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Treadmill training promotes spinal changes leading to locomotor recovery after partial spinal cord injury in cats

Marina Martinez, Hugo Delivet-Mongrain, and Serge Rossignol

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Submitted 3 December 2012; accepted in final form 27 March 2013

AFTER INCOMPLETE SPINAL CORD INJURY (iSCI) in humans and animals, significant spontaneous recovery of sensorimotor functions is frequently observed, even without rehabilitative training or pharmacological treatments (Jiang and Drew 1996; Martinez et al. 2009b, 2011; Martinez and Rossignol 2011). Although the underlying mechanisms enabling the spontaneous recovery are poorly understood, it is actually well known that after iSCIs, adaptive changes occur at all levels of the neuraxis, including the spinal cord below the lesion (Barrière et al. 2008; Martinez et al. 2011, 2012a, 2012b). This last conclusion was reached from studies of a dual spinal lesion paradigm in the cat. In this model, a first partial lesion was performed at thoracic level (T10) and the recovery of locomotion was assessed for 3 wk. To evaluate the potential contribution of spinal changes to this locomotor recovery, the lumbar locomotor circuitry was completely isolated from all remnant supraspinal inputs by performing a complete spinalization at T13, two to three levels below the previous hemisection. The ensuing expression of spinal locomotion was assessed and was taken to reflect intraspinal changes induced by the previous hemisection. Using this strategy, we recently showed that after unilateral thoracic hemisection, the paretic hindlimb progressively recovered spontaneously but the walking pattern remained somewhat deficient, apparent as some kinematic asymmetry and dissymmetrical foot positioning at touchdown (Martinez et al. 2011). Furthermore, the asymmetrical walking pattern observed after hemisection was retained durably after all supraspinal inputs by spinalization were suppressed (Martinez et al. 2012b), demonstrating that the spinal hemisection had induced an asymmetrical reorganization of the spinal locomotor circuitry itself (Martinez et al. 2012b).

The fact that spontaneous changes occur within the spinal cord itself after iSCIs is of great interest considering the high incidence of such lesions in human. The above-described results suggest that neuroplastic changes occur not only in supraspinal structures but also in the spinal cord itself below the lesion. From a clinical perspective, it would be desirable to correct the asymmetrical walking pattern induced by such spinal lesions. In line with this, we have more recently shown that providing daily locomotor training for 3 wk to complete spinal cats previously hemisected for 3 wk but not trained could reverse the walking asymmetry, suggesting that the spinal cord is still amenable to changes imposed by external interventions such as provided by training (Martinez et al. 2012a).

Therefore, our previous studies on the dual spinal lesion paradigm established that after hemisection, locomotion was reexpressed but remained asymmetrical, that some degree of this locomotor asymmetry persisted after spinalization, and that this asymmetry could be reversed by locomotor training in the spinal state. However, none of these studies have evaluated the role of locomotor training provided during the hemisected period on the quality of locomotor recovery in the hemispinal state and, subsequently, the quality of hindlimb locomotion in the spinal state. The present study, which represents a significant development in our studies of this dual spinal lesion paradigm, addresses this important question specifically.

First, we found that locomotor training for a period of 3 wk posthemisection was sufficient to correct the residual deficits of the hindlimb ipsilateral to the lesion and restore symmetrical walking, in contrast to untrained cats. Second, after the com-
complete spinalization, all the trained cats reexpressed bilateral and symmetrical hindlimb locomotion at high speeds, whereas the locomotor pattern of the untrained cats remained asymmetrical and the hindlimb step cycles on the side of the hemisection were still deficient. This study highlights the beneficial role of locomotor training in promoting locomotor recovery after an iSCI by facilitating bilateral and symmetrical adaptive plastic changes within the spinal circuitry itself.

METHODS

Ethical Approval

All procedures followed a protocol approved by the Ethics Committee at the Université de Montréal, according to the Canadian Guide for the Care and Use of Experimental Animals.

Experimental Paradigm

Adult female (n = 16) cats weighing from 2.5 to 3.5 kg were first selected for their ability to walk regularly and continuously for several minutes (10–15 min) on a motor-driven treadmill at different speeds (0.3–1 m/s). Six of these 16 cats were previously used in a recent study in which cats were not trained (Martinez et al. 2012a), and their locomotor performances were reanalyzed for the present study to compare with those of trained cats; they represent the majority of cats included in the untrained group. After control recording sessions (n = 2–3) to obtain baseline kinematic and electromyography (EMG) values for locomotion in the intact state, all cats underwent an iSCI consisting of a hemisection targeting the left side of the spinal cord at T10. Three days after hemisection, a group of 8 cats was trained 7 days a week (trained group), whereas a group of 8 cats was not trained but only evaluated weekly until the third week after hemisection (untrained group) (Fig. 1). For the rest of the time, the cats were confined to their individual cages (104 × 76 × 94 cm), thus limiting sensorimotor experience and self-training. Three weeks after the hemisection, a complete transection of the spinal cord (i.e., spinalization) was performed at T13, i.e., three segments below the hemisection (d0) refers to the day of the hemisection and serves as reference to illustrate changes within the spinal circuitry itself.

