

Original Article

Semiquantitative assessment of hindlimb movement recovery without intervention in adult paraplegic mice

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Study design: Experimental laboratory investigation of hindlimb movement recovery in chronic paraplegic mice.

Objectives: Development of an assessment method to discriminatively quantify motor and locomotor-like movements of paraplegic mice.

Setting: Laval University Medical Center, Quebec, Canada.

Methods: Signs of 'functional recovery' were examined in open-field condition during 1 month in adult mice with a complete spinal cord transection at the low-thoracic level.

Results: None of the mice exhibited hindlimb movements after spinalization. At 7 days, 33% of them displayed weak nonbilaterally alternating movements (NBA). At 14 days, increased NBA were observed and the first bilaterally alternating movements (BA) in 10% of the mice. A progressive increase of movement frequency and amplitude was found after 2–3 weeks. By the end of the month, 86% displayed mixed NBA and BA. However, none of them recovered the ability to stand or bear their own weight with the hindlimbs.

Conclusion: This study reports signs of partial hindlimb movement recovery in chronic paraplegic mice and provides evidence of plasticity in sublesional circuits of neurons occurring in the absence of inputs from the brain, locomotor training or pharmacological treatment. This assessment method can be used to characterize hindlimb movements in complete spinal cord transected mice tested in open-field condition.

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Introduction

In the last 20 years, animal models of spinal cord injury (SCI) have been increasingly used to further understand the pathophysiological changes induced by this type of trauma.¹ These models have allowed the study of potential new treatments and approaches to reduce secondary cellular damage and scar formation or to increase neuronal regeneration and reconnection.² Most recently, efforts have been made to develop murine models of SCI because of the ability to use genetically engineered animals and additional molecular tools in neurotrauma research.³ A number of murine models with different types of injury have already been examined. These include mice with spinal cord contusion, displacement, crush, clip compression, ischemia, and transection.^{4–10} However, it remains unclear from those studies if recovery attributed to descending fiber

regeneration and reconnection across the lesion is not also partially due to spontaneous reorganization and plasticity at the sublesional level.

To answer this question, the present aim is to examine signs of motor and locomotor recovery occurring without intervention in adult mice with a complete spinal cord transection. Evidence of limited spontaneous recovery (ie, no training, no drug treatment of any kind) after complete spinal transection has been reported in the adult cat.^{11,12} However, there is no clear report of spontaneous recovery in spinal rats or mice. We believe that this apparent lack of spontaneous recovery in spinal rodents is due to the use of qualitative assessment methods (Tarlov, BBB, foot-print, grid-walk, etc) that have been designed to assess mainly fine locomotor skills resulting from descending fiber regeneration, but not basic locomotor-like movements resulting from spinal reorganization at the sublesional level.^{13,14} Therefore, we propose to report signs of recovery simply by counting the number of basic motor and locomotor-

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like movements spontaneously occurring without intervention in the hindlimbs of complete paraplegic mice. These values were also combined to obtain representative average scores that can be used to compare overall performances at different time points. These results shall contribute to better characterize hindlimb motor and locomotor-like movements in this animal model and determine if 'functional' recovery without intervention exists in adult mice within 1 month of complete spinal cord transection.

Methods

Subjects and surgical procedures

A total of 31 ($n = 31$) male CD1 mice initially weighing 35–40 g were used for this study. In all, 21 ($n = 21$) of these mice were spinalized and 10 ($n = 10$) were used as control (intact nonspinalized). Spinalization was performed under general anesthesia with isoflurane (2.5%). Microscissors were used to cut intervertebrally the spinal cord at the thoracic level Th9/10. To ensure that complete transection was achieved, the inner vertebral walls were explored and entirely scraped with scissor tips in order to disrupt any small fibers which had not been severed. The opened skin area was sutured and the animals were placed for a few hours on heating pads. Postoperative care provided for 3 days included injection of lactate-Ringer's solution (2 ml/day, s.c.), analgesic (buprenorphine 0.2 mg/kg/day, s.c.) and antibiotic (baytril 50 mg/kg/day, s.c). Complete spinalization was confirmed by (1) initial full paralysis of the hindlimbs, (2) postmortem examination of the spinal cord lesion, and (3) transverse or midsagittal spinal cord sections stained with luxol fast blue/cresyl violet for myelinated axons and for Nissl substance, respectively. Sections were analyzed microscopically for evidence of tissue sparing. Only results from complete spinal animals were kept for analysis. Experimental procedures were in accordance with the Canadian Council for Animal Care guidelines and accepted by the Laval University Research Hospital Animal Care and Use Committee.

