Recovery of hindlimb locomotion after incomplete spinal cord injury in the cat involves spontaneous compensatory changes within the spinal locomotor circuitry

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Martinez M, Delivet-Mongrain H, Leblond H, Rossignol S. Recovery of hindlimb locomotion after incomplete spinal cord injury in the cat involves spontaneous compensatory changes within the spinal locomotor circuitry. J Neurophysiol 106: 1969–1984, 2011. First published July 20, 2011; doi:10.1152/jn.00368.2011.—After incomplete spinal cord injury (SCI), compensatory changes occur throughout the whole neuraxis, including the spinal cord below the lesion, as suggested by previous experiments using a dual SCI paradigm. Indeed, cats submitted to a lateral spinal hemisection at T10-T11 and trained on a treadmill for 3–14 wk re-expressed bilateral hindlimb locomotion as soon as 24 h after spinalization, a process that normally takes 2–3 wk when a complete spinalization is performed without a prior hemisection. In this study, we wanted to ascertain whether similar effects could occur spontaneously without training between the two SCIs and within a short period of 3 wk in 11 cats. One day after the complete spinalization, 9 of the 11 cats were able to re-express hindlimb locomotion either bilaterally (n = 6) or unilaterally on the side of the previous hemisection (n = 3). In these 9 cats, the hindlimb on the side of the previous hemisection (left hindlimb) performed better than the right side in contrast to that observed during the hemispinal period itself. Cats re-expressing the best bilateral hindlimb locomotion after spinalization had the largest initial hemisection and the most prominent locomotor deficits after this first SCI. These results provide evidence that 1) marked reorganization of the spinal locomotor circuitry can occur without specific locomotor training and within a short period of 3 wk; 2) the spinal cord can reorganize in a more or less symmetrical way; and 3) the ability to walk after spinalization depends on the degree of deficits and adaptation observed in the hemispinal period.

IN ADULT CATS, bilateral hindlimb locomotion with plantar foot contact recovers 2–3 wk after a complete T13 spinal cord injury (SCI) (Barbeau and Rossignol 1987). Since there are no longer descending command signals, the locomotor recovery depends unequivocally on the re-expression of a hardwired spinal circuit termed the spinal locomotor pattern generator (Grillner 1973). After such complete SCI, several endogenous factors must contribute to this ability to re-express locomotion, namely, changes in spinal circuits and neurotransmitters (Fouad et al. 2010; Giroux et al. 1999; Murray et al. 2010; Rossignol and Frigon 2011). Locomotor training on a treadmill over 2–3 wk facilitates this re-expression, probably by providing necessary sensory cues to entrain and adapt the spinal circuits. A fundamental question in the field of locomotor recovery after SCI is to know to what extent the same endogenous spinal mechanisms are implicated in the recovery of locomotion after partial SCI. After partial SCI, we can postulate that changes occur at different spinal and supraspinal levels, but it is virtually impossible to identify their separate contributions to recovery. To study this question, we developed a dual spinal lesion paradigm allowing us to identify specific changes occurring at the spinal level itself. In this paradigm, a first partial SCI was performed at thoracic level (T10-T11) and cats were trained to walk on a treadmill for 3–14 wk until their locomotor performance reached a plateau. A subsequent, but this time complete, SCI was then performed two to three spinal segments lower at T13, i.e., at the level of lesions studied in previous experiments on spinal cats (Barbeau and Rossignol 1987; Belanger et al. 1996). Cats submitted to treadmill training between the partial and the complete sections were able to express organized bilateral hindlimb locomotion at speeds up to 0.8 m/s only 24 h after this complete spinalization (Barrière et al. 2008, 2010). As mentioned earlier, this level of locomotor performance usually takes 2–3 wk to occur when a complete spinalization is performed at T13. We concluded that after a partial spinal cord lesion, the recovery of hindlimb locomotion must depend not only on remnant descending inputs but also on changes occurring within the spinal circuits below the lesion, since the complete spinalization revealed the unusual ability of the spinal cat to walk with the hindlimbs at high speed almost immediately after spinalization.

Although we have shown some changes occurring in reflex pathways in this dual lesion paradigm (Frigon et al. 2009), it seemed important to clarify the conditions needed to induce such plastic changes in the spinal cord after a partial SCI. Since the expression of numerous plastic mechanisms can depend on a high level of maintained activity within the neural networks (Fouad and Pearson 2004; Lyskey et al. 2008; Martinez et al. 2009; Thomas and Gorassini 2005), we first asked whether locomotor training was specifically needed to induce this spinal reorganization and, second, whether such changes occur when the delay between the two lesions is short.

In the present study, we used a cohort of 11 cats. First, the interval between the two lesions was limited to only 2 wk, in contrast to the 3- to 14-wk period in our previous work.
(Barrière et al. 2008). We felt that this was the minimal interval needed to obtain good locomotor recovery and to keep the stable electromyography (EMG) signals needed to make reliable comparisons between EMG activity patterns in various states (normal, hemispinal, and spinal), which we were unable to do in previous studies (Barrière et al. 2010). Second, the animals were not trained on the treadmill between the two lesions, although the cats were free to move about in their cages as was the case in our previous studies (Barrière et al. 2008), but were evaluated on the treadmill only once a week. The absence of specific training allowed us to determine the importance of spontaneous intrinsic mechanisms without added extrinsic inputs such as that provided by locomotor training.

The first part of our present study quantifies the kinematic and EMG changes occurring during the 3-wk period between the two spinal lesions. The reason for such detailed study after the partial lesion was to better understand the subsequent locomotor capacity of the cats after the complete spinal lesion. We hypothesized that some aspects of the locomotor behavior documented in the interim period between the two spinal lesions might reflect some of the locomotor capacity found after spinalization as was suspected in the work on reflex changes in similar preparations (Frigon et al. 2009).

The second part of the study deals with the spinal locomotor behavior observed 24 h after the complete spinalization. We needed to know how many of the 11 untrained cats would be able to express locomotion very soon after complete spinalization. We limited our investigation to the first 24 h after the second lesion to remain as close as possible to the state of the spinal cord immediately prior to the second lesion. This work has been presented in abstract form (Martinez et al. 2010).