All surgical procedures for electrode implantation or spinal lesions were done under general anesthesia and aseptic conditions as described in previous works. Animals were first subcutaneously injected with an analgesic (Anafen, 0.01 mg/kg) and premedicated with Atravet (0.1 mg/kg), glycopyrrolate (0.01 mg/kg), and ketamine (10 mg/kg) by intramuscular administration. An endotracheal tube was then inserted to provide gaseous anesthesia (isoflurane 2% in a mixture of 95% O2-5% CO2). Cats were chronically implanted with intramuscular needles to record EMG activity from flexor and extensor hindlimb muscles on both sides. The implanted muscles were sartorius (Srt; hip flexor and knee extensor), semitendinosus (St; knee extensor), gastrocnemius lateralis and medialis (GL and GM; ankle extensors and knee flexors), and tibialis anterior (TA; ankle flexor). Electrodes were led subcutaneously to two 15-pinhead connectors secured to the cranium using acrylic cement. Two to three weeks after EMG implantation, a partial spinal lesion targeted the left part of the cord at T10 was achieved with microscissors, as in previous studies (Martinez et al. 2011, 2012a, 2012b). The same methodology was used for the complete spinalization at T13 except that the lesion gap was filled by firmly pressing the Surgicel to the bottom of the spinal canal. Heart rate and respiration were monitored throughout the surgeries. Before the end of the surgeries, an analgesic (buprenorphine, 0.01 mg/kg) and an antibiotic (Convenia, 8 mg/kg) were administered subcutaneously. Additionally, a patch of fentanyl (25 μg/h) was applied on the skin to alleviate pain for about 5 days.

Training Procedure

After hemisection, 8 cats were trained to locomote 30 min per day at the speed of 0.4 m/s from the 3rd to the 21st day after spinalization (trained group) whereas the locomotor performances of the other 8 cats were only evaluated once a week to prevent any training effect (untrained group) (Fig. 1). From the 3rd to the 7th day after hemisection, cats were unable to express voluntary quadrupedal locomotion such that during this period, cats were trained to walk only with their hindlimbs by using the same procedure as in spinal cats (Barbeau and Rossignol 1987). The forelimbs of cats were kept on a stationary platform fixed at about 2 cm above the treadmill during training. A thin sheet of Plexiglas of 10-cm height was placed longitudinally to separate the hindpaws during stepping. Lateral stabilization and weight support were facilitated by holding the tail. From the second...
hemispinal week, cats were trained to walk with all four limbs by providing cats with perineal stimulation and assistance in body equilibrium when needed. On the third week, perineal stimulation was not necessary anymore to elicit locomotion and assistance in body equilibrium was only provided by holding the tail when needed.

Kinematic and EMG Recordings

During episodes of locomotion, cats were recorded from the left side with a digital video camera and the data were stored on a hard disk. Video images were de-interlaced to yield a resolution of 60 fields/s or 16.6 ms between fields. Reflective markers were placed on the left and right hind foot at the tip of the toes of both hindlimbs to determine the periods of stance and swing. The periods of stance and swing at the fore- and hindlimb levels were determined by visually tagging foot contact and liftoff on video images. The amplified (Lynx-8 amplifiers, Neuralynx) and filtered (bandwidth 100 Hz to 3 kHz) EMG signals were digitized at 1 kHz (NI-6071E, National Instruments) and stored on a computer. Kinematic and EMG recordings were synchronized using a SMPTE (Society of Motion Picture and Television Engineers) time code generator.

Kinematic and EMG Analyses

Step cycle duration represents the time between two successive contacts of the same foot on the treadmill (see Fig. 3A), whereas the stance duration refers to the time between foot contact and toe off which initiates the swing phase (see Fig. 3C). In cases where these kinematic events are not very distinct, we have defined the onset of swing as the onset of forward foot movement and the onset of stance as the onset of backward movement of the foot.

Step length was calculated using stance onset as a trigger point. It was calculated by adding the distance travelled by the toe between two successive paw contacts of the same limb, i.e., distance travelled during the stance and swing phase of a complete step cycle at a given speed. Horizontal movements of the whole cat on the treadmill were taken into account using a hip marker as reference (see Fig. 3B).

Toe position relative to the hip at contact and lift was determined by calculating the mean position (in mm) of the toe relative to the vertical projection of the hip joint on the ground at contact and lift (see Fig. 3D). This measure indicates the extent of forward and backward movements during a locomotor episode.

As the first unilateral hemisection targeted the left side of the cord and is well known to induce an asymmetrical walking pattern (Martinez et al. 2011, 2012a), we described the asymmetry between limbs by comparing the locomotor parameters described above between hindlimbs. The term “symmetry” is used to qualify a similar locomotor pattern between hindlimbs, whereas the term “asymmetry” refers to a different locomotor pattern between hindlimbs. To compare the left and right hindlimb locomotor pattern over time, an asymmetry index (AI) was calculated by quantifying the kinematic parameters described above for the left and right hindlimbs averaged for over 16–20 consecutive step cycles. The AI was calculated for each parameter as (average left – right)/(average left + right). An AI of 0 indicates a perfect symmetry, and in normal conditions, the AI values obtained in all parameters are equal to 0 ± 0.05 (see for instance Fig. 3, right). The AI gives information on the direction of asymmetry. When a parameter is greater on the left than on the right side, the AI will be positive, and vice versa. For example, in case of a step length AI < 0, the step of the right hindlimb will be longer than the left.

EMG burst duration was calculated as the mean time between onset and termination of several single bursts. The EMG bursts onset and offset were visually tagged using homemade software that allows a good level of precision.