Experimental protocol and data analysis

Mice were tested weekly in open-field condition on a normal surface (ie, $60 \times 60 \text{ cm}^2$ on a laboratory bench counter top). Hindlimb movements (ie, mainly constituted of small amplitude movements involving only one or two articulations in this animal model) were counted during 1 min chosen randomly. These movements were counted into two different categories: (1) total number of nonbilaterally alternating movements (NBA); (2) total number of bilaterally alternating movements (BA). Locomotor-like BA movements were defined as a series of flexion–extensions occurring alternatively in both hindlimbs (ie, usually initiated with a flexion since hindlimbs are already in extension, flaccid and dragging behind in spinal mice). NBA consisted of nonlocomotor movements (ie, no bilateral alternation) typically occur-

ring unilaterally such as fast-paw shakes, kicks, twitches, or cramps. Although some other methods have used juxtaposed bouts of videotape-recorded activity to evaluate movement quality^{15,16} (ie, they may be difficult to use for evaluation of movement counts/min), we chose instead to continuously move a small object (eg, pencil, string, etc) in front of the animal to stimulate curiosity and forward progression throughout the 1 min period of testing. This was performed to minimize the variability of movement counts that may be associated with the animal's 'state of arousal'. Movement amplitude was evaluated qualitatively, as for other assessment methods in open-field condition (eg, BBB, adapted motor score, Tarlov, etc), since detailed 2-D kinematic analyses would require movements to be performed on a treadmill.¹⁰ Movement amplitude was characterized by assigning one of three values; 0 – if no movement was observed; 1 – if the amplitude of most movements was less than half the range of motion of normal steps; 2 – if the amplitude of most movements was at least more than half the range of motion of normal steps. 'Movement incidence' was defined as the total number of mice that displayed movements (NBA or BA) over all tested mice of the same group.

An attempt was made to combine these values in order to obtain single scores that would be representative of the overall performances displayed postspinalization (Figure 2). NBA, BA, amplitude and incidence values were simply added up arithmetically and plotted for each day of testing. For example, a combined score of '18' was calculated for one of the mice which displayed 5 NBA, 2 BA and an amplitude of 2 (ie, large) during 1 min of observation. Note that BA is multiplied by a factor of 2 to account for the fact that one BA movement really represents two movements, one in each of the two hindlimbs. Then, by adding together (eg $[5 + (2 \times 2)] \times 2$) and averaging the combined scores of all mice an average combined score (ACOS) was obtained. Note that mice that did not display hindlimb movements were given a score of '0'. Data were analyzed with one-way (*time*) repeated measures ANOVA followed by *post hoc* bonferroni tests using SPSS 11.0 (Chicago, IL, USA). Results were reported as mean \pm standard error (SE).

Results

This study mainly reports signs of hindlimb movement recovery that occur within 1–2 weeks of spinal cord transection in adult paraplegic mice. Figure 1 illustrates the occurrence of movements found in the hindlimbs of spinal mice tested in open-field condition at 1, 7, 14, 21, and 28 days postspinalization. Results showed that none of the mice ($n = 21$) displayed hindlimb movements on the day after surgery (Figure 1a). After 1 week, the mice started to display few NBA of weak amplitude (Figure 1a and c). The number of NBA increased every week almost linearly to reach on average 7.5 ± 1.6 movements/min by the end of the month (Figure 1a). Similar results were found with BA except that their occurrence began

