A dual spinal cord lesion paradigm to study spinal locomotor plasticity in the cat

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After a complete spinal cord injury (SCI) at the lowest thoracic level (T13), adult cats trained to walk on a treadmill can recover hindlimb locomotion within 2–3 weeks, resulting from the activity of a spinal circuitry termed the central pattern generator (CPG). The role of this spinal circuitry in the recovery of locomotion after partial SCIs, when part of descending pathways can still access the CPG, is not yet fully understood. Using a dual spinal lesion paradigm (first hemisection at T10 followed three weeks after by a complete spinalization at T13), we showed that major changes occurred in this locomotor spinal circuitry. These plastic changes at the spinal cord level could participate in the recovery of locomotion after partial SCI. This short review describes the main findings of this paradigm in adult cats.

Keywords: central pattern generator; plasticity; training; spinal cord injury; locomotion

Introduction

Hindlimb locomotion is controlled by a tripartite system that involves dynamic interactions among a spinal neuronal network, supraspinal structures, and peripheral sensory inputs.1–5 Following incomplete spinal cord injury (SCI), this tripartite interaction is perturbed, but animals can recover a surprising degree of locomotor performance, which probably involves widespread plastic changes within remaining ascending and descending pathways,6–9 segmental spinal reflexes,10 and the spinal cord itself.11,12 While many studies emphasize the compensatory role of supraspinal structures in functional recovery after partial SCI, there is little evidence indicating how plastic changes within the spinal circuitry itself can account for some of the locomotor recovery after SCI. This review focuses on locomotor recovery and plasticity after partial SCI by describing some fundamental concepts that emerged from complete SCIs in cats. Adaptation within the spinal locomotor circuitry after partial SCI is then discussed.

Complete spinal lesions and the role of the CPG

In many species, some recovery of hindlimb locomotion can be observed after a complete spinal cord section.1,2 Since locomotion can be expressed in kittens spinalized 7–14 days after birth, that is, before they gain their optimal walking capacities, one can suggest that this behavior is genetically programmed.13–15 After a complete SCI at the level of the last thoracic segment (T13), cats can recover hindlimb locomotion that closely resembles a normal locomotor pattern by using electrical stimulation,16,17 locomotor training,18,19 pharmacological agents,20–24 or a combination of the above.25 This, per se, confirms the concept of spinal generation of locomotion by an intrinsic spinal circuitry at the lumbosacral level, the central pattern generator (CPG). Since descending and ascending pathways are completely destroyed after a complete SCI, some intrinsic changes must have occurred in the CPG to allow the re-expression of hindlimb locomotion. After a complete SCI, the excitability of motoneurons,26,27


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Figure 1. General methodology for the study of locomotion in cats. The animal is placed on the treadmill (arrows within the belt indicate the direction of movement) and is allowed to walk with all four limbs. Pairs of EMG wires are implanted into various muscles (only one pair is represented here) and soldered to a multipin connector cemented to the skull. The EMG signal was filtered and digitized and was then displayed on a computer. The left panel illustrates the EMG trace of the sartorius (hip flexor and knee extensor). Reflective markers are placed at various points on the limb, and the angle measurements are taken in the indicated orientations. For each video field (16.7 milliseconds between fields), the coordinates of the reflective markers are obtained and the hindlimb movement is reconstructed as indicated in the kinematic model (MTP = metatarsophalangeal joint). A seventh point is put on the right hindpaw to obtain kinematic data for this limb and information relative to coordination between hindlimbs. From such data, the swing and stance phases of each cycle can be reconstructed as shown in stick figures and duty cycles. The foot contact and lift are also measured to determine cycle length and duration and also to synchronize EMG events when needed. A digital time code (SMPTE) is used to synchronize video and EMG recordings.

cutaneous reflexes, and receptors of various neurotransmitter systems are changed.

Recovery from partial spinal lesions
Considering that incomplete SCIs are more frequent than complete SCIs in humans, we asked whether the spinal mechanisms observed after complete SCIs also apply to hindlimb locomotion generated after partial SCI. To accurately evaluate the locomotor pattern after spinal lesions, the most objective method consists of using chronic electromyographic recordings combined with synchronized video recordings of movements (Fig. 1). This method permits a powerful quantitative comparison of locomotor performances before and after spinal lesions, as well as its evolution over time. To evaluate the role of descending pathways in locomotor control, studies have investigated the consequences of lesions targeting specific tracts at the spinal level.