Homologous phase coupling of the hindlimbs was calculated from the time between hindlimb contacts divided by the step cycle period of the left hindlimb (see Fig. 5A). The coupling of the right hindlimb was expressed as a phase value of each left hindlimb cycle (i.e., 0 to 1; the full step cycle corresponds to 1) and then averaged over 20 consecutive cycles during treadmill locomotion in each cat. Finally, the mean phase value obtained from all cats in each group was calculated. With this method, a 0.5 phase value indicates a perfect alternating coupling between hindlimbs (see Fig. 5, C and D).

EMG coupling was investigated by taking the left St (knee flexor and hip extensor), which usually discharges with a sharp burst at the end of stance or beginning of swing, as a reference to measure the onset of bursts in different muscles. The coupling between 20 consecutive onsets of muscles was calculated as the time between the 20 consecutive onsets of muscles divided by the corresponding cycle period of the reference muscle (see Figs. 5B and 6A). For example, when measuring the coupling between hip flexor sartorius (Srt) and St (reference muscle), the time point at which Srt began to discharge in relation to St was measured (in ms), and this value was divided by the St cycle duration (see Fig. 6).

Changes in homologous phase coupling and EMG phase relations over time in each group are all expressed as mean phase values (i.e., 0 to 1; the full step cycle corresponds to 1) and are thus illustrated by polar representations (see for instance Fig. 5C). It is important to note that in these graphs, the size of the circles represents the standard deviation (SD) of mean values. For a better representation of the SD, the distance from the origin was taken into account such that the representation of similar SDs appear identical whatever the distance from the origin.

Histology

Three weeks after spinalization, animals were given a lethal dose of intravenous pentobarbital sodium solution. A block of spinal cord from T8 to L1 was extracted and fixed in 10% formalin for several weeks and then transferred to 30% sucrose for 3 days. The spinal cord was frozen, and 40-μm-thick coronal sections centered on the partial and complete lesions were taken for histological examination. Every section was mounted on a slide and stained with cresyl violet. The coronal sections were examined under a microscope to assess all damaged tissue and the total damaged area was calculated (see Fig. 2A).

Statistics

Linear and circular statistical analyses were performed using PASW (PASW Statistics 18.0) and Oriana (3.13; KCS, Isle of Anglesey, UK) software in 16 cats for kinematic parameters and in 12 cats for EMG parameters. To compare the partial lesion size between groups, unpaired t-tests were used. Since we were interested in evaluating the effect of training on the time course of recovery after hemisection, we compared the performances of the two groups of cats over time, i.e., at the intact state, 3 wk after hemisection, and 24 h after spinalization by using two-way repeated-measures ANOVAs for linear values and Watson-Wheeler F-tests for circular values. When the tests indicated a significant effect of group, delay, or both, ANOVAs were supplemented with multiple comparisons (paired t-tests supplemented with a Bonferroni correction). A P value <0.05 was considered statistically significant. Results are means ± SD.

RESULTS

Analysis of the Extent of the Lesion

Since we compared a trained group and an untrained group, it was important to assess and compare the extent of the partial lesion in both groups. As illustrated in Fig. 2A, the hemisections were mainly confined to the left side of the cord in all cats. The lesions spared the most medial part of the left ventral funiculus in the eight untrained cats. In the trained cats, the
hemisections were somewhat more extensive and damaged the most ventromedial part of the cord. It is important to remember that the two groups of cats were done sequentially (untrained group before the trained group) so that the histology of the untrained group was already available. After the histology of this group was examined, it appeared that the ventral part of the cord on the left side was not entirely damaged, and the experimenters just tried to fix this issue with the second group such that it led to larger lesion size. In both groups, some damage was also observed in the dorsal column on the right side. In all cats except one, lesions also damaged part of the gray matter on the right side. Comparisons of the two groups with regard to the percentage of total damaged area (Fig. 2A), including the white and gray matters as well as cavitations, indicated a significant difference between groups such that the lesions were more extensive in the trained group (unpaired t-tests, $P < 0.05$). Indeed, the lesion of the untrained group damaged 42.35% ± 5.03 of the cord, whereas the lesion of the trained group damaged 59.77% ± 9.15 so that the lesions included almost 17% more of the cord cross-sectional area in the trained group, an important consideration to remember later on. We also verified that the second spinal lesion was complete in all cats.

### Overview of the Locomotor Recovery in Trained and Untrained Cats After Incomplete SCI

After the hemispinal lesion on the left side at T10, the left hindlimb was initially paretic in all cats for 3–4 days. In the group of untrained cats, voluntary quadrupedal locomotion on the treadmill and overground was reexpressed as early as 1 wk after hemisection. However, in most of the trained cats, the recovery of voluntary locomotion was delayed such that they did not regain an active pattern of locomotion on the left side before the third hemispinal week, probably due to their larger lesion size. During the first 2 wk, involuntary quadrupedal...
locomotion could be elicited by providing trained cats with perineal stimulation indicating the integrity of spinal locomotor networks. Despite different lesion size, the untrained and trained groups had both regained voluntary quadrupedal locomotion at high speeds (0.7–0.8 m/s) with postural support and body equilibrium 21 days after hemisection. Interestingly, although the untrained cats could readily walk within the first week posthemisection, they also exhibited more deficits (limping pattern and inconsistent plantar foot contact on the side of the lesion) than the trained cats on the third hemispinal week (Fig. 2B) despite smaller lesion size. By contrast, despite larger lesions, the locomotor performances of the trained cats remarkably improved over the 3-wk training period so that their locomotor pattern at 3 wk postlesion closely resembled the pattern in the intact state (see for instance Fig. 2B). Moreover, in untrained cats, the variability of the walking pattern is clearly shown by the increased SD in the excursion of all the joints (Fig. 2B). In trained cats, the mean values and SD observed at the intact and hemispinal state can be superposed for most of the angular excursion.