MATERIAL AND METHODS

General Outline of the Dual Lesion Paradigm

Adult female (n = 8) and male cats (n = 3) weighing from 2.5 to 4.8 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). Thereafter, all cats were engaged in a dual lesion paradigm, as illustrated in Fig. 1A. After control recording sessions (n = 2–3) to obtain baseline EMG and kinematic values for locomotion in the intact state, all cats underwent a hemisection targeting the left side of the spinal cord at T10 (Fig. 1, A and B). To limit the treadmill training effects after spinal hemisection, the treadmill locomotor performances were assessed once a week in all cats for a period of 15–20 min to record EMG and kinematics during quadrupedal walking at different speeds (0.3–1 m/s). For the rest of the time, the cats were confined to their cages. Three weeks after the first partial spinal lesion, a complete transection (i.e., spinalization) of the spinal cord was conducted at T13, i.e., three segments below the hemisection (Fig. 1A). In all spinal cats, hindlimb locomotion was evaluated within 24 h postspinalization while the forelimbs

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Fig. 1. Experimental paradigm and extent of the partial lesion. A: sequence of events used in all 11 cats. Kinematic and electromyography (EMG) recordings were performed in the intact state, once a week after the left thoracic hemisection during a 3-wk period, and finally 24 h after the complete spinal section. Day 0 refers to the day of the hemisection and serves as a reference to illustrate other experimental time points. B: schematic drawings of the hemisections at T10 in individual cats. The damaged tissue is represented in gray and cavitations in black. D, dorsal; V, ventral; L, left; R, right.

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were housed in individual cages (104 for the Care and Use of Experimental Animals. The well-being of the Committee at the Université de Montréal, according to the Canadian Guide
Animal Care

were kept on a stationary platform fixed at about 2 cm above the treadmill.

Animal Care

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. The well-being of the cats was monitored daily and verified regularly by a veterinarian. Cats were housed in individual cages (104 × 76 × 94 cm) with food and water, as in the previous studies (Barrière et al. 2008, 2010). Such housing limits sensorimotor experience and self-training. After the spinal lesions, foam mattresses were placed in the cages and cats were attended to one to two times per day to clean the head connectors, manually express the bladder, and clean the hindquarters when necessary.

Surgical Procedures

All the surgery procedures for electrode implantation or spinal lesions were similar to those described in previous works (Barrière et al. 2008, 2010). With the exception of cat JCA6, all cats were chronically implanted with intramuscular electrodes 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 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-pinhead connectors secured to the cranium using acrylic cement.

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 hard disk. Video images were deinterlaced 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 foot contacts on the treadmill, whereas stance duration refers to the time between foot contact and toe off, which defined swing onset. 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 hindlimb backward movement.

Step length was calculated using stance onset as reference. 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.

Footfall pattern was established by determining successive paw contacts of each limb for several step cycles and plotted as duty cycles. Duty cycles represent the stance and swing phases of each limb graphically.

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 inset, Fig. 4C). This measure determines the quality of forward and backward movements during a locomotor episode.

Homolateral phase coupling is calculated from the time of foot contact of the forelimb and hindlimb on the same side (right or left) expressed in phase value. This measure is expressed as phase values (i.e., 0 to 1) and was calculated as the time between hindlimb and forelimb contacts on the left or right sides divided by the step cycle period of the left and right sides, respectively. For example, in a locomotor cycle, the left homolateral coupling refers to the time point at which the left forelimb contacts the ground with respect to the left hindlimb cycle period. During a locomotor episode containing 12–20 cycles, the mean phase value was calculated. The mean phase values obtained at each delay were then plotted over time, and circular statistics (see below) were used to establish changes of homolateral coupling on the left or right side over time. With this method, a 0.5 phase value indicates a perfect alternating coupling between fore- and hindlimb, whereas a 0 value indicates that the fore- and hindlimb contact the ground at the same time. Since the forelimbs were kept on a stationary platform after spinalization, this measure was only performed during the hemispi nal period.

Homologous phase coupling of the hindlimbs or the forelimbs is calculated from the time of the left and right contact of the limbs of the brachial (forelimbs) and pelvic (hindlimbs) girdles expressed as a phase value of the cycle period of the respective girdle. For example, homologous coupling between hindlimbs represents the time between hindlimb contacts divided by the step cycle period of the left hindlimb. The coupling was expressed as a phase value of the left limb cycle.

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.

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. Because the St cycle duration can vary after spinal lesions, two types of analyses were performed to investigate the EMG coupling. We evaluated the coupling between the consecutive onsets of muscles measured as the time (in ms) between the onset of activity in successive muscles both with and without the changes in St cycle duration taken into account. The phase relations between two muscles were calculated as the time between the consecutive onsets of muscles divided by the cycle period of the reference muscle. 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.

Changes in homolateral, homologous phase coupling and EMG phase relations over time are all expressed as phase values (i.e., 0 to 1) and are thus illustrated by polar representations. It is important to note that in these graphs (see for instance Fig. 3), the size of the circles represents the standard deviation (SD) of mean values and is proportional to the diameter of each circle, which represents each delay. To evaluate the changes occurring after hemisection and spinalization, the delays were plotted on the same polar representation over time. The intact state is represented in the center of the polar representation, and the other time points are depicted on parallel circles with increasing diameter over time. A similar SD value will thus automatically appear larger with increasing distance from the origin, a distance proportional to the number of weeks after injury. Therefore, an increase in size of the SD representation in polar graphs does not mean an increase in variability (larger SD). Conversely, a decrease in the size of the SD with time actually indicates a really significant reduction in variability of the parameters evaluated over time. In Fig. 3A, an identical hypothetical SD value of 0.05 is plotted as gray circles to illustrate that a similar SD appears larger as a function of the
postoperative time, since time progresses from the center to the periphery of the polar plot.

**Histology**

Three weeks after spinalization, animals were given a lethal dose of intravenous pentobarbital sodium solution. A spinal cord segment between T8 and L1 was carefully dissected out and fixed in 10% paraformaldehyde for several weeks. The blocks were cryoprotected by successive transfers into increasing sucrose concentrations (10, 20, and 30%) in 0.1 M phosphate buffer for 72 h at 4°C. The spinal cord was frozen, and 40-μm-thick coronal sections of a spinal cord centered on the lesion were taken for histological examination. Every section was mounted on a slide and stained with cresyl violet (Fig. 1A). The coronal sections were examined under a microscope, and the total damaged area as well as the damaged white matter area in each funiculus (dorsal, lateral, and ventral for each side) were evaluated (see Fig. 9).

