific neuronal populations that drove the motor output in the ST group.

Role of multifunctional spinal networks in 'challenging' training

Neuromuscular activation patterns of fundamentally discrete motor behaviors, such as standing, reaching, and swimming, suggest that these may involve, to a large extent, distinct neuronal circuitries (Bigbee *et al.*, 2007; Girgis *et al.*, 2007; Magnuson *et al.*, 2009; Kuerzi *et al.*, 2010). One could hypothesize that distinct spinal circuits control forward, backward, and sideward stepping behaviors that, in turn, would facilitate stepping only in a given direction. In addition, it would also be reasonable to expect that training the potential for learning a novel motor task is less if only that task is practiced (Bigbee *et al.*, 2007). Our results, however, show that, independent of the nature of the stepping regimen, all animals were able to forward step, although the best forward stepping capability was observed in ST animals.

Alternatively, we hypothesize that stepping in different directions is probably controlled by a multifunctional network of neurons, thereby implying that qualitatively different, but fundamentally similar, motor tasks would engage a broader and more dissimilar neural circuitry than when stepping without these contextual variations. Multifunctional networks have been widely studied in invertebrates and it is well established that, given the appropriate sensory modulation, many motor behaviors are driven by the coordinated activity of a common neuronal pool (Briggman & Kristan, 2008; Berkowitz, 2010). Swimming and crawling behaviors in leeches, for example, are mutually exclusive motor behaviors that are controlled by a common network of neurons (Briggman & Kristan, 2006). Similarly, swimming (a rostrocaudal movement) involves all, plus a larger population, of the neurons that organize the struggling (a more caudorostral pattern) behavior in tadpoles (Soffe, 1996). At a more cellular level, studies have shown that, in the somatogastric nervous system of the crab, at least 20 different neuromodulator patterns elicit similar motor responses of the gastric mill (chewing behavior), and each of these patterns also shares the same core central pattern generator (Saideman *et al.*, 2007). Based on the similarities among muscle synergies in swimming, kicking, and walking behaviors in the frog, D'Avella *et al.* (2003) suggest that there is a sharing of neural circuitries across different motor tasks. Similarly, based on differences in some key kinematics features and muscle activation patterns, it has been suggested that there is involvement of partially overlapping neuronal circuits among different behaviors such as forward (Buford & Smith, 1990), backward (Buford & Smith, 1990), upslope (Carlson-Kuhta *et al.*, 1998), and downslope (Trank & Smith, 1996) stepping in cats,

and forward and backward stepping in dogs (Vilensky & Cook, 2000), and in non-injured human adults (Grasso *et al.*, 1998) and infants (Lamb & Yang, 2000). Interestingly, within 10 days of daily training to walk backward in human subjects, the modulation of the soleus H-reflex is similar to that seen during forward walking (Schneider & Capaday, 2003). This finding further suggests a seemingly interactive feature of the neuronal components underlying similar motor behaviors.

In our present work, as far as we could observe, the same muscles were activated during sideward and forward stepping, although the coordination patterns of the involved motor pools were different. For the TA, but not the MG, during forward stepping there were some similarities in the patterns of bursts of activity among FT, BT, and ST rats, demonstrating that the neuronal circuitries for the three directions of stepping might be composed of at least partially overlapping circuits. Although theoretically these overlapping neural circuits could facilitate or interfere with one another in a training paradigm (Carew *et al.*, 1971; Byrne, 1987), the present results show a clear beneficial effect of the recovery of forward stepping as a result of training with sideward and, to a lesser degree, backward stepping. The observation that backward training did not transfer as effectively to forward stepping as did sideward training leads us to surmise that there is a rather large overlap of circuitries with synergistic potential between forward and sideward stepping compared with forward and backward stepping (Fig. 6). It remains uncertain to what extent the findings in the present study can be generalized to different motor tasks associated with weight-bearing locomotion. We observed improved forward stepping following both backward and sideward training. The degree to which the motor task being trained varies relative to the task being tested is a key unresolved issue. For example, Grasso *et al.* (2004) report that forward step-trained individuals with an SCI were not able to step in a backward direction unless trained to step in a backward direction, but backward step-trained subjects were able to step forward. Overall, our data suggest that, at least after SCI, there are additional advantages in repetitively activating a broader network of neurons associated with stepping rather than activating a more restricted population of neurons involved with stepping in a single direction. A similar potentiation of motor activity has been well described for Aplysia (Antzoulatos *et al.*, 2006). A brief electrical training stimulus that is not sufficient to induce sensitization of the gill withdrawal reflex (generate a greater response to repetitive stimuli) can do so if it is preceded by 4 days of training on the contralateral side. The authors suggested that the sensitization of the gill response on the ipsilateral side was a consequence of a sensitizing, but subthreshold, response mediated via contralateral neurons projecting ipsilaterally. In view of the fact that muscle activation levels during forward stepping were greater in ST and BT rats than in FT rats (Figs. 4 and 5), we postulate that training the locomotor circuitry associated with sideward or backward stepping could have potentiated the activity of the circuitry associated with forward stepping, thereby providing a more expansive circuitry that could serve as a broader source of control of the sensory input that can contribute to improved forward stepping (Fig. 6).