Training Promotes Locomotor Recovery After Incomplete SCI

We extensively quantified the kinematics and EMG characteristics of stepping movements during treadmill locomotion at 0.4 m/s in the trained and untrained groups 3 wk after hemisection and compared the mean values obtained between the two groups.

**Step cycle structure.** After the hemisection on the left side, the right hindlimb remained actively controlled by supraspinal inputs, whereas the left hindlimb has lost most of its supraspinal inputs, thus inducing an asymmetrical control of locomotion. This can be quantified by calculating an AI that compares various locomotor parameters on the left and right side. Because the step cycle is known to be dramatically changed on both sides after spinal hemisections (Martinez et al. 2012b), we first examined the ability of treadmill training to preserve the intrinsic structure of the cycle after such lesions.

In intact cats, the walking pattern is symmetrical, i.e., the step length, cycle, stance, and swing durations are similar on the left and right sides (Fig. 3, A–D, filled bars). In this case, the AI that compares the left and right hindlimb over several mean kinematic values is 0 ± 0.05 (Fig. 3, right). Without training after the hemisection, the left hindlimb exhibited several deficits (Figs. 2B and 3, left hindlimb), whereas the right hindlimb showed little changes (Fig. 3, right hindlimb). Three weeks after the hemisection, the cycle duration remained more or less equal on both sides (Fig. 3A), whereas the step length became asymmetrical (AI < 0, P < 0.05) (Fig. 3B) because the left hindlimb made shorter steps than the right one. In fact, the stance duration was shorter on the left side than on the right (Fig. 3C) to minimize the time spent on the affected hindlimb and a complementary increase of the swing occurred (not shown). Consistently, the transition from extension to flexion in all four joints of the left hindlimb occurred earlier in the step cycle after hemisection (Fig. 2B, left). Such a decrease and increase in stance and swing duration, respectively, was corroborated by a decrease of extensor burst duration (Fig. 4A), whereas the flexor duration increased (Fig. 4B). In addition, the asymmetrical step length (Fig. 3B) was principally due to a significant decrease in the forward placement of the left paw relative to the hip (AI < 0) (Fig. 3D).

In contrast to that in the untrained group, the walking pattern observed in the trained group was symmetrical and similar to that in the intact state (compare the vertical bars for the trained group in Fig. 3, A–C, as well as the horizontal bars in Fig. 3D). The left hindlimb locomotor pattern of the trained cats was more regular than that observed in untrained cats (see SD values in Fig. 2B) and closely resembled the pattern displayed in the intact state (Figs. 2B and 3). The duration of extensor and flexor bursts was also comparable to intact values (Fig. 4). In summary then, significant differences between groups in the step cycle structure and the left hindlimb walking pattern were shown 21 days after hemisection (Figs. 3 and 4), indicating a beneficial role of locomotor training that corrects the hemisection-induced locomotor deficits, leading to a close-to-normal locomotor pattern.

**Coupling between hindlimbs.** A major component characterizing locomotor recovery is the capacity to regain a walking pattern with a normal interlimb coupling. Because the coupling between hindlimbs is greatly affected by a spinal hemisection (Martinez et al. 2011), we evaluated the role of treadmill training in restoring this coordination between hindlimbs (kinematics and EMG in Fig. 5). In the intact state, the right hindlimb contacted the ground at phase 0.5 on the cycle of the left hindlimb in both groups (Fig. 5, A and C). Accordingly, the activity of hindlimb flexor or extensor muscles strictly alternated so that the flexor (St) or extensor (GM) muscles in a limb began to discharge at phase 0.5 of the cycle of the corresponding homologous muscles of the other limb (Fig. 5, B, D, and E). The hindlimb coupling value observed 21 days after hemisection in untrained cats indicated that the right hindlimb contacted the ground earlier than usual within the left hindlimb cycle (0.42 ± 0.05) (Fig. 5A), thus taking a greater share of the weight support than the left hindlimb. These changes in homologous coupling corresponded to changes also in EMG coupling. Indeed, the flexor Srt and extensor GM on the right side were shown to discharge significantly earlier in the cycle of the left Srt and GM, respectively (phase rSrt/lSrt: 0.42 ± 0.09; phase rGM/lGM: 0.41 ± 0.05) (Fig. 5, B, D, and E). Interestingly, the abnormal hindlimb coupling observed in the untrained group was not observed in the trained group (Fig. 5, C–E). In the trained cats, the hindlimb coupling values (0.47 ± 0.04) (Fig. 5A) and the coordination between left and right flexors and extensors (rSrt/lSrt: 0.46 ± 0.08; phase rGM/lGM: 0.46 ± 0.05) (Fig. 5, D and E) reached values similar to those in the intact state.

**Intralimb EMG coupling.** Another important component characterizing locomotor recovery is the capacity to regain a normal coupling between muscle activity within each single limb. The intralimb coupling between the representative hip and knee flexor muscles (Srt and St) was thus assessed and quantified on March 11, 2015 Downloaded from
Fig. 3. Effect of locomotor training on the step cycle structure after hemisection. Insets (left) schematically illustrate the calculation method used for cycle duration (A), step length (B), stance duration (C), and forward placements (D). Changes in the step cycle structure are shown in bar graphs (middle) for the left and right hindlimbs at the intact state and 21 days after hemisection (H21) in both groups. The mean asymmetry indexes calculated for each kinematic parameter were compared between groups at the intact state and 21 days after hemisection (far right). *P < 0.05, statistical differences between the intact and hemispinal states. &P <0.05, statistical differences between groups.
was delayed with respect to the St burst onset such that phase relations between these muscles significantly increased (phase 0.17 ± 0.06) (Fig. 6, A and B). By comparison, the Srt/St coupling remained preserved in the trained group after hemisection (Fig. 6, A and B) and was even similar to intact values.

