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

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Running Head: locomotor training after spinal lesions

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ABSTRACT

After a spinal hemisection at thoracic level in cats, the paretic hindlimb progressively recovers locomotion without treadmill training but asymmetries between hindlimbs persist for several weeks and can even be seen after a further complete spinal transection at T13. To promote optimal locomotor recovery after hemisection, such asymmetrical changes need to be corrected. In the present paper we evaluated if the locomotor deficits induced by a spinal hemisection can be corrected by locomotor training and, if yes, whether the spinal stepping after the complete spinal cord section is also more symmetrical. This would indicate that locomotor training in the hemisected period induces efficient changes in the spinal cord itself. Sixteen adult cats were first submitted to a spinal hemisection at T10. One group received 3 weeks of treadmill training while the second group did not. Detailed kinematic and electromyographic analyses showed that a 3 week-period of locomotor training was sufficient to improve the quality and symmetry of walking of the hindlimbs. Moreover, after performing the complete spinal lesion, all the trained cats re-expressed bilateral and symmetrical hindlimb locomotion within 24 hours. By contrast, the locomotor pattern of the untrained cats remained asymmetrical and the hindlimb on the side of the hemisection was still deficient. This study highlights the beneficial role of locomotor training in facilitating bilateral and symmetrical functional plastic changes within the spinal circuitry and in promoting locomotor recovery after an incomplete spinal cord injury.

Key words: locomotor training, spinal cord injury, recovery, plasticity, cat.
INTRODUCTION

Following 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; Martinez et al. 2011; Martinez and Rossignol 2011). While 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; Martinez et al. 2012a; Martinez et al. 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 10 and the recovery of locomotion was assessed for 3 weeks. 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, 2-3 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 dissymmetric foot positioning at touch down (Martinez et al. 2011). Furthermore, the asymmetrical walking pattern observed after hemisection was retained durably after suppressing all supraspinal inputs by spinalization (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 results suggest that neuroplastic changes occur not only in supraspinal structures but 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 weeks to complete spinal cats previously hemisected for 3 weeks 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 re-expressed 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.

Firstly, we found that locomotor training for a period of 3 weeks post hemisection was sufficient to correct the residual deficits of the hindlimb ipsilateral to the lesion and restore a symmetrical walking, in contrast to untrained cats. Secondly, after the complete spinalization, all the trained cats re-expressed bilateral and symmetrical hindlimb locomotion at high speeds while 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.
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 re-analyzed for the present study to compare with 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 EMG values for locomotion in the intact state, all cats underwent an iSCI consisting in a hemisection targeting the left side of the spinal cord at T10. 3 days after hemisection, a group of 8 cats was trained 7 days a week (trained Group) while a group of 8 cats was not trained but only evaluated weekly until the third week after hemisection (untrained Group) (Fig.1). The rest of the time the cats were confined to their individual cage (104 x 76 x 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. 3 segments below the hemisection. Following the complete spinal lesions, foam mattresses were placed in the cages and cats were attended to 1-2 times per day to clean the head connectors, manually express the bladder, and clean the hindquarters when necessary. Hindlimb locomotion was evaluated within 24 hours post-spinalization.

Surgical procedures

All surgical procedures for electrode implantation or spinal lesions were done under general anaesthesia and aseptic conditions as in previous works. Animals were first subcutaneously injected with an analgesic (Anafen 2mg/kg) and pre-medicated with Atravet 0.1mg/kg, Glycopyrrolate 0.01mg/kg, Ketamine 10 mg/kg; intramuscular administration. An endotracheal tube was then inserted to provide gaseous anaesthesia (Isoflurane 2% in a mixture of 95% O₂ / 5% CO₂). Cats were chronically implanted with intramuscular electrodes to record electromyographic activity (EMG) from flexor and extensor hindlimb muscles on both sides. The implanted muscles were: Sartorius (Srt, hip flexor and knee extensor), Semitendinosus (St, knee flexor and hip extensor), Vastus Lateralis (VL, knee extensor), Gastrocnemius Lateralis and Medialis (GL, GM, ankle
extensors and knee flexors) and Tibialis Anterior (TA, ankle flexor). Electrodes were led subcutaneously to two 15 pin-head 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 micro-scissors, as in previous studies (Martinez et al. 2011; Martinez et al. 2012a; Martinez et al. 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 (25µg/h) was applied on the skin to reduce alleviate pain for about 5 days.