**Statistical Analysis**

Linear and circular statistical analyses were performed using PASW (PASW Statistics 18.0) and Oriana software (3.13; KCS, Isle of Anglesey, UK) in 11 cats for kinematic parameters and in 10 cats for EMG analyses (except for cat JCA6, which was not implanted with EMG electrodes). We first evaluated the effects of the initial partial lesion (postoperative time) on kinematic and EMG parameters by using a one-way ANOVA for linear values and a Watson-Wheeler F-test for circular values. The ANOVA was supplemented with EMG analyses (except for cat JCA6, which was not implanted with EMG electrodes). We first evaluated the effects of the initial partial lesion (postoperative time) on kinematic and EMG parameters by using ANOVA. In all figures, statistical significance between intact and postoperative values is indicated by asterisks. Since 6/11 cats (including cat JCA6) recovered bilateral walking within 24 h, their data were compared with control values as well as the values obtained 21 days after hemisection (i.e., the day before spinalization) using ANOVA. In all figures, statistical differences between the first spinal day and intact state are indicated by asterisks and between the first spinal day and the last hemisplinal day by the symbol #. Since we were interested in evaluating the asymmetries between the left and right hindlimb after hemisection and spinalization, unpaired t-tests were used to compare the kinematic and EMG parameters of the right and left hindlimbs at each pre- and postoperative time. In all figures, these statistically significant differences are indicated by the symbol $. After spinalization, we grouped cats as a function of their locomotor performance and compared their kinematic and EMG performance at each time point using unpaired t-tests. A P value <0.05 was considered statistically significant. Results are means ± SD.

**RESULTS**

**Extent of the Spinal Lesions**

In all cats, we targeted the left side of the spinal cord, and although somewhat variable between cats, the extent of the partial lesion was clearly defined to the left side and always involved a significant portion of the left hemi cord (Fig. 1B). The damaged tissue (gray), including the cavitations (black), represented 40.5 ± 6.3% of the total spinal cord area. In all cases, the lesions spared the most medial part of the left ventral funiculus containing the reticuloo- vestibulospinal fibers, as well as uncrossed corticospinal fibers from the motor cortex ipsilateral to the hemisection. In cats DLE6, DLT1, DLT5, DLE3, DLE4, and DLT4, the dorsal column on the right side was partially damaged, but the right lateral and ventrolateral quadrants were unaffected, indicating the integrity of the corticospinal and rubrospinal tracts on the right side.

**Overview of the Locomotor Recovery in the Dual Lesion Paradigm**

Changes in the locomotor pattern and EMG parameters were quantified in all cats at the same interval (Figs. 1A and 2). After the first hemispinal lesion on the left side at T10, the left hindlimb was initially paretic in all cats for 3–4 days. One week after hemisection (i.e., the first testing session after the hemisection), all cats re-expressed voluntary quadrupedal locomotion despite limping of the hindlimb and inconsistent plantar foot contact on the side of the lesion. No perineal stimulation was necessary to elicit locomotion, but assistance in body equilibrium was provided by holding the tail when needed. Within 2 wk, cats recovered support of the hindquarters, but most exhibited deficits in lateral stabilization throughout the 3-wk period after hemisection. Three weeks after hemisection, cats demonstrated an active hindlimb locomotor pattern on the lesion side and a recovery that gradually approached the locomotor performance of the intact state (Fig. 2, A and B). However, many deficits in EMG activity and hindlimb kinematics still persisted and will be described subsequently.

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. Different types of performances were observed (see Fig. 8 and Table 1, described later). Globally, more than one-half of the cats (6/11) demonstrated a bilateral hindlimb pattern of locomotion with plantar foot placements (as for cat DLT5, Fig. 2C) that could reach up to 0.9 m/s, whereas the other cats (5/11) showed varying degrees of hindlimb recovery from unilateral stepping on the side of the hemisection to no stepping movement.

**Recovery of Locomotor Parameters After Partial and Complete Spinal Lesions**

Various kinematic parameters and EMG signals obtained from 11 cats walking at 0.4 m/s after spinal hemisection and from 6 of the 11 cats that walked after spinalization were analyzed to quantify the spontaneous locomotor recovery during the 3-wk period after the partial lesion as well as 24 h after spinalization (Fig. 2).

**Homolateral coupling.** Most cats maintained a 1:1 cycle period ratio between the fore- and hindlimbs (Fig. 2B), and only these step cycles were used for statistical comparisons and presented in Fig. 3A. Consistent with previous published data (Barrière et al. 2010; Brustein and Rossignol 1998; Jiang and Drew 1996), the homolateral coupling of the limbs on the left and right sides was around 0.19 before the lesion. The left or right forelimb made contact with the ground at phase 0.19 after the corresponding hindlimb contact as shown in the first inner circle of the polar plot. After hemisection, the left forelimb made contact at phase 0.20 ± 0.02 after the left hindlimb contact (Fig. 3A, filled circles). This is clearly illustrated by consistent displacement of the mean coupling values toward the left. However, the homolateral coupling on the right side (Fig. 3A, open circles) remained basically unchanged at 0.19 and was not significantly different from control values, represented by the dotted line.
Different step frequencies between the fore- and hindlimbs was observed only in three sessions in two different cats in which the number of forelimb steps exceeded those of the hindlimbs. These three isolated observations were not included in this group.

**Homologous coupling.** The phase coupling between the left and right limbs at the brachial and pelvic girdles was measured. Whereas the coupling between both forelimbs (Fig. 3B, open circles) remained unchanged after hemisection (i.e., alternating 0.5 phase coupling), the coupling between hindlimbs shifted by a constant amount \(0.40 \pm 0.04\); Fig. 3B, filled circles), indicating that the intact right hindlimb contacted the ground earlier relative to the left foot contact. Thus the right hindlimb phase-advanced its weight support to minimize the contribution of the somewhat incapacitated left hindlimb on the lesioned side. However, immediately after spinalization, the hindlimb coupling shifted again to \(0.53 \pm 0.02\), very close to the intact state value (Fig. 3B, filled circles). Note that the coupling between hindlimbs in the spinal state differs significantly from that observed on the last hemispinal day but not from the intact state.

**Changes in the step cycle structure.** One week after hemisection, the step cycle duration decreased symmetrically in both hindlimbs (Fig. 4A), although the step length differed between hindlimbs (Fig. 4B). This implies that changes within the structure of the cycle must have occurred on both sides to

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Fig. 2. Episodes of treadmill locomotion at different times of the dual lesion paradigm in cat DLT5 walking at 0.4 m/s. Top traces of EMG recordings were obtained bilaterally from hindlimb flexor and extensor muscles at the intact (A), hemispinal (B), and early spinal states (C). Duty cycles (black horizontal bars) below the EMGs illustrate the support periods (stance) of each limb. St, semitendinosus; Srt, sartorius; VL, vastus lateralis; GL, lateral gastrocnemius; GM, median gastrocnemius; TA, tibialis anterior; l, left; r, right; HL, hindlimb; FL, forelimb.