**Summary of the comparison between trained and untrained hemisected cats.** After a hemisection on the left side, asymmetries between left and right hindlimbs occurred in several kinematic and EMG parameters that were not compensated spontaneously without locomotor training. The asymmetrical locomotor pattern resulted mainly from deficits of the left hindlimb kinematics and EMGs. By contrast, providing training after hemisection had, first, a beneficial effect on the quality of the left hindlimb locomotor recovery. Indeed, the normal intrinsic step cycle structure was preserved as early as 3 wk after the incomplete spinal lesion. Second, training maintained a symmetrical coupling between both hindlimbs after hemisection. Despite a larger lesion size on average (Fig. 2) in trained cats and more important initial left hindlimb deficits compared with the untrained cats, the kinematic and EMG values reported after a 3-wk period of training reached values similar to those in the control state.

**Training Promotes Intraspinal Changes After Incomplete SCI**

Three weeks after hemisection, a complete spinalization was performed to remove all supraspinal inputs from the spinal locomotor circuits and evaluate the spinal locomotor capacity of cats that had been treadmill trained or not during the hemisected period. It was reasoned that the locomotor capacity after spinalization could be indicative of intraspinal changes that occurred in the lumbosacral cord when cats were recovering locomotion after hemisection and could potentially suggest that training during that period makes a difference in the quality of the purely spinal locomotion.

Training promotes the reexpression of bilateral walking after spinalization. After spinalization, locomotion was elicited by weak stimulation of the perineum (light touch or bending the proximal tail) or stronger stimulation (constant pressure or light pinch) when necessary. In untrained cats, the locomotor pattern displayed 1 day after spinalization was variable, as previously reported (Martinez et al. 2011). Although 5 of the cats could walk with both hindlimbs (i.e., 60%), 2 cats displayed a unilateral locomotion on the side of the previous hemisection and another cat was not able to walk at all (Table 1). By contrast, in the present study, 100% of trained cats could reexpress a bilateral hindlimb stepping (Fig. 7A, Table 1). More interestingly, the walking pattern displayed by the hindlimb previously impacted by the hemisection was still deficient in untrained cats, whereas it was well organized in trained cats (Fig. 7B, Table 1). As assessed by measuring the angular excursion of the four hindlimb joints, the magnitude of angular excursion was clearly higher in trained cats than in untrained cats (Fig. 7B), and their locomotor pattern was more regular (Fig. 7B, compare SD values for untrained and trained groups). The maximal speed reached by the trained group on the first spinal day was significantly higher than that of the untrained group (Fig. 7A). In addition, the perineal stimulation required to elicit locomotion was of weaker intensity for the majority of trained cats compared with that for untrained cats, suggesting a greater excitability of the spinal locomotor circuitry in trained cats compared with untrained cats (Table 1).

**Beneficial effect of training on the quality of hindlimb locomotion after spinalization.** In the 5/8 untrained cats able to walk after spinalization and in the trained group, the AI values for cycle duration (Fig. 8A), step length (Fig. 8B), stance (Fig. 8C), and toe position at contact (Fig. 8D) were similar to control values. However, despite their ability to display a bilateral locomotor pattern, the untrained cats exhibited a significant paw drag and smaller joint excursion amplitude (Fig. 7B). By contrast, the quality of the left hindlimb walking pattern was clearly better and less variable in the trained cats (Fig. 7B).

The well-organized kinematic output observed in the trained cats was corroborated by the EMG coupling values. In the trained group, the coupling between hindlimbs (Fig. 9A) and the coupling between homologous flexors (Fig. 9B) and exten-

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**Fig. 4.** Effect of training on burst durations of extensors and flexors after hemisection. Shown are changes in ankle extensor (gastrocnemius lateralis and medialis, GM; A) and knee flexor (semitendinosus, St; B) burst durations (as a percentage of intact value) of the left and right hindlimbs in untrained and trained groups 21 days after hemisection. *P <0.05, statistical differences between the intact and hemisected values. &P <0.05, statistical differences between groups.
sors (Fig. 9C) remained unchanged and comparable to the intact and hemispinal state. By contrast, the EMG coupling values obtained from the untrained group after spinalization were different from those obtained in the trained group (Fig. 9, A–C). In the untrained cats, the hindlimb coupling (Fig. 9A) and EMG phase relation values (Fig. 9, B and C) obtained after spinalization shifted compared with those observed after hemisection.

In addition, the intralimb coupling phase values between the hip and knee flexor muscles (Srt and St) were shown to differ between groups after spinalization on the left (Fig. 10A) and right side (Fig. 10B). Whereas the Srt/St coupling remained unchanged on both sides and comparable to the intact and hemispinal state in the trained group, changes between the third hemispinal week and the first spinal day occurred in the untrained group (Fig. 10A and B). In the untrained cats, the hip flexors Srt discharged before the knee flexors St on both sides (Fig. 10A and B). This earlier initiation of the hip swing could account for the foot drag observed in both hindlimbs in the untrained cats after spinalization (Fig. 7B). Conversely, the persistence of a normal Srt/St coupling in the trained cats may have accounted for the absence of foot drag after spinalization (Fig. 7B).

