Dual Spinal Lesion Paradigm in the Cat: Evolution of the Kinematic Locomotor Pattern

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Barrière G, Frigon A, Leblond H, Provencher J, Rossignol S. Dual spinal lesion paradigm in the cat: Evolution of the kinematic locomotor pattern. J Neurophysiol 104: 1119–1133, 2010. First published June 23, 2010; doi:10.1152/jn.00255.2010. The recovery of voluntary quadrupedal locomotion after an incomplete spinal cord injury can involve different levels of the CNS, including the spinal locomotor circuitry. The latter conclusion was reached using a dual spinal lesion paradigm in which a low thoracic partial spinal lesion is followed, several weeks later, by a complete spinal transection (i.e., spinalization). In this dual spinal lesion paradigm, cats can express hindlimb walking 1 day after spinalization, a process that normally takes several weeks, suggesting that the locomotor circuitry within the lumbo-sacral spinal cord had been modified after the partial lesion. Here we detail the evolution of the kinematic locomotor pattern throughout the dual spinal lesion paradigm in five cats to gain further insight into putative neurophysiological mechanisms involved in locomotor recovery after a partial spinal lesion. All cats recovered voluntary quadrupedal locomotion with treadmill training (3–5 days/wk) over several weeks. After the partial lesion, the locomotor pattern was characterized by several left/right asymmetries in various kinematic parameters, such as homolateral and homologous interlimb coupling, cycle duration, and swing/stance durations. When no further locomotor improvement was observed, cats were spinalized. After spinalization, the hindlimb locomotor pattern rapidly reappeared, but left/right asymmetries in swing/stance durations observed after the partial lesion could disappear or reverse. It is concluded that, after a partial spinal lesion, the hindlimb locomotor pattern was actively maintained by new dynamic interactions between spinal and supraspinal levels but also by intrinsic changes within the spinal cord.

INTRODUCTION

After a complete spinal transection (i.e., spinalization), hindlimb locomotor movements can be re-expressed, as documented in several animal species (Delcomyn 1980; Grillner 1981; Rossignol 1996). In cats, recovery of a bilateral hindlimb locomotor pattern with plantar foot placement and prolonged periods of hindquarter weight support takes a minimum of 2–3 weeks of treadmill training (Barbeau and Rossignol 1987; Lovely et al. 1986). This clearly emphasizes the role played by an intrinsic spinal circuitry and afferent feedback for the expression of hindlimb locomotion after spinalization (Grillner 1981; Rossignol 1996, 2006; Rossignol et al. 2006).

However, is the intrinsic spinal circuitry also involved in the recovery of hindlimb locomotion after a partial spinal lesion, in which spared descending supraspinal and/or propriospinal pathways can still access the spinal cord? We recently showed that the spinal locomotor circuitry was indeed critical for the recovery of hindlimb locomotion after a partial spinal lesion using a dual spinal lesion paradigm (Barrière et al. 2008). First a hemisection of the left spinal cord was performed at thoracic levels (T10–T11), and cats were trained on a treadmill for several weeks until they reached a stable level of voluntary quadrupedal locomotion. The spinal cord was completely sectioned at T13 (same level as in previous work on spinal cats) (Barbeau and Rossignol 1987; Belanger et al. 1996). Remarkably, within 24 h after spinalization, these previously hemisectioned cats could walk with their hindlimbs at high speeds (>0.8 m/s) and with bilateral plantar foot contact. This is in sharp contrast to the few weeks needed to reach such performance in cats not partially lesioned before spinalization (Barbeau and Rossignol 1987). Therefore during the period of locomotor training after the partial spinal lesion, plastic changes occurred in the lumbo-sacral spinal cord, configuring the locomotor circuitry so that it could operate with limited supraspinal influences.

This study provides greater detail concerning the evolution of the locomotor pattern over the weeks after the partial spinal lesion and subsequent spinalization. A detailed kinematic analysis is used to gain further insight as to the neurophysiological mechanisms involved in locomotor recovery after an incomplete spinal cord injury. It is hypothesized that kinematic changes reflect functional plasticity within spinal circuits, which can be shown by performing a spinalization following several weeks of recovery after a partial spinal lesion. Indeed, after the partial lesion, new dynamic interactions were established between supraspinal and spinal structures, leading to adaptive changes in locomotor kinematics. Changes occurring within the spinal cord were shown by the spinalization and remained maintained over time, highlighting the tremendous plastic changes within the spinal cord.