Different step frequencies between the fore- and hindlimbs was only observed in three sessions in two different cats in which the number of forelimb steps exceeded those of the hindlimbs. These three isolated observations were not included in this group.

Fig. 3. Interlimb coupling throughout the dual lesion paradigm during treadmill locomotion at 0.4 m/s. A: mean left (filled circles) and right (open circles) foreand hindlimb phase coupling in the intact and hemispinal states at 7 (Hemi 7), 14 (Hemi 14), and 21 days (Hemi 21) in 11 cats. Time 0 represents the left or right hindfoot contact, respectively, for the left and right homolateral coupling. Gray circles represent a similar SD of 0.05 plotted at the intact and spinal states to illustrate the fact that a similar SD appears larger as a function of the postoperative time. B: mean hindlimb (filled circles) and forelimb (open circles) coupling in the intact and hemispinal states (11 cats), as well as 24 h after spinalization (spinal; 6 cats, hindlimb coupling only). Time 0 represents the left hind- or forefoot contact, respectively, for the hind- and forelimb coupling. Statistical differences between pre- and postoperative values are indicated by asterisks. Statistical differences between the last hemispinal and the first spinal delay are indicated by the symbol #.
compensate for the asymmetry. The decrease in step length observed 1 wk after hemisection was principally due to a decrease in the forward placement of both paws relative to the hip, and the changes were more prominent in the left hindlimb on the side of the lesion (Fig. 4C). Moreover, the decreased cycle duration observed on both sides after hemisection (Fig. 4A) resulted from complementary changes within the two hindlimbs' subphases (Fig. 4D). The shorter cycle duration of the left hindlimb (Fig. 4A, filled circles) resulted from a decrease in stance duration and an increase in the swing (Fig. 4D, filled circles); it was strictly the opposite for the right hindlimb (Fig. 4D, open circles). After the second week posthemispinal lesion, the step cycle duration of both hindlimbs returned to control values (Fig. 4A).

In addition, the step length of the right hindlimb (Fig. 4B, open circles) could now perform proper forward placement rostral to the hip (Fig. 4C, black bars). In contrast to the right hindlimb, the left hindlimb step length remained shorter until the third postoperative week (Fig. 4B, filled circles) and was accompanied by a deficit in forward limb placement (Fig. 4C, gray bars). The stance duration/cycle on the right side (Fig. 4D, open circles) returned to control values, whereas it remained lower on the left side (Fig. 4D, filled circles). The opposite effect was observed for the swing phase. Despite the gradual recovery of the left hindlimb kinematics after hemisection, residual deficits persisted until the third week just before spinalization. In contrast, the right hindlimb deficits recovered to control...
values by the third week. The persistent left hindlimb deficits contributed to left/right asymmetries in step length, paw placement, and step duration over the entire hemispinal period (Fig. 4, B–D).

Immediately after spinalization (i.e., 24 h), the step cycle duration and the step length of both hindlimbs decreased significantly compared with the final day before spinalization (Fig. 4, A and B). A decrease in the forward step length was observed on both sides and also in backward paw placement for the right hindlimb (Fig. 4C). Interestingly, the persistent deficits observed in the left hindlimb in swing and stance durations after hemisection disappeared and the cycles became comparable to control values (Fig. 4D). In contrast, the stance duration decreased and the swing duration increased for the right hindlimb after spinalization (Fig. 4D). The results obtained after spinalization suggest that the compensatory changes observed after hemisection reversed after spinalization such that the left hindlimb (i.e., the side of the previous hemisection) displayed better performances than the right hindlimb.

To summarize, the hemisection on the left side resulted in left/right asymmetries persisting over the entire hemispinal period (Figs. 3B and 4). Prominent and persistent deficits were reported in the left hindlimb kinematics and had not recovered after 3 wk, i.e., just before the second complete spinal section. By contrast, the right hindlimb performance improved over time and even returned to control values on the third week (Fig. 4). The day after spinalization, the right hindlimb underwent more drastic changes than the left such that the left/right asymmetries observed after hemisection tended to reverse (Fig. 3B and Fig. 4, C and D), indicating that the left hindlimb appeared to walk with larger and smooth steps than the right hindlimb and resembled the normal walking pattern.

Changes in EMG Activity

EMGs of several flexor and extensor muscles of both hindlimbs were obtained from 10 cats walking at 0.4 m/s after spinal hemisection and from 5 of these 10 cats that walked after spinalization. As illustrated in Fig. 2, all the EMG signals maintained a good signal-to-noise ratio during the entire testing period except for TA, in which the ratio was inconsistent.

Burst duration. After hemisection, the flexor burst duration of St and Srt muscles increased on the left side (Fig. 5, A and B, filled symbols), whereas burst duration of the extensors VL and GM diminished concurrently (Fig. 5, C and D, filled symbols). Conversely, the activity of the homologous flexors of the right hindlimb tended to decrease (Fig. 5, A and B, open symbols), and the extensors tended to increase after hemisection (Fig. 5, C and D, open symbols). Despite a gradual recovery observed on both sides throughout the hemispinal period, asymmetries in flexor and extensor burst duration between the left and the right hindlimb were noted during the entire hemispinal period, probably contributing in part to the changes observed in phase duration after hemisection (Fig. 4D).

After spinalization, EMG activity again changed. The St burst duration of the left hindlimb returned to preoperative control values compared with the final day after hemispinal section (Fig. 5A, filled symbols). The principal changes were observed in extensor activity on the right side (Fig. 5, C and D). After spinalization, the extensor activity decreased in both hindlimbs and was even lower than control and hemispinal values (Fig. 5, C and D). Moreover, the asymmetries between the two hindlimbs previously observed during the hemispinal period were abolished, confirming in part the global return of symmetrical subphase duration after spinalization (Fig. 4D).

Intralimb EMG coupling. The intralimb coupling between muscle activity, especially between the representative hip and knee flexor muscles (Srt and St) and between the knee flexor (St) and the extensor VL, are illustrated in Fig. 6 using real-time values or phase values (see METHODS).