Summary of spinal cats trained or untrained during the preceding hemisected period. As early as 24 h after spinalization, 100% of cats trained to locomote on a treadmill for a 3-wk period after hemisection reexpressed a bilateral and well-organized locomotor pattern at high speeds. By contrast, 60% of untrained cats reexpressed a bilateral walking pattern that remained deficient in many aspects as left hindlimb paw drag and abnormal intra- and interlimb EMG coupling resumed. More importantly, the symmetrical and coordinated locomotor pattern observed in the trained cats after hemisection was preserved after spinalization.

DISCUSSION

In the current study we examined the effect of locomotor training on locomotor recovery after a low thoracic hemisection affecting the left hindlimb, and we determined if such a
procedure induced intraspinal changes within the spinal circuitry below the hemisection. We first showed that in contrast to the untrained cats, which displayed an asymmetrical walking pattern 3 wk after hemisection, a 3-wk period of treadmill training after hemisection led to the recovery of a symmetrical walking pattern. Second, we demonstrated that the locomotor recovery induced by training after hemisection was mainly attributable to bilateral functional changes within the spinal cord, because the spinal locomotor pattern displayed by the trained cats after the remnant supraspinal inputs were suppressed remained symmetrical and coordinated, whereas those of the untrained cats remained deficient and asymmetrical.

**Lesion Size in Trained and Untrained Cats**

In the present study, trained cats exhibited better locomotor performances than untrained cats as early as 3 wk after hemisection despite larger lesions. Indeed, the lesions of the trained cats were more extensive than those of the untrained cats and were shown to damage entirely the left hemicord as

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<th>Table 1. Locomotor patterns characteristics of the left and right hindlimbs in 8 untrained and 8 trained cats assessed 24 h after spinalization</th>
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As early as 24 h after the second spinal lesion (T13), hindlimb locomotion was evaluated on the treadmill with the forelimbs placed on a fixed platform. Locomotion was elicited by weak stimulation of the perineum (light touch or bending the proximal tail) or stronger stimulation (constant pressure or light pinch) when necessary. Different types of performances were observed in the untrained cats. Five of 8 cats (~60%) demonstrated a bilateral hindlimb pattern of locomotion with plantar foot placements that could reach $\leq 0.9$ m/s, whereas 2/8 cats displayed unilateral stepping on the side of the hemisection and 1/8 cat exhibited no stepping movement. By contrast to untrained cats, 100% of trained cats displayed a bilateral hindlimb pattern of locomotion with plantar foot placements that could reach $\leq 1.1$ m/s.

Fig. 6. Effect of training on the coupling between flexor muscles after hemisection. Intralimb coupling between hip (Srt) and knee (St) flexors was calculated by using top traces of EMG recordings (A), and the Srt/St coupling values on the left (B) and right sides (C) are expressed in phase relations. Time 0 represents the Srt muscle burst onset (B and C). *P < 0.05, statistical differences between the intact and hemispinal values. &P < 0.05, statistical differences between groups.
well as part of the right hemicord, especially the gray matter. In the untrained cats, the lesions were more restricted to the left side of the cord and left part of the ventral pathways intact. Although such a difference is mainly imputable to methodological concerns in our study, it is an important factor to take into account because the lesion size is known to influence the recovery process.

It has been demonstrated that SCI severity is clearly correlated with locomotor recovery such that greater motor deficits are related to greater SCI severity as measured by biomechanical parameters and the degree of tissue sparing (Basso et al. 1995, 1996; Fehlings and Tator 1995; Kloos et al. 2005; Li et al. 2006; Martinez et al. 2009a). More specifically, the extent of spinal white matter damage is strongly related with the

Fig. 7. Effect of training on the reexpression and quality of hindlimb locomotion after spinalization. A: after spinalization, 8/8 trained cats reexpressed a bilateral hindlimb locomotion compared with 5/8 untrained cats, and the maximal speed displayed by the trained group was significantly higher than that displayed by the untrained group (\(P < 0.05\)). B: angular excursion of the 4 left hindlimb joints averaged over 20 consecutive cycles during treadmill locomotion at 0.4 m/s 24 h after spinalization in the untrained (black lines) and trained groups (gray lines). Averaged angular excursions are represented with solid lines, and the dotted lines on either side of the solid lines represent SD. Flexion always corresponds to downward deflections of the angular traces. The vertical dotted line represents the onset of ankle flexion and determines the onset of the swing phase. The vertical solid line represents the foot contact of the paw, and all the angles were synchronized on foot contact. Compared with untrained cats, which exhibited left hindlimb deficits corresponding to smaller joint excursion amplitude, the quality of the left hindlimb walking pattern observed in the trained cats after spinalization was better and less variable.

Fig. 8. Effect of training on the step cycle structure after spinalization. Shown are comparisons of the mean asymmetry indexes calculated for cycle duration (A), step length (B), stance duration (C), and toe position at contact (D) between groups 21 days after hemisection and 1 day after spinalization (Spinal 1). *\(P < 0.05\), statistical differences between the intact and hemispinal values.
number, localization, and amount of damaged spinal tracts and thus with locomotor deficits (Li et al. 2006), whereas the quantity of gray matter lost after contusion poorly correlates with locomotor deficits (Magnuson et al. 2005). In accordance, the trained cats, which exhibited initially more severe locomotor deficits, had on average a more extensive lesion size, thus suggesting that the amount of spared tissue in the untrained cats had a beneficial effect on locomotor performance. Particularly, the ventral part of the cord was completely damaged on one side in the trained cats, whereas it was partially left intact in the untrained cats.