Dorsal/dorsolateral spinal tracts. After the dorsal/dorsolateral SCI’s bilateral effect on the cortico- and rubrospinal fibers, cats can recover voluntary quadrupedal locomotion while maintaining their weight and equilibrium after a 3- to 10-day period. They, however, have some long-lasting deficits such as foot drag (resulting from impaired coupling between hip and knee flexors) and they lose the capacity for anticipatory modifications when negotiating obstacles placed on the treadmill. Despite these defects, the cats do walk remarkably well with all four limbs on the treadmill, suggesting that the cortico- and rubrospinal tracts do not appear critical for the control of treadmill locomotion but are important for skilled locomotion.

Ventral/ventrolateral spinal tracts. Vestibulo- and reticulospinal pathways traveling in the ventral part of the cord are known to be, respectively, involved in postural control and initiation of locomotion. After small ventral/ventrolateral lesions, cats can walk voluntarily at speeds of up to 0.7 m/s with all four limbs one to three days after the lesion. With larger lesions, sparing only part of dorsal columns, cats behave initially as complete spinal cats. However, with regular treadmill training, all cats could...
regain voluntary quadrupedal locomotion but, with the largest lesions, the animals could not walk faster than 0.4 m/s even after several weeks of training. The important observation here is that, even with extensive bilateral lesions to ventral pathways, cats could initiate and maintain voluntary quadrupedal locomotion, suggesting that the ventral tracts do not appear essential for triggering hindlimb locomotion.

Hemisections. In contrast to bilateral dorsal or ventral lesions of the cord, unilateral hemisections damage the dorsal end ventral tracts on one side, while the other side remains intact. The functional consequences of such lesions mainly depend on the extent of the lesion. Indeed, we showed in cats hemisectioned at thoracic level 10 that the smaller the lesion the faster the locomotor recovery.\(^{1,11,32}\) During the first two to three days after a nearly complete hemisection on one side, the hindlimb ipsilateral to the lesion shows flaccid paresis and animals adopt a tripod gait, such that they require help for body support and stabilization. One week after hemisection, voluntary quadrupedal locomotion reappeared despite limping of the hindlimb and inconsistent plantar foot contact on the side of the lesion. Stepping activity greatly improves during the first three weeks posthemisection, but some deficits were shown to persist at this postoperative time. To minimize the time spent on the effected hindlimb, the support time was reduced on the side of the lesion while the swing phase was increased. Concomitantly, the burst duration of extensors decreased while that of the flexors increased on the side of the lesion.\(^{12}\) By plotting the relationships between the cycle and its subphases (stance and swing) at various treadmill speeds, we showed that the hemisection had induced profound changes in the intrinsic structure of the cycle on both sides and had thus altered the neural control of locomotion.\(^{33}\) Moreover, forelimb/hindlimb coordination on the side of the lesion and left/right hindlimb coupling was altered and cats exhibited an asymmetrical gait.\(^{12}\) The ipsilateral descending reflexes (scratch, vestibular drop reflex) were lost after hemisection and skilled locomotion such as ladder walking was also impaired durably on the side of the lesion.\(^{34–36}\) Altogether, although a gradual recovery of walking occurs after a spinal hemisection, some deficits associated with dorsal (postural deficits and altered coordination) and ventral lesions (impairied skilled locomotion) persist.

From the studies on partial spinal lesions described previously, we can conclude that although there are some deficits due to the interruption of specific tracts, animals can in most cases regain a functional locomotor pattern. Several nonexclusive mechanisms occurring at different levels of the neuraxis probably participate in locomotor recovery after a partial SCI such as physiological and/or anatomical reorganizations within remnant pathways could somehow take over the functions of the damaged spinal cord.\(^{37–43}\) However, an alternative interpretation is that after a partial SCI, the CPG below the lesion assumes a greater role in generating the hindlimb locomotor pattern. In this case, the plastic changes in descending pathways would serve to reorganize functionally the spinal cord and modify new input–output characteristics of the spinal CPG. This interpretation requires demonstrating that changes occur within the CPG after a partial lesion, which is discussed next.