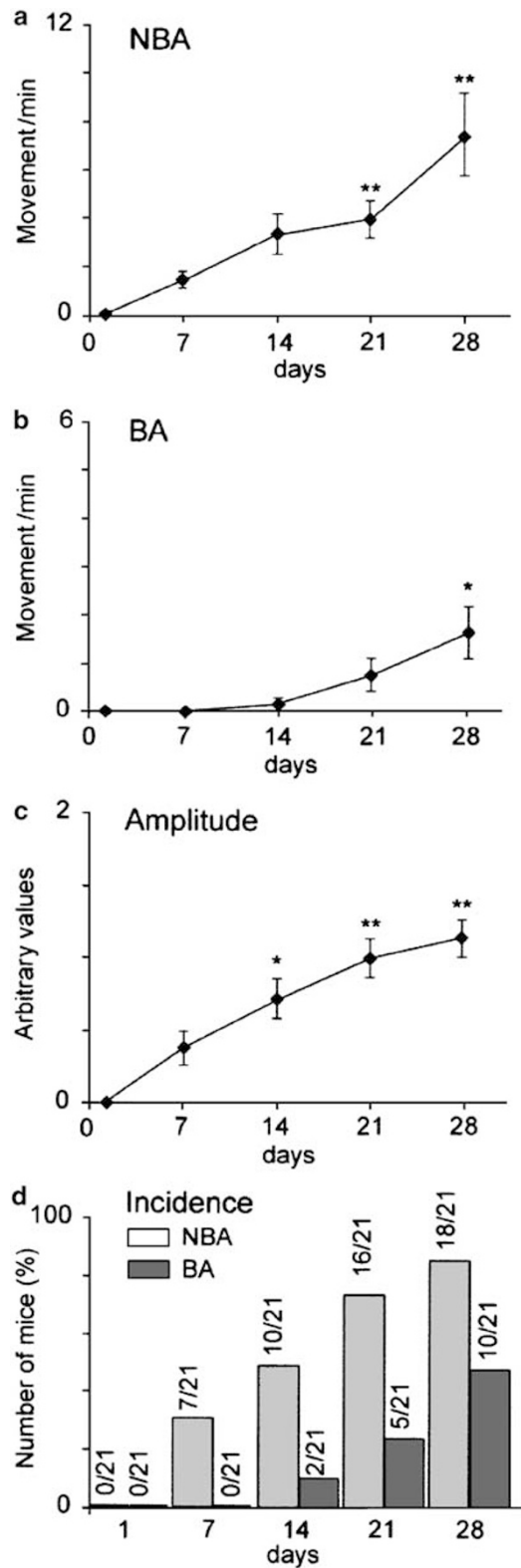


Figure 1 Hindlimb movements observed weekly during 1 month post-transection. (a) Number of NBA. (b) Number of BA. (c) Arbitrary amplitude values for NBA and BA. (d) Incidence (number of mice) of hindlimb movements for NBA and BA. * $P < 0.05$, ** $P < 0.005$, $n = 21$

2 weeks after spinalization rather than after 1 week as for NBA (Figure 1b). On average ($n = 21$ spinal mice) for BA, 1.6 ± 0.49 movements/min (ie, one BA representing one flexion–extension on one side followed by one flexion–extension on the other side) were found at 28 days postspinalization (Figure 1b). Amplitude of these movements (NBA and BA) progressively increased throughout the month with average values ranging from 0 to 1.1 ± 0.1 (Figure 1c). The number of mice (incidence) displaying spontaneous movements steadily increased from 33% ($n = 11/21$) at 7 days to 86% ($n = 18/21$) with NBA or BA at 28 days postspinalization.

Applied to the results illustrated in Figure 1, average combined scores (ACOS) at 1, 7, 14, 21, and 28 days of spinalization ranged from 0 to 14.6 in open-field condition (Figure 2). We found ACOS ranging from 0 to 14.6 that were progressively increasing from day-1 to day-28 postspinalization. An ACOS of ‘0’ was obtained on the day after spinalization while an ACOS of only ‘0.9’ was found at 7 days, nicely reflecting the fact that only small amplitude NBA displayed by a few mice were found at that stage. Steadily increasing ACOS of 3.8, 7.9, and 14.6 were found at 14, 21, and 28 days postspinalization, respectively (Figure 2). Again, this progressive time-dependent increase of ACOS closely reflected the steady increase of NBA and BA as well as a quasilinear increased number of mice (incidence) which displayed movements (Figure 1a–d). Note that these ACOS remained in the low-range portion of scores obtained from normal mice. We found, indeed, that nonspinal mice ($n = 10$) tested with the same method obtained an ACOS of 309 ± 20 .

Discussion

These results demonstrate the existence of motor and locomotor-like movements occurring without intervention in the hindlimbs of complete paraplegic mice. All

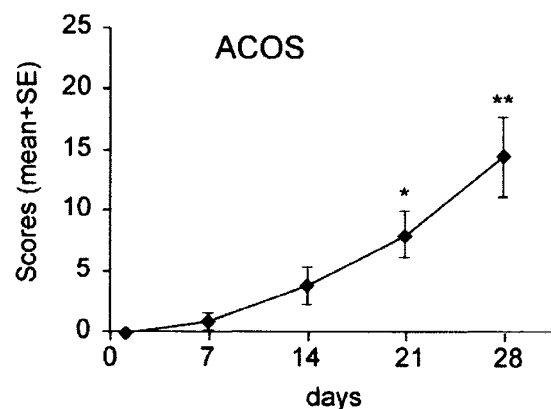


Figure 2 Average combined scores: Data presented in Figure 1 were simply added up ($NBA + (BA \times 2) \times Amplitude$) for each mouse. Results were then averaged ($n = 21$ mice) for each of the five groups (days). * $P < 0.05$, ** $P < 0.005$, $n = 21$

parameters representative of this recovery (ie, NBA, BA, amplitude, incidence) were found to quasilinearly increase throughout the first month postspinalization. This method of assessment was therefore sensitive and discriminative enough to report a time-dependent increase in hindlimb movements during that period of time post-transection. This is in contrast with results from behavioral and qualitative assessment studies that have reported little to no spontaneous hindlimb movements in mice several weeks after spinalization. For example, with the 5-point scale Tarlov test, no signs of recovery were detected several weeks after severe SCI.¹⁷ Also, no meaningful movements were reported on a 10-point scale,¹⁸ while a score of 2 was found with the BBB 21-point scale¹⁷ in spinal mice few days to few weeks after spinalization. Similar results were reported with these methods in complete spinal rats.^{13,18}