**Training procedure**

After hemisection, 8 cats were trained to locomote 30 minutes per day at the speed of 0.4 m/s from the 3rd to the 21st day after spinalization (trained Group) while the locomotor performances of the 8 other 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 lift off on video images. The amplified (Neuralynx, Lynx-8 amplifiers) and filtered (bandwidth 100 Hz to 3 kHz) EMG signals were digitized at 1 kHz (National Instruments, NI-6071E) 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 (Fig. 3A) whereas the stance duration refers to the time between foot contact and toe off which initiates the swing phase (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 (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 (Fig. 3D). This measure indicates the extent of forward and backward movements during a locomotor episode.

*Evaluation of the left/right asymmetries.* 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; Martinez et al. 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 while 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: \((\text{average left} - \text{right}) / (\text{average left} + \text{right})\). An AI of 0 indicates a perfect symmetry and in normal conditions, the AIs obtained in all parameters are equal to 0 ± 0.05 (see for instance Fig. 3 right panels). 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 (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 (Fig. 5C-D).

**EMG coupling.** The left semitendinosus (St, knee flexor and hip extensor), which usually discharges with a sharp burst at the end of stance or beginning of swing, was taken as a reference to measure the onset of different muscles’ bursts. 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 (Fig. 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 (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 (Fig. 2A).

**Statistics**

Linear and circular statistical analyses were performed using PASW (PASW Statistics 18.0) and Oriana (3.13, KCS, UK) software in 16 cats for kinematic parameters and 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 thus compared the performances of the two groups of cats over time i.e. at the intact state, 3 weeks after hemisection (hemispinal 21) and 24 hours after spinalization (spinal 1) 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). In all figures, statistical significance between groups is indicated by &. Statistical significance
between delays is indicated by *. A p value of < 0.05 was considered statistically significant. Results are presented as means ± standard deviation (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 8 untrained cats. In the trained cats, the hemisections were somewhat more extensive and damaged the most ventro-medial 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 examining the histology of this group, 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 grey matter on the right side. Comparisons of the 2 groups with regards to the percentage of total damaged area (Fig. 2A), including the white and grey matters as well as cavitations, indicated 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 while 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 re-expressed as early as one week 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 two weeks, 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 post hemisection, 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-week training period so that their locomotor pattern at 3 weeks post lesion 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 weeks 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 while the left has lost most of its supraspinal inputs thus inducing an asymmetrical control of locomotion. This can be quantified by calculating an asymmetry index (AI) which compares various locomotor parameters on the left and right side. As 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. that the step length, cycle, stance and swing durations are similar on the left and right side (black rectangles in Fig. 3A-D). In this case, the asymmetry index which compares the left and right hindlimb over several mean kinematic values is $0 \pm 0.05$ (Fig. 3, right panels). Without training after the hemisection, the left hindlimb exhibited several deficits (Fig. 2B and 3, see left hindlimb) while the right hindlimb showed little changes (Fig. 3, see right hindlimb). Three weeks after the hemisection, the cycle duration remained more or less equal on both sides (Fig. 3A) while the step length became asymmetric ($AI < 0$, $p < 0.05$) (Fig. 3B, see *) 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, see *) 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 panel). Such a decrease and increase in stance and swing duration respectively was corroborated by a decrease of extensor burst duration (Fig. 4A, see *) while that of the flexor duration increased (Fig. 4B, see *). 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, see *).