METHODS

General methodology

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. Surgical procedures for spinal lesions, implantation of electrodes to record EMG, and animal care were described in detail (Barrière et al. 2008). The expressions “intact,” “partial lesion,” and “spinalization” refer, respectively, to the control state before any lesion, to the period...
after the partial lesion at T_{10-T_{11}} up to the spinalization, and to the period after the complete spinal section at T_{13}.

**General outline of the dual lesion paradigm**

Adult female cats (n = 5; 2.4–3.5 kg) were first selected for their ability to walk regularly and continuously for several minutes (10–15 min) on a motor-driven treadmill at a speed of 0.4 m/s. Thereafter, all cats underwent the dual spinal lesion paradigm, as shown in Fig. 1B. After control recording sessions (n = 3–10) to obtain baseline EMG and kinematic values for locomotion in the intact state, cats underwent a partial spinal lesion targeting the left side of the spinal cord at T_{10-T_{11}}. All cats were intensively trained on a treadmill after the partial lesion for a period ranging from 21 to 95 days. The training regimen consisted of 20–30 min of quadrupedal locomotion on the treadmill, 3–5 days/wk. During each session, the maximal treadmill speed that animals could maintain for several successive steps was evaluated. When no more noticeable changes in the various locomotor parameters (cycle duration, step length, etc.) were observed from day to day, a complete transection of the spinal cord (i.e., spinalization) was performed at T_{13} (2–3 segments below the partial lesion, as shown in Fig. 1A). In all cats, hindlimb locomotion was evaluated as early as 24 h after spinalization, with the forelimbs kept on a stationary platform fixed ~2 cm above the treadmill. After spinalization, all five cats were again regularly trained on the treadmill, and recordings were made for 3–4 more weeks. At the conclusion of the experiments, the spinal cord was removed and processed (40-μm serial spinal cord sections using cresyl violet) to assess the extent of the partial lesion (Fig. 1A).

**Kinematic and EMG recordings**

During episodes of locomotion, cats were recorded from the left side with a digital video camera, and data were stored on DVD support. Video images were de-interlaced to yield 60 fields/s or 16.6 ms between fields. Reflective markers were placed on the left and right hindfeet at the tip of the toes. Periods of stance and swing for the fore- and hindlimbs were determined by visually tagging foot contacts and lift-offs from video recordings. 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 computer. Kinematics and EMG were synchronized using a SMPTE (Society of Motion Picture and Television Engineers) time code generator.

Step cycle duration (or period) was measured as the time between two successive stance onsets (i.e., foot contacts). Stance duration was calculated as the time between foot contacts and toe offs (i.e., swing onset). The onset of swing was defined as the onset of forward foot movement until the next foot contact.

Step length was calculated using stance onset as reference. It was measured by adding the distance traveled by the toe during two successive paw contacts of the same limb (i.e., the distance traveled by the limb during the stance and swing phases of a complete step cycle). Horizontal movements of the whole cat on the treadmill were taken into account using a hip marker as reference.

Footfall patterns were established by determining successive paw contacts of each limb for several step cycles and plotted either as duty cycles or foot fall diagrams that indicate the number of paws in contact with the ground in the various subphases of the step cycle. From this, the percentage of the cycle during which the cats had two, three, or four feet in contact with the belt (respectively, defined as bipod, tripod, or tetrapod contact patterns) was determined.

Homolateral phase coupling 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 side, respectively. During a locomotor episode, the mean phase relationship was used to plot changes over time, and circular statistics were used to establish changes in the mean direction of the vectors representing the phase of various events relative to the normalized cycle. With this method, a 0 value indicates that the kinematic events occurred simultaneously with the trigger event used to define the cycle (foot contact), whereas a value of 0.5 indicates that the events occurred out-of-phase in an alternate manner.

Homologous phase coupling of the hindlimbs or the forelimbs was calculated from the time of left and right contact of the limbs at each girdle divided by the cycle period of the respective girdle using the left limb as reference.

Foot drag is often observed after a spinal lesion. Foot drag was quantified as the percentage of time that a given hindpaw was touching the treadmill belt during swing and also as the percentage of steps within a sequence (i.e., 10–20 cycles) in which foot drag could be observed.