The lack of discriminative results with these qualitative assessment methods may be explained by the fact that some of their criteria are either not relevant or cannot be easily assessed in complete paraplegic mice. These criteria include (1) toe drag and clearance which cannot be steadily detected in spinal mice according to some researchers,¹⁴ (2) two-joint *versus* three-joint movements that cannot be easily determined in mice (personal observations), and (3) forelimb–hindlimb coordination which is irrelevant in complete spinal rodents since descending fibers do not regenerate after a complete spinal transection without grafts or treatments.² Consequently, the use of these methods can only lead to an underestimation or failure to detect the progressive increase of hindlimb movements shown in this study to occur without intervention in paraplegic mice within the first month of spinal transection. This said, and despite the problems mentioned above, qualitative assessment methods such as the BBB locomotor scale have been shown to satisfactorily detect progressive ‘functional’ recovery in mice with incomplete SCI.^{15,16} However, as mentioned earlier, only a nonsignificant and steady (ie plateau-like) score of 2 was reported with the BBB method in complete spinal transected mice,^{15,16} which is indicative of insufficient sensitivity and discriminative power at least in complete paraplegic animals.

An additional advantage of the present method is that only a few straightforward and ‘easy-to-assess’ criteria were sufficient to report the progressively increasing recovery found in complete paraplegic mice. For this reason, and also because this method is largely quantitative rather than qualitative, only one observer suffices to assess the type (ie NBA *versus* BA), the incidence and the overall amplitude of hindlimb movements performed by these animals. Consequently, the use of several observers or of videocamera recording, kinematic systems and off-line analyses is not mandatory. However, ‘off-line’ kinematic analysis methods may be useful for completely untrained observers or if higher levels of recovery are to be found due to treatments or training.¹⁰ Altogether, this method offers fair advantages over other methods for ‘drug-screening’

studies where large number of protocols or treatments are to be tested and compared in complete spinal cord transected animals (eg, effects of various types of training and drug treatment on central pattern generator activation).^{11–13}

The present results are comparable to what was reported with a similar approach in complete spinal cats. Indeed, spontaneous recovery was clearly demonstrated in untrained cats, which were shown to step on a motor-driven treadmill shortly after spinal transection.¹² After only 1–2 weeks post-transection, the number of steps counted in these cats revealed that some of them performed up to 25 steps (ie, unilaterally counted) during 45 s on a treadmill running at 0.1 m/s.¹¹ The apparent higher level of spontaneous recovery in cats when compared with mice may result from the use of a motor-driven treadmill and of tail stimulation to entrain rhythmic hindlimb movements.^{12,19} This is supported by recent results reporting that similar conditions (ie, tail pinching and treadmill) can also occasionally trigger rather complete step cycles in mice two weeks after spinal cord transection in some cases.¹⁰

The cellular mechanisms responsible for the motor and locomotor-like recovery in complete spinal animals remain largely unknown. The progressive return of hindlimb movements over time could result from time-dependent plastic changes in networks of the lumbo-sacral spinal cord that can generate walking in the absence of supraspinal input (ie, CPG²⁰). Participation of these spinal locomotor networks in spontaneous recovery is further supported by the fact that neurons below the transection site do survive in paraplegic mice with only limited atrophy several weeks after transection. Furthermore, spontaneous episodes of fictive locomotion were reported in mice *in vitro* isolated spinal cords²¹ and signs of increased CPG excitability were found in neonatal rats 1 week after spinal transection.¹⁹ Many biochemical changes and gene-regulated processes found at the sublesional level could be involved in motor recovery. For instance, the return of some functions and reflexes may be influenced by the sprouting of primary afferent fibers²² and propriospinal neurons²³ or by increased glycinergic and gabaergic inhibition.²⁴

To examine higher levels of functional recovery such as after regeneration and reconnection across the lesion (ie, induced by grafts or drug treatments), it may be best to combine methods that are specifically designed to assess basic or fine locomotor movements (BBB, grid-walking, foot-print, etc). The idea of combining several methods based on the level of recovery was indeed proposed and successfully tested by others.^{25,26}

In conclusion, this study reports time-dependently occurring hindlimb motor and locomotor-like movements in untrained, nonstimulated (no intervention) and complete paraplegic animals suggesting that some form of plasticity and reorganization of the locomotor networks take place at the lumbo-sacral spinal cord level shortly after spinal cord transection in adult mice.

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