In addition, although a larger amount of spared inputs is generally associated with a better recovery, few studies have demonstrated a negative role of preserving a larger amount of tissue on locomotor recovery and/or spinal plasticity. It has been shown that in health and disease, descending inputs are involved in shaping and remodeling the spinal cord (Chen et al. 2002, 2006, 2007; Chen and Wolpaw 2002; Wang et al. 2012) and probably play a beneficial role in locomotor recovery (Thomas and Gorassini 2005) by allowing the spinal circuitry to remain in an active state after iSCI. By contrast, some studies have also reported that the disruption of some supraspinal pathways after SCI could lead to maladaptive plasticity in spinal sensory (Ferguson et al. 2012) and reflex pathways (Tan et al. 2012), but to our knowledge, none of these studies have shown a correlation between the number of spared pathways and the occurrence of maladaptive events.

Thus, because the trained group recovered better than the untrained group, one can hypothesize that training had an important impact because it allowed cats with extensive lesions and important initial deficits to recover better with only a 3-wk training period compared with cats with smaller lesions and better initial performance.

**Training Promotes Locomotor Recovery and Intraspinal Changes After Incomplete SCI**

By comparing the locomotor pattern displayed by eight untrained and eight trained cats after hemisection and spinalization, we could hypothesize on the role of treadmill training in the locomotor recovery and the potential role of intraspinal plasticity. Our results show that in untrained cats, the hemisection on the left side led to an asymmetrical locomotor pattern that was not fully compensated 3 wk after hemisection. To compensate for the loss of descending inputs accessing to the spinal cord on the side of the lesion, several kinematic and EMG changes occurred, mainly on the side of the hemisection. To limit the load on the affected hindlimb, the untrained cats reduced their stance phase duration whereas their swing phase increased, and these kinematic changes were accompanied by corresponding changes in extensor and flexor muscles burst duration. The coupling between hindlimbs was also modified, as previously reported (Martinez et al. 2011). By contrast to findings in untrained cats, submitting cats to daily treadmill training from the 3rd to the 21st day after hemisection led to the recovery of kinematic and EMG parameters. Despite more extensive lesions and more important initial deficits, the trained cats displayed a hindlimb locomotor pattern similar to the intact state as early as 3 wk after hemisection. Despite contradictory results in the literature, improvement of locomotor recovery by training after partial lesions has been previously reported in rodents (Goldshmit et al. 2008; Martinez et al. 2009b; Singh et al. 2011; Thota et al. 2001), humans (Visintin et al. 1991), and, in only one study, in cats (Barrière et al. 2009B).

In the majority of studies, however, the mechanisms subserving experience-induced recovery are difficult to assess, especially the intraspinal changes that occur after an iSCI. By performing a complete SCI after an hemisection and comparing the locomotor pattern displayed by animals in both condi-
tions, we can assess more accurately the intraspinal changes promoted by the incomplete SCI and training.

We first showed that after the complete SCI, 100% of trained cats were able to walk with their hindlimbs at high speeds by contrast to 60% of untrained cats. This result shows that submitting cats to training after hemisection facilitates the reexpression of bilateral locomotion after the total disruption of supraspinal inputs, suggesting that the training procedure primes the spinal circuitry to “walk.” This result confirms, in a large cohort of cats, the preliminary results obtained previously (Barrière et al. 2008). In this previous study, three cats were submitted to treadmill training between a spinal hemisection and a spinalization and two cats were not trained between the two lesions. Although the present study and that of Barrière et al. (2008) are not directly comparable because the interlimb period between the lesions varied between cats and was longer than in the present study, the previous study also showed that only the three trained cats reexpressed a bilateral hindlimb pattern of locomotion after spinalization, whereas the two others expressed a unilateral stepping pattern on the side of the previous hemisection. The results previously reported by Barrière et al. (2008) and those reported in the present article in a larger cohort of cats suggest that without training, the hemisection induced a new left/right balance in the spinal circuits and that the training procedure restored or maintained this left/right balance in the spinal locomotor circuitry after hemisection. Second, we showed that despite the bilateral locomotion observed in 60% of untrained and 100% of trained cats after spinalization, the interlimb and intralimb EMG coupling was abnormal in untrained cats, whereas it was similar to control values in trained cats. This result suggests that training had participated in maintaining the spinal circuits in a functional state.

Although the reexpression of locomotion after spinalization is a strong indicator of the excitability within spinal networks, we previously showed, in untrained cats, that this could be influenced by the size of the hemisection (Martinez et al. 2011). Because our two groups of cats exhibited somewhat different lesion size more extensively affecting the ventromedial spinal quadrant, we had to compare the quality of hindlimb locomotion displayed by both groups before and after the complete SCI to evaluate the type of intraspinal changes induced by training after the hemisection.

Of great interest was the fact that despite larger hemisections, the locomotor pattern of the trained cats recovered within a short period of 3 wk after hemisection and remained unchanged after spinalization, showing that the training-related recovery observed after hemisection was mainly attributable to intraspinal changes. By contrast to the robust and well-coordinated pattern of trained cats, untrained cats exhibited locomotor deficits at the same time points despite smaller lesions. Consistent with our recent work (Martinez et al. 2011), the changes observed between left and right flexors and extensors coupling and the Srt/St intralimb coupling 3 wk after hemisection in untrained cats reversed 24 h after spinalization, suggesting that without training, the hemisection has induced a new left/right balance in the spinal circuits. By contrast, the intralimb and interlimb EMG coupling of trained cats after spinalization was comparable to intact values, demonstrating the beneficial role of treadmill training on maintaining a good left/right balance between the CPG networks.