**Statistical analysis**

All statistical analyses were performed using a minimum of 10 values. Changes and asymmetries in the coupling between limbs were analyzed with circular statistics (Watson-Williams F-test) using Oriana (KCS). Other statistical analyses (1-way ANOVA, unpaired t-test) were performed using Prism software (Graphpad Software). The significance threshold was set at P < 0.05 in all cases. Insofar as our experiments are based on a dual lesion paradigm involving three different states, statistical analyses in one state were performed using the previous state as reference. Thus data obtained in the partial spinal state were compared with the intact state, and in all figures, this statistical significance is indicated by asterisks. Data collected after spinalization were compared with the values obtained during the last experiment performed in the partial spinal state, and in all figures, this statistical significance is indicated by the symbol #. All graphical representations are means ± SD.
RESULTS

Extent of the partial spinal lesions

Although the aim was to perform a unilateral hemisection of the left spinal cord, postmortem histological examination of serial spinal sections showed that the extent of the partial lesion varied between cats (Fig. 1A). We can only speculate as to the tracts that were damaged based on known anatomy (see Fig. 3 in Rossignol et al. 2009). In cats GB5 and GB8, the partial lesion was mostly confined to the left dorsal/dorsolateral region, which contains

FIG. 2. Episodes of treadmill locomotion at different time points in the dual spinal lesion paradigm in cats GB5 and GB6. Top traces are EMGs obtained bilaterally from hindlimb flexor and extensor muscles in the intact (A and E), partial (B and F), early (C and G), and late (D and H) spinal states. Duty cycles (black horizontal bars) below the EMGs show the support periods (i.e., stance) of each limb. Numbers above duty cycles in B and F indicate the sequential order of cycles in hindlimbs and forelimbs and show the maintenance (GB5) and loss (GB6) of antero-posterior coupling in these cats following the partial lesion. l, left; r, right; Srt, sartorius; VL, vastus lateralis; St, semitendinosus; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; HL, hindlimb; FL, forelimb; l, left; r, right.
corticospinal and rubrospinal tracts. In cats GB3 and GB7, the left lateral hemisection was nearly complete, affecting the dorsal/dorsolateral quadrant and most of the ventral/ventrolateral descending reticulo- and vestibulospinal pathways. In both cases, however, part of the ventromedial spinal cord remained intact. Finally, cat GB6 showed extensive bilateral damage to the spinal cord in which only the ventromedial region on the right side was spared. Despite this variability, in all cats, the left side of the spinal cord was clearly more damaged than the right side. From a functional point of view, cats GB5, GB7, and GB8 had several similarities whereas cats GB6 and GB3 were more similar to each other, as will be seen later. The subsequent spinalization at T13 was complete because the spinal lesion gap and the bottom of the vertebral canal were seen under microscope. In addition, axonal regrowth was prevented by densely packing the lesion with Surgicel.

Overview of locomotor recovery

All cats re-expressed voluntary quadrupedal locomotion after the partial lesion, despite variability in lesion extent. In the first few days after the partial lesion, the left hindlimb did not walk. However, within the first week, four of the five cats recovered voluntary quadrupedal locomotion on the treadmill. In cat GB6, which had the largest partial lesion (Fig. 1A), both hindlimbs were initially paretic and, although slight perineal stimulation could elicit bilateral hindlimb locomotion within

**FIG. 3.** Footfall patterns at various time points for cats GB5 and GB6. A–C: footfall diagrams in cat GB5 in the intact state, 1 and 18 days after the partial lesion. Two consecutive steps are shown for each condition. Filled horizontal rectangles represent the stance phase of each limb; left (l) and right (r) hindlimb (HL) and forelimb (FL), respectively. Vertical lines divide the step cycles according to the number of supporting limbs, shown, as well, in the bottom in which circles represent contact with the treadmill (black) or not (gray). D and E: quantification of the gait pattern after the partial lesion in cats GB5 and GB6. Stack bar representation of the total percent of time the cat is supported by 2 (light gray), 3 (dark gray), or 4 (black) limbs. Percentages were calculated from a range of 11–43 step cycles in cat GB5 and 13–59 step cycles in cat GB6.
the first week, voluntary quadrupedal locomotion recovered only after 2 wk of treadmill training. Following the partial lesion, two groups of cats could be distinguished based on gross observation of locomotor episodes. In a first group (GB5, GB7, and GB8), episodes of quadrupedal locomotion were characterized by the maintenance of a 1:1 coupling of the fore- and hindlimbs, whereas in the other group (GB3 and GB6), we observed a forelimb/hindlimb uncoupling that persisted over time.