First, the coupling between the onset of the hip flexor St and the knee flexor St was measured as a function of time using one-way ANOVA (Fig. 6, A–C). Normally, St begins to discharge at around 50 ms after St (Fig. 6, A and C), corresponding to a delay of around 5% of the step cycle (i.e., phase 0.05 ± 0.02) (Fig. 6B, dotted line from the origin). This normally ensures that the knee is flexed before the hip and that the foot has left the ground before the limb moves forward. One week after hemisection, this delay was maintained in the left hindlimb (P > 0.05) but tended to decrease for the right hindlimb, resulting in significant asymmetries in flexor coupling between the left and right hindlimbs (P < 0.05, unpaired t-test) (Fig. 6A). By the third hemispinal week, the left St burst onset was delayed with respect to the St burst onset (71.6 ± 95.8 ms; P < 0.05) (Fig. 6, A and C), and the phase relations between these muscles tended to increase (phase 0.14 ± 0.08) (Fig. 6B). At this time, asymmetries in phase relations between the left and right hindlimbs were noted (P < 0.05, unpaired t-test) (Fig. 6B). After spinalization, this phase relation changed in both real time and phase values for the two hindlimbs so that the hip flexors (Srt) discharged before the knee flexors (St), especially on the left side (−63.4 ± 38.7 ms; phase −0.08 ± 0.04) (Fig. 6, A–C). This earlier initiation of the hip swing could account for the foot drag observed in both hindlimbs after spinalization.

Second, we investigated the intralimb coupling between the antagonist muscles of the knee (extensor VL and flexor St) as a function of time using a one-way ANOVA (Fig. 6, D–F). Normally, St activity begins a few milliseconds after VL activity begins at the end of the stance phase, whereas VL bursts begin at the end of the swing phase. In intact cats, the delay between the onset of VL and St bursts is around 35% of the cycle (i.e., phase 0.35 ± 0.04 of the cycle defined by St onset) (Fig. 6E), equivalent to 330 ms (Fig. 6, D and F). This delay was generally preserved on both sides after hemisection (Fig. 6D, P > 0.05). However, as the VL tended to become active later in the cycle on the left side and to become active earlier on the right side (Fig. 6E), asymmetries between the left and right hindlimb were observed during the hemispinal period (Fig. 6, D and E, P < 0.05). The changes in VL/St coupling described above can account, in part, for the respective prolonged stance phase and diminished swing phase observed on the left and right side after hemisection (Fig. 4D). After spinalization, the VL/St coupling was similar to control values on the right side (phase 0.37 ± 0.10) (Fig. 6, D and E, P > 0.05). On the left side, new changes occurred such that the VL/St phase value was greatly reduced (phase 0.24 ± 0.09) (Fig. 6E) and VL onset began 185.6 ±
98.5 ms after St onset \((P < 0.05)\) (Fig. 6, D and F). These changes can explain, in part, the decrease in swing duration observed in the left hindlimb after spinalization (Fig. 4D) but cannot solely account for its return to control values.

**Interlimb EMG coupling.** In normal conditions, the activity of hindlimb flexor or extensor muscles alternate strictly so that the extensor or flexor muscles in alimb begin to discharge at phase 0.5 of the cycle of the corresponding homologous muscles of the other limb (Fig. 7, B, D, and F). The coupling between the right and left hip flexors (rSrt/lSrt), knee flexors (rSt/lSt), and knee extensors (rVL/lVL) were investigated as a function of time after the spinal lesions using one-way ANOVA (Fig. 7). In the intact state, the right Srt, St, and VL contracted at about 450 ms after the left corresponding muscles (Fig. 7, A, C, and E). After hemisection, these temporal relations were preserved (Fig. 7, A, C, and E) but some phase relations were changed (Fig. 7, B, D, and F). By the third hemispinal week, the right Srt discharged earlier in the cycle of the left Srt (phase 0.44 ± 0.09; \(P < 0.05\)) (Fig. 7B). The right VL also tended to contract earlier in the cycle of the left VL (hemispinal 21: phase 0.44 ± 0.06) (Fig. 7F), and changes in phase relations closely followed the modifications in homologous coupling observed during the hemispinal period (Fig. 3B).

After spinalization, the changes observed in rSrt/lSrt and rVL/lVL couplings after hemisection reversed such that the right Srt and VL muscles became active earlier with reference to the left Srt and VL (phase rSrt/lSrt: 0.52 ± 0.07, rVL/lVL: 0.56 ± 0.02; \(P < 0.05\)) (Fig. 7, B and F). The latter observation probably accounts in part for the homologous coupling changes after spinalization (Fig. 3B).

The correspondence between the overall changes in kinematics and the changes for the EMG coupling described in this report implies that the muscles chosen are representa-
Interindividual Differences in Re-expression of Locomotion After Spinalization

After spinalization, 6/11 cats were able to express a bilateral hindlimb pattern of locomotion with bilateral foot placement (Table 1). Alternate locomotor movements and well-organized EMG activities were observed on both sides (Figs. 2C and 8A) for a minimum of 10 consecutive steps. These cats could all walk with both hindlimbs at the minimal speed of 0.4 m/s with gentle perineal stimulation and up to 0.9 m/s in certain cats (Table 1). This result contrasts with the 2–3 wk of treadmill training normally necessary to obtain a similar level of recovery in spinal cats (Barbeau and Rossignol 1987). In one cat (cat DLE3), bilateral locomotor EMG activity was observed, especially in flexors, but these bursts were too weak to produce significant movements and foot placement, and the hindlimbs remained mainly in an extended position with the paws well behind the vertical projection of the hip joint.

The three other cats (cats DLE2, DLE5, and DLT4) expressed unilateral spinal locomotion on the previously hemisected side (Fig. 8B), as observed by Barrière et al. (2008). Furthermore, the locomotor pattern of the left hindlimb was...
well developed (Fig. 8B) and shared many characteristics of normal stepping locomotion (Fig. 2A). Despite well-synchronized rhythmic locomotor EMG activity, the right hindlimb did not step and remained most of the time in an extended position. Finally, only one cat failed to show either locomotor movements or locomotor EMG activity despite intense perineal stimulation (cat DLE4, Fig. 8C), similar to a complete spinal section (Barbeau and Rossignol 1987).

To further investigate these interindividual differences in the re-expression of the hindlimb walking pattern observed on the first day after spinalization (Fig. 8), we asked whether some characteristics of the bilateral hindlimb locomotion seen after hemisection were different. We classified cats into two groups according to their locomotor behavior after spinalization (Table 1). To be included in group 1 (i.e., bilateral stepping), cats had to exhibit an alternate activity between the hindlimbs (as shown in Figs. 2C and 8A) for a minimum of 10 consecutive step cycles. Moreover, forward movements had to be sufficient to place the foot in front of the hip (contrary to cat DLE3, that just expressed bilateral EMG locomotor rhythm without advancing the limb). By taking these criteria into account, six cats were included in group 1 (see Table 1). The five other cats were assigned to group 2. Because cat JCA6 was not implanted with EMG electrodes and was included in the first group, only five cats were included in this group for EMG analyses.