Taken together, these results demonstrate that submitting cats to locomotor training after hemisection promotes a better locomotor recovery and that this is probably in great part due to bilateral functional changes within the spinal locomotor circuitry.

Mechanisms of Activity-Dependent Plasticity Within Spinal Networks After Incomplete SCI

After a complete SCI at low thoracic level (T13), treadmill training was shown to reactivate the lumbar locomotor networks through a reinforcement of activity-dependent sensory feedback (Barbeau and Rossignol 1987; Cote and Gossard 2004; Lovely et al. 1986; Rossignol and Frigon 2011) and thus promote locomotor recovery in the absence of supraspinal inputs. After i SCI, the situation is more complex, since supraspinal structures and the spinal cord remain partially connected through remnant descending pathways and the interactions between peripheral sensory inputs, supraspinal structures, and the spinal cord are reorganized (Martinez and Rossignol 2011; Rossignol et al. 2009). In this case, locomotor training can act on these different targets known to control and modulate locomotor activity.

In view of the early locomotor recovery observed in the trained cats after hemisection, it is unlikely that collateral sprouting, even if observable a few days after neural injury (Ballermann and Fouad 2006), is an underlying mechanism, because the time course of such a structural reorganization would not lead to the emergence of functional changes before the third week post-SCI (Fouad et al. 2001; Ghosh et al. 2010; Murray and Goldberger 1974; Weidner et al. 2001). Rather, it is well established that use-dependent activity within existing sensory and descending pathways controlling the hindlimb musculature is modified through training (Edgerton et al. 2001), leading to a strengthening of the neural pathways that sustain activation of locomotion-generating spinal circuitry. The fact that in the present study 100% of trained vs. 60% of untrained cats displayed a bilateral and well-organized walking pattern 24 h after spinalization suggests that training has promoted bilateral spinal changes and/or has maintained the spinal circuits in a functional state after hemisection. However, we currently do not know if these spinal changes are promoted by supraspinal inputs, sensory inputs, or both. In the untrained cats, the level of sensory inputs is clearly diminished after hemisection, and the fact that 60% of them could walk after spinalization suggests that changes in the spinal cord occurring during the hemisedected period are most probably largely dependent on remnant descending inputs that could imprint changes in the spinal cord. In the case of trained cats, the level of sensory, and probably supraspinal, inputs is increased after hemisection. Indeed, when training is performed after iSCI, remnant descending fibers can increase their activity to compensate for the unilateral spinal lesion (Thomas and Gorassini 2005) and can thus induce bilateral changes within spinal sensorimotor pathways. Moreover, these spinal changes can be induced by the repetitive bilateral phasic cutaneous and proprioceptive feedback provided by locomotor training, as previously described in spinal cats with or without previous hemisection (Courtine et al. 2009; Harkema et al. 1997; Martinez et al. 2012a; Rossignol et al. 2006; Rossignol and Frigon 2011). An alternative and probably concomitant phenomenon
contributing to functional recovery is the global enhancement of the spinal locomotor circuitry excitability by training. In the chronic spinal cat, studies have shown that training could act on the central pattern generator (CPG) excitability by modulating spinal cord inhibitory circuits (de Leon et al. 1999). In addition, training might induce changes in the firing threshold and conduction velocity of motoneurons [mechanisms explored in a series of studies on monosynaptic stretch reflex, which can be modulated in both animals and humans (Wang et al. 2012; Wolpaw 1997)] that might be crucial in the adaptation of the CPG excitability. Finally, neuromodulators such as monoamines or neuropeptides can influence the functional status of the CPG circuitry (Ribotta et al. 2000). As recently demonstrated, changes in the excitability of monamines receptors can be profound because these can become constitutive after SCI, which will lead to a state of hyperexcitability of motoneurons that can facilitate locomotor recovery (Harvey et al. 2006; Murray et al. 2010).

**General Conclusion**

In this study, we have provided evidence that treadmill training can promote the recovery of locomotor performances after an iSCI by promoting bilateral functional changes within the spinal locomotor circuitry. This is the first study highlighting the beneficial role of locomotor training on locomotor recovery and plasticity after iSCI in such a large cohort of cats. This is a necessary proof of principle that justifies the promotion of such a locomotor training procedure in humans with iSCI to induce objective physiological changes in the spinal cord.

**ACKNOWLEDGMENTS**

We are very grateful to H. Leblond and P. Drapeau for their competent help in this work.

**GRANTS**

This work was funded by a Canada Research Chair on the Spinal Cord, by the SensoriMotor Rehabilitation Research Team (ERRSM) as part of the Regenerative Medicine and Nanomedicine Strategic Initiative of the Canadian Institute for Health Research (CIHR), as well as by an individual grant from the CIHR to S. Rossignol. M. Martinez was funded by a postdoctoral fellowship from ERRSM and Fonds Québécois de la Recherche sur la Nature et les Technologies,

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

M.M. and S.R. conception and design of research; M.M. and H.D.-M. performed experiments; M.M. and H.D.-M. analyzed data; M.M. interpreted results of experiments; M.M. prepared figures; M.M. drafted manuscript; M.M. and S.R. edited and revised manuscript; M.M., H.D.-M., and S.R. approved final version of manuscript.

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