The two types of quadrupedal locomotor patterns are shown in Fig. 2, showing one cat from each group (GB5 and GB6) walking at a comfortable speed of 0.4 m/s before (Fig. 2, A and E) and 16 or 18 days after the partial lesion (Fig. 2, B and F). In cat GB6, the fore- and hindlimbs walked at two different rhythms (note the difference in duty cycles between Fig. 2, E and F), whereas in cat GB5, the same rhythm was maintained in the fore- and hindlimbs (compare duty cycles in Fig. 2, A and B). Therefore the locomotor recovery of all four limbs can involve different degrees of intergirdle coordination after the partial lesion, which is further documented below. In addition, cats from the smaller lesion group recovered the ability to maintain lateral equilibrium during locomotion and walk unassisted. In contrast, in cats GB3 and GB6, lateral stability was generally impaired. Despite the fact that they could occasionally walk unassisted for several step cycles, this postural deficit never completely recovered, and the experimenter was required to gently hold the tail, to provide postural assistance, the majority of the time.

**Footfall patterns and coupling of fore- and hindlimbs**

Figure 3, A–C, shows typical consecutive footfall patterns in the intact state and for 2 selected days after the partial lesion. In the intact state, the pattern of paw contact is accomplished primarily by an alternating tripod and bipod foot contact patterns with a sequence of left hindlimb, left forelimb, right hindlimb, and right forelimb contact (Fig. 3A). The footfall pattern changes after the partial lesion. In cat GB5, because of the phase advance of the left forelimb relative to the left hindlimb, there is a general tendency to have more bipod patterns, going from ~15% in the intact state to close to 40% of the cycle 16–17 days after the partial lesion (Fig. 3D). Although more variable, a tetrapod foot contact pattern is also seen more frequently after the partial lesion. In cat GB6 (Fig. 3D), after the partial lesion, there is initially a greater proportion of bipod contact patterns, which gradually decreases over time.

In Fig. 3, A and C, the change in quadrupedal coupling pattern is caused principally by a shift in the coupling between limbs on the left side, because this coupling was practically unchanged on the right side, although right hindlimb stance was somewhat longer. This shift brings both the left forelimb and hindlimb to contact the ground more or less at the same time, as in a pacing gait (i.e., homolateral synchrony). Thus cat GB5 adopted a pacing gait on the left while maintaining the usual fore-hindlimb coupling on the right side, which resulted in an overall uncoordinated gait. However, these changes maintained a similar step frequency in the fore- and hindlimbs.

Figure 4 shows in detail the homolateral coupling in three cats (GB5, GB8, and GB7) that maintained a 1:1 cycle period ratio between the fore- and hindlimbs after the partial lesion. In cats GB5 and GB8, homolateral coupling on the left and right sides was around 0.2 before the partial lesion (i.e., the left forelimb made contact with the ground at phase 0.2 after left hindlimb contact in the normalized step cycle, defined by successive left hindlimb contacts). After the partial spinal lesion, homolateral coupling on the left side decreased significantly in cats GB5 and GB8, so that a pacing pattern was observed most of the time on this side. Cat GB7, which had a
larger partial lesion, showed a shift in homolateral coupling on the left and right sides, leading to pacing on the right side and even a reversal of homolateral coupling on the left side, so that the left forelimb contacted the ground before the left hindlimb (i.e., a negative phase value), a pattern that was maintained throughout the post-partial lesion period. This type of pattern was also briefly observed early after the partial lesion in GB8. However, despite these changes in homolateral coupling, the same cycle period was maintained between the fore- and hindlimbs, as shown in Fig. 5A for GB5.

On the other hand, cats GB3 and GB6 did not maintain a 1:1 coordination between the fore- and hindlimbs. This is shown in Fig. 5B for cat GB6. During the first 2 wk after the partial lesion, there was an initial decrease in cycle period in the fore- and hindlimbs, but over time, the forelimbs maintained a higher cadence, whereas hindlimb cycle period increased, reaching values significantly higher than controls. A more important functional disconnection between centers controlling the fore- and hindlimb rhythms in cat GB6 could be caused by the large partial lesion (see Fig. 1).

Finally, the coupling between left and right forelimbs and between left and right hindlimbs were measured. Figure 6, A–E, clearly shows that the coupling between the two forelimbs (△) remained practically unchanged after the partial lesion in all cats, with values around 0.5 phase, meaning that there was a sustained out-of-phase coupling between forelimbs. The situation differed for hindlimb coupling, depending on the cat. In cats GB5, GB7, and GB8 (i.e., the group of cats that maintained a 1:1 ratio between the fore- and hindlimbs), hindlimb coupling shifted downward, below 0.5, indicating that, relative to left foot contact, the right hindlimb contacted the ground earlier than in the control period. Cats GB3 and GB6, on the other hand, showed a normal alternating coupling close to control values over time after the partial lesion. However, it should be remembered that, in these two cats, the step frequency was higher in the forelimbs than in the hindlimbs.