Fig. 7. Changes in EMG interlimb coupling in the intact state and hemispinal states (10 cats) and 24 h after spinalization (5 cats) during treadmill locomotion at 0.4 m/s. Coupling between left and right Srt, St, and VL muscles is expressed in duration (in ms; A, C, and E, respectively) or in phase relations (B, D, and F, respectively). Statistical differences between pre- and postoperative values are indicated by asterisks. Statistical differences between the last hemispinal and the first spinal delay are indicated by the symbol #. Statistical differences between the left and right hindlimbs are indicated by the symbol §.
Lesion size. The differences observed between the two groups could result from the lesion size and the amount of remnant descending pathways. We thus compared the two groups in regard to the percentage of damaged area, including the white and gray matter as well as cavitations, relative to the entire surface of the spinal cord by using unpaired t-tests. No difference between groups was noted (Fig. 9B, P > 0.05).

Because the quantity of gray matter lost has been shown to poorly correlate with locomotor deficits (Magnuson et al. 2005), we also quantified the extent of spinal white matter damage in specific funiculi. As illustrated in Fig. 9A, the left side of the spinal cord was divided into three parts as an attempt to delineate spinal white matter areas known a priori to contain descending fiber tracts that may subserve specific functions. The dorsal funiculus (1) contains the dorsal column tract that conveys sensory information from periphery to the contralateral somatosensory cortex. The lateral funiculus (2) contains the descending cortico- and rubrospinal tracts (Fig. 9A, light gray) and is mostly involved in voluntary and fine motor control. The ventral funiculus (3) contains the reticulo- (Fig. 9A, dark gray) and vestibulospinal tracts (Fig. 9A, black), respectively, from the reticular formation and Deiter’s nucleus, which are known to be involved in posture and locomotion as well as some direct corticospinal fibers. Unpaired t-tests indicated no difference between groups in regard to the percentage of damage in dorsal and lateral funiculi (P > 0.05). However, the left ventral funiculus was shown to be more damaged in group 1 (33.78 ± 20.17%) compared with group 2 (12.37 ± 18.35%) (P < 0.05; see Fig. 9B). Therefore, group 1, expressing the best locomotor performance after spinalization, exhibited more damage to the left ventral white matter at the time of hemisection. It is well known that the fibers travelling in the ventral funiculus (reticulo- and vestibulospinal fibers) are greatly involved in locomotion and also influence the spinal locomotor circuitry activity. This result indicated that damaging a large number of ventral fibers with the first hemisection

Table 1. Locomotor pattern characteristics of the left and right hindlimbs in 11 cats assessed 24 h after spinalization and cats’ group inclusion

<table>
<thead>
<tr>
<th>Cat</th>
<th>Maximal speed, m/s</th>
<th>No. of consecutive steps</th>
<th>Plantar foot placement</th>
<th>Right Hindlimb</th>
<th>Maximal speed, m/s</th>
<th>No. of consecutive steps</th>
<th>Plantar foot placement</th>
<th>Perineal Stimulation</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLE1</td>
<td>0.9</td>
<td>&gt;10</td>
<td>X</td>
<td>0.9</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLE6</td>
<td>0.8</td>
<td>&gt;10</td>
<td>X</td>
<td>0.8</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLT1</td>
<td>0.6</td>
<td>&gt;10</td>
<td>X</td>
<td>0.6</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>JCA6</td>
<td>0.8</td>
<td>&gt;10</td>
<td>X</td>
<td>0.8</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLT5</td>
<td>0.5</td>
<td>&gt;10</td>
<td>X</td>
<td>0.5</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLT3</td>
<td>0.5</td>
<td>&gt;10</td>
<td>X</td>
<td>0.5</td>
<td>&gt;10</td>
<td>X</td>
<td>Gentle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLE5</td>
<td>0.3</td>
<td>&gt;10</td>
<td>X</td>
<td>0.3</td>
<td>&gt;10</td>
<td>X</td>
<td>High</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DLT4</td>
<td>0.3</td>
<td>&lt;10</td>
<td>X</td>
<td>0.3</td>
<td>&lt;10</td>
<td>X</td>
<td>High</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DLE2</td>
<td>0.3</td>
<td>&lt;10</td>
<td>X</td>
<td>0.3</td>
<td>&lt;10</td>
<td>X</td>
<td>High</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DLE3</td>
<td>0.3</td>
<td>&gt;10</td>
<td>X</td>
<td>0.3</td>
<td>&gt;10</td>
<td>X</td>
<td>High</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DLE4</td>
<td>0</td>
<td>&lt;10</td>
<td>X</td>
<td>0</td>
<td>&lt;10</td>
<td>X</td>
<td>High</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. Interindividual differences in the hindlimb walking pattern and EMG activity assessed 24 h after the complete spinal section. A–C, top: stick representations of typical swing and stance phases of the left hindlimb reconstructed from video recordings during treadmill locomotion. Bottom: EMG bursts of selected muscles in the left (i) and right (co) hindlimbs are represented. HL traces show the associated duty cycles for both hindlimbs in case of bilateral (A) and unilateral stepping (B). In the absence of locomotion (C), the continuous lines indicate that the paws are always in contact with the treadmill.
led to better locomotor performance after spinalization (for further details, see DISCUSSION).

Walking pattern and EMG activity. We compared the two groups regarding the kinematic and EMG changes occurring during the hemispinal period. All the kinematic and EMG parameters previously described were compared in the two groups of cats by using unpaired t-tests at the intact and hemispinal state. One-way ANOVAs were also used to evaluate the performances of each group over time. For reasons of simplification, only the salient features of the results are described.

Overall, comparisons between the two groups of kinematic parameters and EMG burst duration during the intact and hemispinal period indicated only small differences. Indeed, some kinematic parameters such as step length and forward placement of the left hindlimb were more affected in group 1 compared with group 2 at 3 wk after hemisection (step length: group 1, 338.3 ± 8.3 mm, group 2, 399.0 ± 40.6 mm; toe position at contact: group 1, 56.4 ± 16.7 mm, group 2, 89.0 ± 18.8 mm). Interestingly, cats in group 1 exhibited more kinematic deficits than cats in group 2 just before the spinalization.

The parameters that most differentiated the two groups during the hemispinal period were the intra- and interlimb muscle coupling (Fig. 10). By comparing the two groups over the same parameters as presented in Figs. 6 and 7, we observed that the changes in intra- and interlimb EMG coupling previously observed in the entire group of 11 cats were only attributable to the cats of group 1 (Fig. 10, gray circles). Indeed, these couplings, measured in real time (not shown) or in phase values, remained unchanged in group 2 during the entire hemispinal period. Moreover, the changes observed in group 1 involved both hindlimbs in many cases (Fig. 10), despite the fact that the right side was more dramatically impacted (Fig. 10, right panels).