**Role of the CPG after partial spinal lesions: the dual lesion paradigm**

A dual lesion paradigm has been developed\(^{11}\) to study the changes occurring within the lumbar spinal locomotor circuitry (CPG) after a partial spinal lesion. In this paradigm, a unilateral hemisection of the cord is first performed at T10–11, well above the CPG located in the lumbo-sacral segments, and is followed, several weeks later, by a second complete spinal lesion two spinal segments below the first one at T13 (Fig. 2) (i.e., at the level where the complete spinal lesions were usually made\(^{18,25,44}\)) to isolate the spinal CPG from its supraspinal influences. The main idea of this paradigm is that if intrinsic changes occurred within the spinal cord itself during locomotor recovery after the initial hemisection, these changes could probably be retained and expressed very early after a second and complete spinalization a few segments below. In the first study with this paradigm, cats were trained to walk on the treadmill during the interim between the two lesions and the second complete lesion was performed only when the locomotor performances of the cats reached a plateau of locomotor performance. The first major finding was that within 24 h (i.e., the first testing session) following complete spinalization, cats could reexpress...
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Figure 2. The dual lesion paradigm. (A) In intact cats, locomotion is controlled and modulated by descending inputs (red arrows) from supraspinal structures that interact with the spinal CPG (in gray) to coordinate the left (lHL, in red) and right (rHL, in green) hindlimb activity. (B) After a unilateral hemisection targeting the left part of the spinal cord at T10, descending inputs are disconnected from the CPG on the left side (red dotted arrows). While the right hindlimb remained actively controlled by the remnant supraspinal fibers, the left hindlimb is mainly under the control of the partially deafferented CPG. (C) Three weeks after the hemisection, a complete spinal section is performed at T13, removing all the supraspinal inputs to the CPG, such that the left and right hindlimbs are mainly under the control of the CPG completely devoid of supraspinal inputs.

hindlimb locomotion with plantar foot placement at high speeds (up to 1.0 m/s) without any pharmacological stimulation.\(^{11,32}\) It should again be recalled that under normal circumstances the expression of spinal locomotion requires two to three weeks of intense treadmill training in spinal cats.\(^{18}\) This result indicates that the hemisection had induced changes within the spinal circuitry below the lesion, such that it was already primed to reexpress locomotion after the complete SCI. As these cats were trained to walk during the interim between the two lesions and that this interim period varied among cats, it was important to standardize the conditions required to induce plastic changes in the spinal locomotor circuitry after hemisection.

Could such intraspinal changes occur spontaneously after the first partial lesion—that is, in the absence of training and within a short period of time between the two lesions? To address this question, the interim period between the two lesions was limited to a short period of three weeks in a cohort of 11 cats.\(^{12}\) To limit self-training that could influence locomotor recovery after hemisection, cats were kept in individual cages and their locomotor performances were recorded only once a week on the treadmill. Such housing condition limits sensorimotor experience and contrasts with the daily treadmill training procedure used in previous studies.\(^{11,32}\) Interestingly, 24 h after spinalization, 6/11 cats (55%) expressed a bilateral hindlimb locomotion, 3/11 expressed a unilateral pattern on the side of the previous hemisection, and 2/11 were not able to walk. These results indicate that the spinal circuitry below the hemisection can reorganize spontaneously and within a relatively short period of time after hemisection.

This result raised the question of the type of reorganizations occurring within the spinal locomotor circuitry after hemisection. To determine how the spinal circuits reorganize after hemisection, we investigated, over the entire dual lesion paradigm, the evolution of specific locomotor parameters mainly controlled by the spinal locomotor circuitry after hemisection. We measured the various subcomponents of the step cycle (swing, stance) (see, for instance, Fig. 3A) and plotted the relationships between the cycle and its subphases at various treadmill speeds. First, the results showed that after a hemisection, the intrinsic structure of the cycle is altered in both hindlimbs, such that the cycle period can change by adapting the duration of each subphase according to the speed. Second, removing all supraspinal inputs by spinalization revealed that the changes observed after hemisection were retained for a long time but...
Figure 3. Effect of locomotor training on hindlimb kinematics in completely spinalized cats previously submitted to a spinal hemisection on the left side. (A) Illustrations of the kinematic measures. Step cycle duration is the time between two successive contacts of the same foot on the treadmill, whereas the stance duration refers to the time between foot contact and toe off that initiates the swing phase. Step length is the distance traveled during the stance and swing phase of a complete step cycle. Toe position relative to the hip at contact 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. As the first spinal lesion (the hemisection on the left side) is known to induce an asymmetrical walking pattern, the locomotor parameters described previously were compared between hindlimbs by calculating an asymmetry index (AI). An AI of 0 indicates a perfect symmetry, and in normal conditions, the AIs obtained in all parameters are equal to 0 ± 0.05. The AI gives information on the direction of asymmetry. When a parameter is greater on the left than on the right side, the AI will be positive and vice versa. For example, in the case of a step length AI < 0, the step of the right hindlimb will be longer than the left. (B) Evolution of the asymmetry indices calculated for cycle duration, step length, stance duration, and toe position at contact in cats trained to locomote after spinalization and untrained cats over the entire dual lesion paradigm. In the intact state, the two groups of cats exhibited a symmetrical walking pattern (AIs = 0 ± 0.05). Twenty-one days after a hemisection on the left side of the cord, both groups displayed an asymmetrical walking pattern due to the deficits of the left hindlimb (side of the lesion). Note that both groups were comparable before the spinalization and the beginning of training. Twenty-one days after spinalization, the asymmetrical walking pattern displayed by all cats after hemisection was retained in untrained cats, while it reversed in trained cats. Statistical differences between delays are indicated by the symbol (*). Statistical differences between groups are indicated by the symbol (#). L, left; R, right.
only on the side of the previous hemisection, thus demonstrating a durable asymmetrical reorganization at the spinal level resulting from the previous partial spinal lesion. In addition, the asymmetrical walking pattern observed after hemisection was also retained by cats after the complete SCI, such that the hindlimb on the side of the previous hemisection kept its deficits after spinalization. Moreover, some changes that occurred in simple cutaneous reflexes after the partial lesion were also retained following spinalization. These carry-over changes that result from the previous partial SCI indicated that the spinal cord has an intrinsic capacity to be imprinted by past experiences.