In summary, after the partial spinal lesion at T10–T11, cats could adopt two different quadrupedal walking strategies, which depended on whether a 1:1 coordination between the forelimbs and hindlimbs was maintained.

**Hindlimb kinematics after partial and complete spinal lesions**

**Step lengths of the hindlimbs.** Figure 7A shows that the step length (see METHODS) of the left hindlimb was decreased for the first 4 days after the partial lesion, whereas right hindlimb step length was similar throughout the partial spinal lesion period in cat GB5. However, the right hindlimb generally performed steps of longer lengths compared with the left hindlimb. Of interest, after spinalization, the asymmetrical step length pattern was reversed in the first 2 wk so that the left hindlimb performed longer steps than the right hindlimb.

In cat GB6, the step lengths of both hindlimbs decreased symmetrically after the partial lesion before increasing above control levels (Fig. 7B), although the increase was greater on the right side. After spinalization, hindlimb step lengths were decreased bilaterally compared with the last day of the partial lesion and there was no significant difference between hindlimbs (Fig. 7D). Therefore after smaller partial lesions, an asymmetry in step length could persist and be reversed after spinalization, whereas with larger partial lesions, changes in step length were more symmetrical.

**Duration of hindlimb step cycles and their subphases (stance and swing).** There was an asymmetry in step length between hindlimbs for cat GB5 after the partial lesion (Fig. 7A), although step cycle duration was similar bilaterally (Fig. 5A), indicating changes within subphases of the cycle. Figure 8, A–C, shows that the shorter cycle duration of the left hindlimb resulted from a decreased stance duration proportionally greater than the increase in swing duration. On the right side, stance phase duration was more or less constant throughout the partial lesion period, but swing phase duration decreased. This implies that changes in the velocity of the subphases of the cycle, most likely swing, occurred bilaterally to maintain left/right coordination. After day 5, there was a persistent asymmetry between hindlimbs (Fig. 8B): stance phase duration of the right hindlimb remained near control values, whereas stance phase duration on the left side remained decreased. As expected, swing phase duration on the left side remained longer than control values, whereas swing phase duration on the right side remained shorter. Cats GB5, GB7, and GB8 generally followed this pattern.

After spinalization in cat GB5, cycle period was decreased (Fig. 8D), because of a decrease in both stance (Fig. 8E) and swing (Fig. 8F) phase durations. Interestingly, stance phase...
duration on the left side remained the same throughout the partial and complete spinal periods. However, on the right side, stance phase duration decreased after spinalization. Thus after the partial spinal lesion, stance phase duration decreased on the left side but remained unchanged on the right side. In contrast, after spinalization, stance phase duration decreased on the right side but remained unchanged on the left side compared with the last partial lesion day. As for swing phases, there was a complete reversal between the partial lesion and complete spinal lesion periods, with longer swing phase durations on the right side compared with the left.

A similar analysis was performed in cat GB6, which had the largest partial lesion. Cycle duration initially decreased after the partial lesion but returned to, and exceeded, control values in subsequent weeks (Fig. 9A). The initial decrease and subsequent increase was achieved by a bilateral reduction of stance (Fig. 9B) and increase of swing (Fig. 9C) phase durations. Essentially, the cat was making coordinated shorter steps on both sides early after the lesion followed by longer symmetrical steps on both sides after 3 wk. After spinalization, cycle period was permanently reduced (Fig. 9D) because of a decrease in stance (Fig. 9E) and swing (Fig. 9F) phase durations bilaterally, after some initial variability in the first few days. A similar pattern was found in GB3, although initial changes were much smaller following the partial lesion (data not shown).

**PAW DRAG.** The evaluation of the subphases of the step cycle must take into account paw drag of the hindlimbs that can be observed after the partial and complete spinal lesions. As explained in METHODS, swing onset was determined by the forward movement of the foot and the onset of stance by foot contact. In normal (i.e., spinal intact) cats, these events correspond to moments of foot contact or foot lift-off, which can be