More specifically, the left (Fig. 10A) and right (Fig. 10B) Srt/St phase relations and the right VL/St phase relations (Fig. 10D) were significantly altered by the hemisection only in group 1. Because the VL/St coupling of the left hindlimb was little affected by the hemisection in the entire group (see also Fig. 6E), it was reasonable to investigate comparable couplings between groups (Fig. 10C). Corroborating results were observed for the interlimb EMG coupling between the left/right VL (Fig. 10E) and left/right Srt (Fig. 10F) that were respectively changed from the second and third hemispinal week only in group 1 (group 1 vs. group 2: \( P < 0.05 \), unpaired t-tests).

Together, the results obtained by comparing the EMG activity in the two groups during the hemispinal period indicated that changes in intra- and interlimb coupling occurred exclusively in group 1 and especially on the right side, i.e., contralateral to the hemisection. Moreover, cats in group 1 had larger ventral lesions than those in group 2 and presented more kinematic deficits on the left side after hemisection. To summarize, the changes of the locomotor pattern during the hemispinal period as well as the lesion size seemed to have an impact on the subsequent locomotor performance after the complete spinalization such that cats with the largest ventral lesions and more prominent kinematic and EMG changes after hemisection (group 1) exhibited the best locomotor performances after spinalization.

DISCUSSION

In the present work, we investigated the conditions in which the spinal circuitry for locomotion changes below a partial spinal lesion and account for the observation that cats can walk immediately after a complete spinalization (Barrière et al. 2008, 2010). We investigated whether spinal plasticity can occur without specific locomotor training after the partial SCI and within a short period of time (3 wk). We showed that the majority of cats (6 of 11 cats; group 1) were able to express a bilateral walking pattern as early as 24 h after the second complete SCI, although the other cats (5 cats; group 2) expressed various degrees of locomotor movements such as unilateral stepping on the side of the hemisection (3 cats) or no stepping at all (2 cats). Interestingly, cats recovering bilateral hindlimb locomotion best after spinalization had the largest partial spinal lesions and displayed the most prominent kinematic and EMG coupling deficits after the first lesion. These results provide evidence that 1) marked reorganization of the spinal locomotor circuitry occurred spontaneously (i.e., without training) within a short period of time; 2) the spinal cord can reorganize in a more or less symmetrical way; and 3) the ability to walk after spinalization depends on the degree of adaptation observed in the hemispinal period.

Spontaneous Locomotor Recovery Following the Spinal Hemisection

After the left hemisection at T10, compensatory kinematic and EMG changes occurred in both hindlimbs and persisted throughout the 3-wk hemispinal period in all 11 cats. The shorter step cycle duration of the ipsilateral hindlimb resulted from a shorter stance phase and a prolonged swing phase, together with corresponding changes in extensor and flexor muscles burst duration. To compensate for the left hindlimb deficits, mirror compensatory alterations were seen in the contralateral hindlimb. The shortened stance phase in the hindlimb of the lesioned side has also been reported after thoracic or lumbar hemisections (Barrière et al. 2010; Eidelberg et al. 1986; Helgren and Goldberger 1993; Kuhtz-Buschbeck et al. 1996) as well as bilaterally after spinalization.
In addition, the coupling between hindlimbs was modified in all cats, as previously reported after unilateral SCI (Barrière et al. 2010; Kuhtz-Buschbeck et al. 1996). The coordination between fore- and hindlimb on the side of the partial SCI was also affected (Barrière et al. 2010; Bem et al. 1995; Helgren and Goldberger 1993; Kuhtz-Buschbeck et al. 1996) and is probably due to the unilateral interruption of various pathways that couple the fore- and hindlimb locomotor circuits (Bareyre et al. 2004; Courtine et al. 2008; English 1980; Kato et al. 1984; Sherrington and Laslett 1903).

**Mechanisms of Locomotor Recovery After Incomplete SCI**

Since the recovery of locomotion after partial SCI could involve reorganization at both supraspinal and spinal levels, these observations made after complete spinalization removing all supraspinal inputs should have major implications. Twenty-four hours after the complete SCI, six cats expressed bilateral hindlimb locomotion (group 1), and some were able to walk at high speeds (up to 0.8–0.9 m/s) with a spinal cord that had been subjected to a hemisection 3 wk earlier, whereas three others expressed a unilateral walking pattern on the side of the