However, can a spinal cord previously modified by past experience (such as a hemisection) again adapt to new demands, or are these changes immutable? To address this question, the performance of cats subjected to treadmill training after the complete SCI were compared with that of untrained cats during the entire dual lesion paradigm. While without training the asymmetrical locomotion induced by the previous unilateral hemisection was retained for three weeks after the subsequent spinalization, training cats after spinalization reversed the asymmetrical locomotor pattern induced, suggesting that new plastic changes occurred within the spinal cord in response to training. Moreover, training cats after spinalization was shown to improve the locomotor performance of the hindlimb previously affected by the hemisection. These results demonstrated that a spinal cord previously modified by past experience (such as after a hemisection) can remarkably adapt to new demands imposed by locomotor training and suggest that locomotor training can be beneficial in the spinal state regardless of the previous experience of the spinal cord.

Considering the role of locomotor training in shaping intraspinal plasticity in cats previously submitted to spinal hemisection, can the locomotor deficits and asymmetrical intraspinal changes observed after hemisection be compensated by locomotor training? In the most recent study, we evaluated the effect of treadmill training on locomotor plasticity after hemisection. We compared the functional locomotor outcome of eight cats trained to locomote for three weeks after hemisection, and eight untrained cats that served as control. The activity-induced intraspinal plasticity was then assessed by comparing the locomotor performances of both groups of cats 24 h after the subsequent spinalization. We confirmed that a three-week period of locomotor training after hemisection had a beneficial role in the recovery of voluntary quadrupedal locomotion. Furthermore, locomotor training enhanced plasticity in the spinal cord below the lesion because 100% of the trained cats reexpressed a high level of bilateral hindlimb locomotion immediately after spinalization compared to 60% of untrained cats. This study highlighted the beneficial role of locomotor training on facilitating adaptive plastic changes within the spinal circuitry and in promoting locomotor recovery after hemisection.

From these studies using a dual spinal lesion paradigm, we demonstrated that (1) the spinal locomotor circuitry has an intrinsic potential of plasticity; (2) this plasticity most likely participates in the recovery of locomotion after partial SCIs; (3) the spinal cord is a flexible integration center capable of adapting and shaping its motor circuits in order to optimize function with available inputs (i.e., descending and sensory); and (4) locomotor training has a powerful effect on spinal plasticity and acts positively on spinal circuits by reestablishing kinematic parameters approaching the normal state.

Clinical implications

For years, several concepts relative to the notion of a spinal locomotor CPG have been obtained from cat models of SCI. This review highlights the notion that the spinal locomotor CPG plays a prominent role in the recovery of locomotion after complete but also after incomplete SCIs. The question of whether such notions apply to humans, especially when such a strong role is considered for a spinal cord CPG, has been debated. Although the existence of a spinal CPG in humans cannot be directly and stringently demonstrated as in animal models, there are a number of indications that the isolated spinal cord of humans also contains rhythmogenic capabilities. Consequently, targeting intrinsic spinal circuits together with remaining descending and sensory pathways should be a prime approach for rehabilitative strategies in humans with incomplete SCI, which is the concept of locomotor training.

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References