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**Fig. 6.** Fore- and hindlimb left/right coupling at different time points after the partial lesion in all cats. A–E: mean forelimb (FL, △) and hindlimb (HL, ○) left/right phase relationships were calculated from treadmill contact events using the left side as reference. F: schematic representation of the phase relationships calculated between the fore- and hindlimbs. Black and white asterisks indicate phase relationships that were significantly different from control (intact) at the fore- and hindlimb levels, respectively.
Fig. 11, caused by a change in coupling between hindlimbs. Indeed, pedal coupling was nonetheless altered, which was primarily, and cats GB5, GB7 forelimbs and hindlimbs (lesion in cats that maintained a same cycle duration in the perfectly alternating pattern. As shown, after the partial coupling between hindlimbs is around 0.5, which is typical of for some days appearing as clear outliers. The dag was largely the same before and after spinalization, except GB5 had it been trained longer. In month of training, which could have also been the case for the partial lesion was less pronounced and subsided after 1 more or less the same overall pattern except that paw drag after hindlimbs dragged during swing for most step cycles. Of interest is that both the right and left same on the left side as it was before, but now the right hindlimb also drags. Of interest is that both the right and left hindlimbs dragged during swing for most step cycles. GB8 had more or less the same overall pattern except that paw drag after the partial lesion was less pronounced and subsided after 1 month of training, which could have also been the case for GB5 had it been trained longer. In cat GB6, the pattern of foot drag was largely the same before and after spinalization, except for some days appearing as clear outliers.

INTERLIMB COUPLING OF THE HINDQUARTERS. The normal phase coupling between hindlimbs is around 0.5, which is typical of a perfectly alternating pattern. As shown, after the partial lesion in cats that maintained a same cycle duration in the forelimbs and hindlimbs (cats GB5, GB7, and GB8), quadrupedal coupling was nonetheless altered, which was primarily caused by a change in coupling between hindlimbs. Indeed, Fig. 11, A–C, shows that the coupling phase stabilized at around 0.4 after the partial lesion. However, early after spinalization, the coupling remarkably returned to a value of 0.5, as in the intact state (Figs. 11, F–H). Only cat GB8 developed another hindlimb asymmetry in phase coupling some 10 days after spinalization (see decreasing phase coupling). In cats that had two different step cycle frequencies in the forelimbs and hindlimbs, hindlimb phase coupling remained around 0.5 after the partial and complete lesions (Fig. 11, D, E, I, and J).

To summarize, after the partial lesion, there were several left/right asymmetries in certain parameters of the locomotor pattern that persisted over time. After spinalization, all cats could walk with their hindlimbs at the first recording session (i.e., within 24 h) in a coordinated pattern. However, left/right asymmetries observed after the partial lesion often reversed (i.e., from right to left) after spinalization, which indicates that complex dynamic interactions were involved in locomotor recovery after the partial spinal lesion.

DISCUSSION

The aims of this study were to evaluate changes in locomotor kinematics of all four limbs after a partial spinal cord lesion at the thoracic level and to study how hindlimb locomotor parameters evolved after a second but complete spinal section two segments below the partial lesion. After spinalization, not only could the cats perform hindlimb walking within 24 h (i.e., the 1st testing session) but left/right compensatory changes observed after the partial lesion (e.g., interlimb coupling, swing/stance durations) were often reversed. The “reversal” of
Compensatory changes after the partial lesion

Kinematic changes after the partial spinal lesion indicate that cats adopted two very different adaptive strategies. In one strategy, a 1:1 coordination between girdles was maintained by reorganizing homolateral coupling of the fore- and hindlimbs, as well as hindlimb coupling. In the other strategy, the fore- and hindlimbs were uncoupled and walked at different rhythms, which can lead to more stumbling, as seen with partial but large bilateral ventral spinal lesions (Brustein and Rossignol 1998). Although the partial spinal lesions were unavoidably variable between animals, cats could be classified as favoring one strategy over the other based on lesion extent. With smaller lesions a 1:1 ratio between the fore- and hindlimbs was maintained, whereas larger lesions led to an uncoupling between the fore- and hindlimbs, even though some descending tracts remained on the right side.

Some of the deficits and adaptations observed were consistent with previous studies investigating locomotor recovery after specific spinal lesions. For instance, the loss of corticospinal and rubrospinal pathways altered the coupling between hip and knee flexor muscles and induced foot drag (Jiang and Drew 1996; Rossignol et al. 1999), a deficit that is not observed after ventral/ventrolateral lesions (Brustein and Rossignol 1998). In addition, damage to the dorsal columns may also partly account for changes in forelimb–hindlimb coordination, as previously discussed (English 1980; Rossignol et al. 2004). However, the major deficit in forelimb–hindlimb coupling in cats GB3 and GB6, associated with reduced weight support, inferred by reduced stance phases, resembled those reported after ventrolateral spinal lesions affecting vestibulo- and reticulospinal pathways (Brustein and Rossignol 1998). Finally, damage to propriospinal pathways may also have important effects.
caused by the importance of these pathways, at least for quadrupedal locomotion in rodents and cats (Courtine et al. 2008, 2009; Cowley et al. 2008; Kato et al. 1984; Sherrington and Laslett 1903; Zaporozhets et al. 2006).