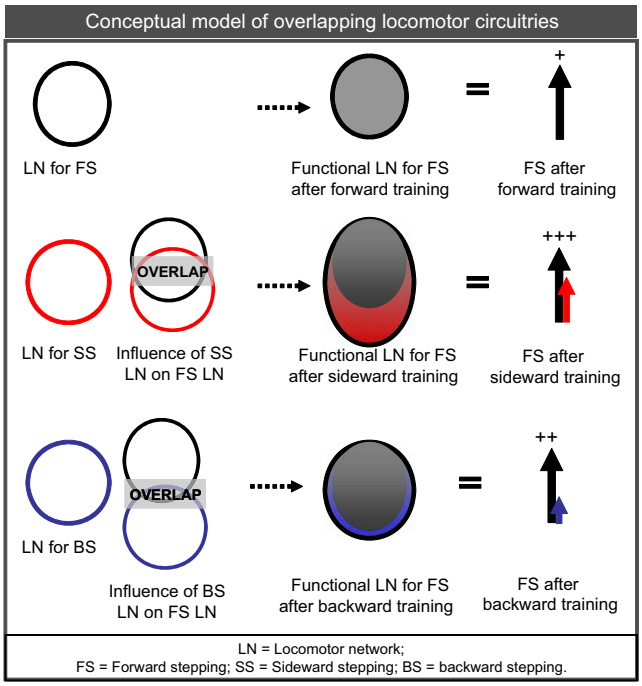

FIG. 6. Conceptual model explaining our study hypothesis. Theoretically, sideward and backward training engaged not only the circuitry necessary for sideward and backward stepping but also involved those circuits that generate forward stepping, suggesting the contribution of a multifunctional network of neurons in evoking stepping in different directions. The circles indicate locomotor networks (LNs) for forward (FS, black), sideward (SS, red) and backward (BS, blue) stepping. The LN for FS is partially complemented by activity from the LN for SS and BS after sideward and backward training, respectively. Additionally, the extent of overlap of circles indicates that sideward stepping circuitry overlaps to a larger extent with the forward stepping circuitry than backward stepping circuitry overlaps with the forward stepping circuitry. Accordingly, neuronal activation for FS is greater after sideward training (vertical arrow with +++) than after backward training (vertical arrow with ++) and forward training (vertical arrow with +).

Conclusion

In summary, our data demonstrate a positive transfer of one motor task to another by imposing qualitatively altering features of a specific oscillatory motor task. This implies that there are overlapping neuronal elements that can be used to provide more precise control of another qualitatively similar motor task. Therefore, training a fundamentally similar but more expansive circuitry appears to enable better motor performance than training a specific task that engages a more limited circuitry. Testing this hypothesis will require extensive neurophysiological and kinematics data that can be tightly linked to the activation of specific interneurons and motoneurons during the motor tasks of interest. The clinical implication of this work directly impacts motor rehabilitation after central nervous system injury. Motor recovery following an SCI, for example, is often limited by the scarce availability of physical rehabilitation strategies. Our data clearly highlight the importance of adding variations to a task for better performance and suggest that one motor task can facilitate the performance of another task as long as there is some degree of neural network sharing between the different motor tasks, thereby expanding the existing physical therapies available to enhance motor recovery in patients with a neurological injury. Additionally, this work raises questions that necessitate a much deeper understanding of the neurological mechanisms underlying the addition of 'challenges' to task-specific physical rehabilitation strategies.

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Abbreviations

BT, backward step trained; Con, control; EMG, electromyography; FT, forward step trained; MG, medial gastrocnemius; SCI, spinal cord injury; St, semitendinosus; ST, sideward step trained; TA, tibialis anterior; VL, vastus lateralis.

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