In contrast to the untrained Group, the walking pattern observed in the trained Group was symmetrical and similar to the intact state (compare the vertical bars for the trained group in Fig. 3A, B and 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 (Fig. 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 (Fig. 3-4, see &) indicating a beneficial of locomotor training which 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. As the coupling between hindlimbs is greatly affected by a spinal hemisection (Martinez et al. 2011), we thus 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. 5A 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. 5B, 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. 5B, D and E). Interestingly, the abnormal hindlimb coupling observed in the untrained Group was not observed in the trained Group (Fig. 5C-E, see &). 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. 5D-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 using phase values (Fig. 6). Normally, Srt begins to discharge after the onset of St at around phase 0.06 of the St step cycle (Fig. 6A). This ensures that the knee is flexed before the hip and that the foot has left the ground before the limb moves.
forward. Three weeks after hemisection, changes in the coupling of flexor muscles were observed on the left side in the untrained Group (Fig. 6A-B) while the right hindlimb flexors coupling remained unchanged by comparison to the intact state (Fig. 6C). The left Srt burst onset of the untrained Group was delayed with respect to the St burst onset such that phase relations between these muscles significantly increased (phase 0.17 ± 0.06) (Fig. 6A-B). By comparison, the Srt/St coupling remained preserved in the trained Group after hemisection (Fig. 6A-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 firstly 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 weeks after the incomplete spinal lesion. Secondly, training maintained a symmetrical coupling between both hindlimbs after hemisection. Despite a larger lesion size on the average (Fig. 2) in trained cats and more important initial left hindlimb deficits compared to the untrained cats, the kinematic and EMG values reported after a three week 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 studying 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 potentially suggest that training during that period makes a difference in the quality of the purely spinal locomotion.
Training promotes the re-expression 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 one day after spinalization was variable, as previously reported (Martinez et al. 2011). While 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 re-express 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 while 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, see &). In addition, the perineal stimulation required to elicit locomotion was of weaker intensity for the majority of trained cats by comparison to untrained cats suggesting a greater excitability of the spinal locomotor circuitry in trained cats by comparison to 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 AIs 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 with the EMG coupling values. In the trained Group, the coupling between hindlimbs (Fig. 9A) and the coupling between homologous flexors (Fig. 9B) and extensors (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. 9A-C, see &). In the untrained cats, the hindlimb coupling (Fig. 9A, see *) and EMG phase relations (Fig. 9B-C, see *) values obtained after spinalization shifted by comparison to that 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, see &) and right (Fig. 10B, see &) side. 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-B, see *). In the untrained cats, the hip flexors Srt discharged before the knee flexors St on both sides (Fig. 10A-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 account 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 hours after spinalization, 100% of cats trained to locomote on a treadmill for a 3 week period after hemisection re-expressed a bilateral and well organized locomotor pattern at high speeds. By contrast, 60% of untrained cats re-expressed a bilateral walking pattern which 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 evaluated 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 weeks after hemisection, a three week 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 suppressing the remnant supraspinal inputs remained symmetrical and coordinated while that 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 weeks after hemisection despite larger lesions. Indeed, the lesions of the trained cats were more extensive than the untrained cats and were shown to damage entirely the left hemicord as well as part of the right hemicord, especially the grey matter. In the untrained cats, the lesions were more restricted to the left side of the cord and left intact part of the ventral pathways. While 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; Basso et al. 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 number, localization, and amount of damaged spinal tracts and thus with locomotor deficits (Li et al. 2006) while 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 while it was partially left intact in the untrained cats.

In addition, while a larger amount of spared inputs is generally associated with a better recovery, little 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; Chen et al. 2006; Chen et al. 2007; Chen and Wolpaw 2002; Wang et al. 2012) and probably play a beneficial role on locomotor recovery (Thomas and Gorassini 2005) by allowing the spinal circuitry to remain in an active state after incomplete SCI. 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, as the trained group recovered better than 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 week training period compared to 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 8 untrained and 8 trained cats after hemisection and spinalization, we could hypothesize on the role of treadmill training on 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 weeks 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 while 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 that observed in untrained cats, submitting cats to daily treadmill training from the 3rd to the 21st day after hemisection lead to the recovery of kinematic and EMG parameters. In spite of 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 weeks after hemisection. Despite contradictory results in the literature, improvement of locomotor recovery by training after partial lesions has been previously reported in rodents (Goldshmidt 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. 2008). In the majority of studies however, the mechanisms subserving experience-induced recovery are difficult to assess and especially the intraspinal changes that occur after an incomplete SCI. By performing a complete SCI after an hemisection and comparing the locomotor pattern displayed by animals in both conditions, 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 the 60% of untrained cats. This result shows that submitting cats to training after hemisection facilitates the re-expression 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, 3 cats were submitted to treadmill training between a spinal hemisection and a spinalization and 2 cats were not trained between the two lesions. While the present study and that of Barrière at al. (2008) are not directly comparable because the interim period between the lesions varied between cats and was longer than in the present study, it also showed that only the 3 trained cats re-expressed a bilateral hindlimb pattern of locomotion after spinalization while the 2 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 herein 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 while 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 re-expression 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). As our two groups of cats exhibited somewhat different lesion size affecting more the ventro-medial 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 weeks after hemisection and remained unchanged after spinalization showing that the training-related recovery observed after hemisection was mainly imputable 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. Consistently 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 weeks after hemisection in untrained cats reversed 24 hours 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 incomplete 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, as 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 versus 60% of untrained cats displayed a bilateral and well organized walking pattern 24 hours 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, 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 hemisected 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 of supraspinal inputs is increased after hemisection. Indeed, when training is performed after incomplete SCI, 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 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 both in animals and humans (Wang et al. 2012; Wolpaw 1997)- 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 monoamines receptors can be profound as 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 provide evidence that treadmill training can promote the recovery of locomotor performances after an incomplete SCI 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 a so
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.