Compensatory changes after spinalization

All cats walked on the treadmill at a very early stage after spinalization, as recently shown (Barrière et al. 2008), which indicates that the spinal locomotor circuitry was configured in such a way that it could operate without descending influences from supraspinal or long propriospinal sources. However, one of the key observations of this work is that left/right asymmetries seen after the partial spinal lesion disappeared or even reversed in direction after the complete spinal transection. Examples of this can be found in Figs. 7, A and C, and 8, C and F.

Reversals of left/right asymmetries after spinalization indicate that the optimization of locomotion after an incomplete spinal cord injury is achieved through new dynamic interactions between supraspinal and spinal circuits. The power of the present dual spinal lesion approach is to underscore that some of the changes that occurred at the spinal level were maintained or induced by descending pathways. These changes were only shown by the spinalization.

Mechanisms of spinal asymmetries

The mechanisms possibly responsible for the left/right asymmetries in the spinal cord may fall in three different categories.

ASYMMETRIC DESCENDING INPUTS. An operant conditioning paradigm in which animals learn to unilaterally decrease or increase H-reflex amplitude in an ankle extensor induces an asymmetric “memory trace” of the learned task within the spinal cord, shown by a spinalization, in anesthetized monkeys (Wolpaw and Lee 1989). Operant conditioning depends strongly on descending pathways, especially corticospinal inputs (Chen and Wolpaw 2002; Chen et al. 2002, 2006), which are thought to trigger enduring asymmetrical
changes within the spinal cord. Of particular interest was the demonstration that the same operant conditioning paradigm could compensate for an asymmetrical hindlimb coupling in rats with a unilateral thoracic spinal lesion (Chen et al. 2006).

Anatomical reorganization of connection patterns between supraspinal centers and the spinal cord can also lead to the formation of new neural circuits (Ballermann and Fouad 2006; Bareyre et al. 2004; Belhaj-Saif and Cheney 2000; Courtoine et al. 2008; Fouad et al. 2001; Raineteau et al. 2002). From a theoretical point of view, such mechanisms could “re-route” descending information through undamaged descending pathways. Second, compensatory mechanisms occurring within supraspinal structures may also be involved (Raineteau and Schwab 2001). For example, increased corticospinal efficacy paralleled the improvement of locomotor movements after cutaneous denervation of the hindpaw in the cat (Bretzner and Drew 2005) and after several weeks of locomotor training in incomplete spinal cord–injured human patients (Thomas and Gorassini 2005).

The reversal of certain locomotor parameters, such as paw drag and stance/swing durations, suggests that some of the adaptation after the partial lesion was maintained by remaining descending pathways, from supraspinal or propriospinal sources on the ipsi and/or contralateral sides. Therefore the paw drag on the left may recover because of intact contralateral pathways, and the spinalization shows that descending pathways were actively maintaining this compensation.

FIG. 10. Hindlimb paw drag after the partial lesion and spinalization in cats GB5, GB8, and GB6. Open and filled squares represent the percentage of the step cycle during which the paw drags along the treadmill during swing in the left and right hindlimbs, respectively, in cats GB5 (A), GB8 (B), and GB6 (C). Open and filled circles indicate the mean duration of the paw drag relative to swing duration. Hindlimb paw drag was calculated from 11–44, 14–51, and 15–53 step cycles in cats GB5, GB8, and GB6, respectively.
ASYMMETRICAL REFLEX PATHWAYS. Asymmetrical changes in sensorimotor integration at the spinal cord level may contribute to the asymmetrical output expressed after spinalization. From an anatomical point of view, sprouting of sensory afferent terminals on the side of the lesion was shown in hemisected animals (Goldberger and Murray 1974; Helgren and Goldberger 1993). These asymmetrical inputs could compensate for the loss of descending excitatory inputs to the cord. This hypothesis is strengthened by the observation that segmental reflexes became asymmetric after a spinal hemisection in rats or cats (Hultborn and Malmsten 1983a,b; Malmsten 1983; Muir et al. 1998).
Of great interest was the finding that bilateral cutaneous reflex responses were modified asymmetrically in the cats used in these experiments (GB3, GB6, GB7, and GB8) after the partial spinal lesion and that the asymmetry persisted for some days after spinalization, before reflex responses increased symmetrically on both sides (Frigon et al. 2009). Reflex changes over the course of the dual lesion paradigm also provide some indication of how different pathways are regulated before and after spinal cord injury. For instance, longer-latency excitatory responses (P2) in hindlimb flexor muscles, evoked by stimulating the ipsilateral superficial peroneal (SP) nerve, are depressed after a partial lesion, whereas P2 responses in ipsilateral extensors are unchanged or increased. Thus a given cutaneous reflex pathway has differential projections to flexors and extensors, with a strong supraspinal contribution to flexor muscles, which was also shown after a complete spinal transection (Frigon and Rossignol 2008a). The apparition of short-latency excitatory responses (P1) during the stance phase, instead of the more common inhibitory responses (N1), also showed that interactions between cutaneous reflex pathways and the spinal CPG central pattern generator (CPG) are modified after a partial (Frigon et al. 2009) or complete (Frigon and Rossignol 2008a) spinal lesion. Evidently, these changes could be caused by the loss of specific projections from supraspinal structures, which are known to influence reflex and spinal excitability (Holmqvist and Lundberg 1961).