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**Fig. 10. Comparisons of the EMG intra- and interlimb coupling between groups along the dual lesion paradigm during treadmill locomotion at 0.4 m/s.** A–D: Srt/St and VL/St phase relations in the left (A and C, respectively) and right hindlimb (B and D, respectively). E and F: coupling between left and right VL and Srt muscles expressed in phase relations, respectively. Statistical differences between the pre- and postoperative values are indicated by asterisks. Statistical differences between the last hemispinal and the first spinal delay are indicated by the symbol #. Statistical differences between groups 1 and 2 are indicated by the symbol &.
first hemisection, as previously observed (Barrière et al. 2008). Interestingly, for the cats of group 1, the bilateral locomotor pattern observed was not strictly symmetric such that the hindlimb on the side of the first SCI performed globally better than the right. Reversals of left/right asymmetries between the hemispinal and spinal states indicate that new dynamic interactions between supraspinal and spinal circuits occurred after partial SCI to optimize locomotion. The common point between these two groups was their ability to walk with their left hindlimb, thus suggesting the first unilateral spinal lesion promoted a reorganization and/or changes of activity within the caudal spinal circuitry and especially on the side of the first lesion such that it was already able to walk after the spinalization. Indeed, it has been previously demonstrated that anatomical and functional asymmetric changes can take place within the spinal cord itself after partial spinal lesions (Frigon et al. 2009; Helgren and Goldberger 1993; Hultborn and Malmsten 1983a, 1983b; Murray and Goldberger 1974) or can involve reorganization of connections between the supraspinal structures and the spinal cord (Ballermann and Fouad 2006; Courtine et al. 2008; Fouad et al. 2001; Ghosh et al. 2009; Raineteau et al. 2002; Rosenzweig et al. 2010), thus re-rerouting descending pathways by undamaged descending pathways. The divergent point between groups was their differential ability to use their right hindlimb. By comparing these two groups during the hemispinal period, we found that cats in group 1 had more prominent kinematic and EMG coupling deficits compared with cats in group 2 after hemisection and that the changes in EMG coupling occurred significantly more on the right side (i.e., the unaffected hindlimb). Because group 1 also exhibited the largest ventral lesions, this probably accounts in part for the greater deficits observed in the left hindlimb EMG coupling and kinematics. Such changes in EMG intralimb coupling of the affected hindlimb observed in group 1 have previously been reported after a spinal hemisection disrupting all the tracts on one side (Kuhtz-Buschbeck et al. 1996). Thus, in cats of group 1, the multiple disruption of major reticulospinal and vestibulospinal fibers, which play a major role in coordinating the flexor and extensor muscle activity by their direct access to the spinal central pattern generator (CPG) (Drew and Rossignol 1984; Gossard et al. 1996; Orlovsky 1970, 1972a, 1972b; Perreault et al. 1994; Russell and Zajac 1979), probably account for the changes in EMG coupling on the side of the hemisection and thus in the generation of the locomotor pattern. By contrast, in the three cats of group 2 displaying a unilateral walking pattern after spinalization, no evident changes in EMG coupling were noted, thus suggesting that the presence of a higher number of remnant supraspinal inputs reaching the spinal circuitry could be sufficient for triggering the CPG after hemisection. The EMG changes observed on the unaffected side (right side) in the first group could be explained by behavioral adaptations. Indeed, to compensate for the prominent left hindlimb deficits and the larger partial SCI on the left side, it is likely that the right hindlimb had to adapt more intensively to assume the weight support, thus leading to EMG changes on the right side. Such an overuse of the right hindlimb can stimulate plasticity (Bland et al. 2000; Ghosh et al. 2009; Girgis et al. 2007; Humm et al. 1998; Kozlowski et al. 1996; Krajacic et al. 2010; Maier et al. 2008) and probably affect the spinal reorganization, especially on the right side. In return, the right side of the spinal cord provides important inputs to the lesion side via commissural interneurons whose activity is influenced by the moving hindlimb and supraspinal structures (Jankowska 2008). Moreover, one can hypothesize that damaging a large number of ventral descending fibers unilaterally will probably lead to a greater takeover of function by the spinal CPG on that side than would be the case of more restricted lesion, thus enabling the CPG to work more autonomously and be already primed to re-express bilateral hindlimb locomotion immediately after spinalization.

Role of Locomotor Training and Delay During the Interlesion Period

The two types of spinal locomotor patterns reported presently after spinalization (i.e., unilateral vs. bilateral) differed from that previously observed in cats trained to walk after the first partial SCI (Barrière et al. 2008, 2010). Indeed, these trained cats all expressed a bilateral stepping pattern after the second complete SCI (Barrière et al. 2008, 2010). In this previous study (Barrière et al. 2008), the two untrained cats expressed a unilateral walking pattern on the side of the previous partial lesion, as did some cats in the present study. The main differences between these two previous studies and the present one are 1) the level of locomotor activity after the first partial lesion (no training in the present study vs. 3–14 wk of training in the previous one, except for 2 cats) and 2) the delay between the two lesions that was limited to 3 wk in the present study and extended until the performance of the cats no longer improved.

With respect to the impact of training after hemisection, it is well established that use-dependent activity within existing pathways controlling the hindlimb musculature is modified through training, leading to a strengthening of the neural pathways that sustain activation of locomotor-generating spinal circuitry (Rossignol et al. 2009). Moreover, the repetitive sensory inputs provided by the treadmill have been shown to facilitate the recovery of locomotor patterns (Barbeau and Rossignol 1987; de Leon et al. 1998; Edgerton et al. 2008; Harkema 2001; Lovely et al. 1986; Van de Crommert et al. 1998). In the present study, our untrained cats were free to move in their cage during the hemispinal period and were not forced to use their two hindlimbs concomitantly, as was the case with cats trained daily to walk in a straight path on treadmill. In the present case, our cats probably developed behavioral strategies to compensate for the left hindlimb deficits by using the right hindlimb more. Such an increased use of the unaffected hindlimb could impact in a more or less symmetrical way the spinal reorganization, in contrast to cats that were trained to use both their hindlimbs during the repetitive training sessions. Indeed, one can hypothesize that treadmill training leads to a more symmetrical spinal reorganization and a bilateral stepping pattern after spinalization by forcing the two hindlimbs to walk after hemisection.

Another main difference between these studies and the present one was the time between the two spinal lesions, which was limited to 3 wk in the present study. Such an interlesion period was too short to allow the cats to reach their plateau of recovery and thus could have impacted the full completion of plastic mechanisms. Usually, anatomical and/or structural changes such as sprouting of fibers take a minimum of 3 wk to stabilize (Ballermann and Fouad 2006; Bareyre et al. 2004;
Fouad et al. 2001; Ghosh et al. 2009, 2010; Raineteau et al. 2002; Rosenzweig et al. 2010). Although these latter mechanisms cannot be discounted, it is more likely that the remnant fibers had increased their activity to compensate for the unilateral spinal lesion (Thomas and Gorassini 2005). This increased activity could be proportional to the hindlimb use, thus influencing reshaping of the spinal circuitry. The observation that the asymmetries observed during the hemispatial period could actually reversed in direction after spinalization is in accordance with this latter hypothesis (see also Barrière et al. 2010). The adaptations observed after hemisection appeared to be mainly maintained by remaining descending pathways, thus creating a new left/right balance within the spinal circuits. In addition, physiological changes in synaptic weighting can occur within the CPG itself and could account for the establishment of a new balance between left and right sides (Sakurai and Katz 2009). Investigations are in progress to study the impact of locomotor training during the same base period.

In conclusion, after thoracic hemisection, both remnant descending pathways and spinal mechanisms must participate in the re-expression of hindlimb locomotion. Recovery of voluntary quadrupedal locomotion occurred in the absence of specific locomotor training, but asymmetries between left and right hindlimbs were shown to persist 5 wk after the hemisection. This period of time, despite its brevity, was sufficient to promote changes within the spinal locomotor circuitry, as revealed by the ability of the majority of cats to walk after spinalization with one or two hindlimbs. The different locomotormechanisms observed after spinalization provide evidence that asymmetrical reorganization can actually occur within the spinal networks. We also conclude that the ability to walk after spinalization depends on the degree of deficits and adaptation observed in the hemispatial period. Moreover, locomotor training appeared to not be decisive for the re-expression of spinal locomotion but seemed to maximize reorganization of the spinal circuitry in a more symmetrical way. This study again highlights the important intrinsic capacity of the locomotor system to reorganize and optimize its function after incomplete SCI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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