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DISCLOSURES

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

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**FIGURE LEGENDS**

**Figure 1. Experimental paradigm.** After control recording sessions (n = 2-3) to obtain baseline kinematic values for locomotion in the intact state, 16 cats underwent a hemisection targeting the left side of the spinal cord at T10. Three days after hemisection, 8 of the 16 cats were trained to locomote 30 minutes per day until the 21st day (trained Group) while the locomotor performance of the 8 other cats was only evaluated once a week to prevent any training effect (untrained Group). 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. From the second hemispinal week, cats were trained to walk with all four limbs by providing, when needed for some of the cats, perineal stimulation and assistance in body equilibrium. Three weeks after the hemisection, a complete transection of the spinal cord (i.e. spinalization) was performed at T13, i.e. 3 segments below the hemisection and removed all remaining supraspinal inputs to the spinal locomotor circuitry. The hindlimb locomotor performance of both groups was evaluated 24 hours after spinalization. Day 0 (d0) refers to the day of the hemisection and serves as reference to illustrate other experimental time points. L, left; R, right.

Figure 2. Comparison of the partial lesion size and left hindlimb locomotor pattern between groups. A: schematic drawings of the largest and smallest hemisections reported in all cats (in grey respectively bordered with black and grey dotted lines) and comparisons of the extent (in %) of the total damaged area in the 2 groups. Statistical differences between groups are indicated by the symbol &. B: angular excursion of the four left hindlimb joints averaged over 20 consecutive cycles during treadmill locomotion at 0.4 m/s. in the intact state (dotted lines) and 21 days after hemisection (solid lines) in the untrained (left panel) and trained (right panel) groups. Averaged angular excursions are represented with solid lines and the dotted lines on either side of the solid lines represent the standard deviation. 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 are synchronized on foot contact. By comparison to the untrained cats which exhibited left hindlimb deficits corresponding to a precocious transition from extension to flexion in the four left hindlimb joints in the step cycle after hemisection, the left hindlimb walking pattern observed in the trained cats after spinalization was close to intact values and was less variable.

Figure 3. Effect of locomotor training on the step cycle structure after hemisection. The first panel schematically illustrates 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 the left (first panel) and right (second panel) hindlimbs at the intact state and 21 days after hemisection (H21) in both groups. The mean asymmetry indices calculated for each kinematic parameter were compared between groups at the intact state and 21 days after hemisection (fourth panel). Statistical differences between the intact and hemispinal states are indicated by the symbol *\. Statistical differences between groups are indicated by the symbol &\. 

**Figure 4. Effect of training on extensors’ and flexors’ bursts duration after hemisection.** Changes in ankle extensor (GM) \((A)\) and knee flexor (St) \((B)\) bursts duration (in percentage of intact value) of the left and right hindlimbs in untrained and trained groups 21 days after hemisection. Statistical differences between intact and hemispinal values are indicated by *\. Statistical differences between groups are indicated by the symbol &\. 

**Figure 5. Effect of training on the coupling between hindlimbs after hemisection.** Interlimb coupling was calculated by using kinematic events (hindlimb contact and step cycle) \((A)\) or top traces of EMG recordings in homologous muscles \((B)\). Mean coupling between hindlimbs and between left and right Srt and GM muscles are expressed in phase relations where 1 represents the full step cycle \((C, D \text{ and } E\) respectively) and are compared between groups at the intact state and 21 days after hemisection (H21). Time 0 represents the right hindlimb contact \((C)\) or the right muscle burst onset \((D-E)\). Statistical differences between intact and hemispinal values are indicated by *\. Statistical differences between groups are indicated by the symbol &\. 

**Figure 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 \((C)\) side are expressed in phase relations. Time 0 represents the Srt muscle burst onset \((B-C)\).
Statistical differences between intact and hemispinal values are indicated by *. Statistical differences between groups are indicated by the symbol &. H21: hemispinal 21 days.