UNCOVERING ASYMMETRIES WITHIN SPINAL CIRCUITS. There are a number of examples showing asymmetrical changes imprinted within the spinal cord that are shown after a complete spinal transection.

For example, we performed a spinalization before or after a unilateral partial denervation of ankle extensor muscles (Frigon and Rossignol 2008b, 2009). It was shown that the spinal network could compensate for the denervation in cats that previously recovered hindlimb locomotion after spinalization. On the other hand, the re-expression of hindlimb locomotion could be drastically impaired and even antagonized in cats that had recovered from a denervation before spinalization. These experiments clearly highlight the impact of past experiences in the recovery of locomotion and that major spinal cord changes were uncovered by the spinalization.

Cats with a unilateral denervation of ankle flexors on one side will appear to have a more or less normal symmetrical hindlimb locomotor pattern. However, after spinalization, the locomotor pattern, which is usually symmetrical, is dominated by very large hyperflexions on the side of the ankle flexor denervation, thus hampering locomotion (Carrier et al. 1997). This suggests that, throughout the recovery period after the denervation, supraspinal and spinal mechanisms were at play to maintain a symmetrical locomotor pattern and that the asymmetry was shown only after spinalization (Carrier et al. 1997). Similarly, complete cutaneous denervation of the hindpaws may be largely offset in otherwise normal cats but spinalization shows underlying changes that have occurred in the spinal cord. These changes may affect the ability of the cat to place the foot or even to express symmetrical walking and cutaneous reflexes (Bouyer and Rossignol 2003a,b).

What was so striking in this series of experiments was the observation that compensatory changes that occurred after the partial lesion could actually reverse in direction after spinalization, suggesting that a new left/right equilibrium was achieved in the spinal cord and that new dynamic interactions had been established with supraspinal descending pathways.

Intrinsic changes within the spinal cord are reminiscent of the Bechterew effect, a well-known phenomenon in the vestibular field. After removing the labyrinth on one side, an asymmetrical posture developed, which included limb flexion on the lesioned side and extension on the intact side. This asymmetry normalized over time. A subsequent lesion of the labyrinth on the other side induced a postural reaction on the newly lesioned side, although all vestibular inputs were now absent. Later studies showed that circuit and biochemical plasticity at different level of the CNS, including the spinal cord, may contribute to vestibular compensation (Deliagina 1997a,b; Dieringer 1995; Pavlova and Deliagina 2003; Pavlova et al. 2004; Straka and Dieringer 1995; Vibert et al. 1999a,b). Of particular interest is a paper (Galiana et al. 1984) showing how a simple change in the weighting of excitability between the vestibular nuclei could account for the Bechterew phenomenon.

Recent work in an invertebrate CPG also shows how a new balance between left and right sides can be established in the escape behavior of tritonia (Sakurai and Katz 2009). In this preparation, it is possible to study in detail changes in CPG connectivity after a lesion disconnecting the swim circuit on both sides. The work elegantly shows that the return of the behavior, only a few hours after the lesion, results from physiological changes in synaptic weighting and sign in ganglionic circuits before any anatomical changes (sprouting or regeneration) could occur. Consequently, there are potentially several left/right circuits in the spinal cord of higher vertebrates that are modified after unilateral lesions. It is probable that this new intraspinal balance is induced and maintained by descending circuits and afferent inputs to optimize function and symmetry. The spinalization allows us to unmask some of the changes that occurred within the spinal cord.

In conclusion, the main point is that the spinal cord again achieved in the spinal cord and that new dynamic interactions had been established with supraspinal descending pathways.

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DISCLOSURES

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

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