**Figure 7. Effect of training on the re-expression and quality of hindlimb locomotion after spinalization.** *A*: after spinalization, 8/8 trained cats re-expressed a bilateral hindlimb locomotion by comparison to 5/8 untrained cats and the maximal speed displayed by the trained Group was significantly higher than that of the untrained Group (see &). *B*: angular excursion of the four left hindlimb joints averaged over 20 consecutive cycles during treadmill locomotion at 0.4 m/s. 24 hours after spinalization in the untrained (black lines) and trained (grey lines) groups. Averaged angular excursions are represented with solid lines and the dotted lines on either side of the solid lines represent the standard deviation. 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. By comparison to 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.

**Figure 8. Effect of training on the step cycle structure after spinalization.** Comparison of the mean asymmetry indices calculated for cycle duration (*A*), step length (*B*), stance duration (*C*) and toe position at contact (*D*) between groups 21 days after hemisection (H21) and one day after spinalization (Spinal 1). Statistical differences between hemispinal and spinal values are indicated by *.

**Figure 9. Effect of training on the coupling between hindlimbs after spinalization.** Comparison of the coupling between hindlimbs (*A*) and the coupling between homologous flexors Srt (*B*) and extensors GM (*C*) between groups 21 days after hemisection (H21) and one day after spinalization (Spinal 1). Statistical differences between hemispinal and spinal
values are indicated by *. Statistical differences between groups are indicated by the symbol &.

**Figure 10. Effect of training on the coupling between flexor muscles after spinalization.** Comparison of the Srt/St coupling on the left (A) and right (B) side between groups 21 days after hemisection (H21) and one day after spinalization (Spinal 1). Statistical differences between hemispinal and spinal values are indicated by *. Statistical differences between groups are indicated by the symbol &.

**TABLES**

**Table 1. Locomotor patterns characteristics of the left and right hindlimbs in 8 untrained and 8 trained cats assessed 24 hours after spinalization.** As early as 24 hours 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. 5/8 cats (about 60%) demonstrated a bilateral hindlimb pattern of locomotion with plantar foot placements that could reach up to 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 that observed in untrained cats, 100% of trained cats displayed a bilateral hindlimb pattern of locomotion with plantar foot placements that could reach up to 1.1 m/s.
**Figure 1**

- **Hemisection - T10**
  - **Left (L) - Right (R) Control**
  - **NO TREADMILL TRAINING**
  - Untrained Group

- **Complete section - T13**
  - **Left (L) - Right (R) Control**
  - **DAILY TREADMILL TRAINING**
  - Trained Group

- **Time Points**
  - d0, d21, d22
  - d3: Hindlimb training
  - d7: Quadrupedal training

- **n = 8**

---

The diagram illustrates the experimental setup involving hemisection or complete section at T10 or T13 respectively, with a control group not undergoing treadmill training. The trained group undergoes daily treadmill training. The timeline includes specific days for hindlimb and quadrupedal training.
Figure 2

A

Untrained Group  
Trained Group

---

Minimal extent of the lesion
Maximal extent of the lesion

---

B

Unintimated Group  
Trained Group

Joint angle degrees

Intact  
Hemispinal 21 days - Untrained Group  
Hemispinal 21 days - Trained Group

Foot contact

Phase of step cycle

Hip  
Knee  
Ankle  
MTP

Extension  
Flexion

0 12

Intact  
Hemispinal 21 days - Untrained Group  
Hemispinal 21 days - Trained Group

Stance  
Swing

0 1

0 2
Figure 3
Figure 4

A. Extensor burst duration (GM)

B. Flexor burst duration (St)
Figure 5

A. Coupling between hindlimbs

Intact

Untrained cat
Hemispinal 21

Trained cat
Hemispinal 21

B. Coupling between hip flexors Srt

C. Right contact

D. Right Srt burst onset

E. Right GM burst onset

Alternating coupling

Figure 5
Figure 6
A  Re-expression of locomotion after spinalization

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B  Angular excursion of the four left hindlimb joints

---

Figure 7
Figure 8
Figure 9
Figure 10
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<th>Plantar foot placement</th>
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**Table 1**

Locomotor patterns characteristics of the left and right hindlimbs in 8 untrained and 8 trained cats assessed 24 hours after spinalization.