Mid-Lumbar Segments Are Needed for the Expression of Locomotion in Chronic Spinal Cats

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Langlet, C., H. Leblond, and S. Rossignol. Mid-lumbar segments are needed for the expression of locomotion in chronic spinal cats. J Neurophysiol 93: 2474–2488, 2005. First published January 12, 2005; doi:10.1152/jn.00909.2004. In acute experiments performed in decerebrated and spinalized (T13) cats, an intraspinal injection of clonidine, a noradrenergic agonist, restricted to mid-lumbar segments L3–L4, can induce hindlimb locomotion, whereas yohimbine, a noradrenergic antagonist, can block spinal locomotion, and a second spinal lesion at L4 can abolish all locomotor activity. In the present study, we investigated whether the abolition of locomotion after this second spinal lesion was due to an acute spinal shock or to the functional disconnection of the rostral and caudal lumbar segments. In seven cats, first spinalized at T13 and having recovered treadmill locomotion, a second transection was performed at lower lumbar levels. Video and electromyographic recordings were used to evaluate locomotor performance. Results show that after a second transection at L2 or rostral L4 levels, spinal locomotion was maintained; when the second lesion was performed at caudal L3 or L4, all locomotor activity was abolished even after several weeks of attempted locomotor training; vigorous fast paw shakes (FPS) were observed in all cases; and after an intraperitoneal injection of clonidine in cats with a second transection below L4, perineal stimulation induced hyperextension of the hindlimbs but no locomotion. Considering that the main motoneuron pools of the hindlimbs are caudal to L4 and are still functional for the specific expression of spinal locomotion but not necessarily for other rhythmic motor patterns.

INTRODUCTION

After a complete spinal transection at the lowest thoracic level, several animal species are capable of walking with the hindlimbs over a treadmill (Rossignol et al. 2000), a behavior that is essentially generated by a spinal “central pattern generator” (CPG) (Grillner 1981). The question studied here concerns the segmental distribution of this locomotor network and whether it is regionalized or distributed throughout the spinal cord of the cat. In the in vitro neonatal rat, the thoracolumbar cord constitutes a key area for locomotor pattern generation (Cazalets 2000) although the sacral cord also has autonomous rhythmic capabilities (Cazalets and Bertrand 2000a; Lev-Tov et al. 2000). Other works also suggest a regionalized organization of rhythmicity based on the segmental distribution of various serotonin (5-HT) receptors (Jordan and Schmidt 2002). Chronic lesions of the gray matter at the level of L2 by kainic acid results in paraplegia in adult rats (Magnuson et al. 1999). Other studies in which spinal lesions and neurotransmitters were also used on various spinal segments suggest that the rhythmonic potential is distributed throughout the spinal cord, with rostral lumbar segments being more excitable (Kjaerulff and Kiehn 1996).

It was suggested that, in cats, the rostral lumbar segments L3–L5 play a leading role during scratching but that rhythmogenicity was present in lower L6–S1 segments (Deliagina et al. 1983), a conclusion also reached in studies of “fictive locomotion” induced by L-3,4-dihydroxyphenylalanine (l-DOPA) in acute spinal cats (Grillner and Zangger 1979). We have shown potent effects on locomotion of small 100-μl injections of α2-noradrenergic agonist (clonidine) and antagonist (yohimbine) through a chronically implanted intrathecal cannula that delivered the drugs in a limited region around L1–L4 (Giroux et al. 2001). We also demonstrated that in decerebrated cats spinalized at T13, intraspinal microinjections of clonidine at L3–L4 segments could evoke a locomotor pattern that could be abolished by a further microinjection of yohimbine. Similarly, after an intravenous injection of clonidine, locomotion was blocked by microinjections of yohimbine in the L3 or the L4 segment (Marcoux and Rossignol 2000). Finally, we also showed that locomotion, evoked by intravenous or intrathecal injections of clonidine, disappeared after a second lesion at L4, suggesting that the integrity of the segments above L4 was necessary to trigger and to maintain spinal locomotion. However, we could not determine whether the abolition of locomotion was due to depressive effect on the cord of this second acute spinal lesion or to the disconnection of the mid-lumbar segments from the lower lumbar segments. The present work presents a study of this problem using a double chronic spinal lesion. Cats were first spinalized at T13 and trained to regain spontaneous spinal locomotion on the treadmill. Then a single second spinal lesion was performed at various lower lumbar levels. Our results show that walking capabilities remained after lesions at L2 or rostral L4 but was permanently abolished after spinal transections that disconnected the midlumbar segments (caudal L3 or L4) from the L5–S1 segments. However, other rhythmic patterns such as fast paw shakes could be elicited even though locomotion could no longer be evoked. Because the main motoneuron pools of the hindlimbs are located in the L5–S1 segments (Vanderhorst and Holstege 1997) and because they can be recruited in rhythmic patterns other than locomotion after lesions at L3–L4, it is concluded...
that the midlumbar segments provide essential inputs to organize the locomotor pattern in hindlimb motoneurons. Part of this work has been published in abstract form (Langlet and Rossignol 2002).

**METHODS**

**Chronic experiments**

**GENERAL PROTOCOL.** First, cooperative adult cats ($n = 7$) were trained to walk on a motor-driven treadmill at different speeds (0.3–0.6 m/s) every day during 1 mo. Once the cats were able to walk at a constant speed for 15–20 min, intramuscular electrodes were implanted chronically in various flexor and extensor muscles of the hindlimbs. After implantation, locomotion was recorded to establish baseline values in the intact state. The cats were then spinalized at the last thoracic vertebra ($T_{13}$, labeled A in Fig. 1) and trained to walk on the treadmill every day for 3–4 wk without any drugs. When they regained a stable locomotor pattern, a second spinalization was performed at $L_2$ ($n = 2$; cats $B$ and $C$), $L_3$ ($n = 2$, cats $D$ and $E$), $L_4$ ($n = 2$; cats $F$ and $G$), and $L_5$ ($n = 1$, cat $H$) and, again, the ability to walk was documented for several weeks. In Table 1, each cat is identified by a letter and the level of the second lesion is shown for each cat as well as the number of days between the first spinal section at $T_{13}$ and the second spinal section plus the number of days the animals were kept after the second lesion. At the end of the experimental series, an acute experiment was carried out in the cats that were spinalized at $L_2$ and at rostral $L_1$ to perform other acute lesions. Two other cats, spinalized only at $T_{13}$ and trained to walk on the treadmill for 1 mo, were also studied in acute experiments. All procedures were approved by the Ethics Committee of Université de Montréal.

**IMPLANTATION OF CHRONIC ELECTROMYOGRAPHIC (EMG) ELECTRODES.** All surgical procedures were performed in aseptic conditions and under general anesthesia. The cats received buprenorphine (0.01 mg/kg sc) 1 h before surgery. They were premedicated with ketamine (10 mg/kg), acepromazine maleate (Atravet, 0.1 mg/kg), and glycopyrrolate (0.01 mg/kg) injected intramuscularly and anesthetized by inhalation of isoflurane 1–3% in 95% O$_2$ maintained through an endotracheal tube. Lactate Ringer solution was administered during surgery through an intravenous catheter in the cephalic vein. The body temperature and heart rate were monitored throughout the surgery. The animal was secured in a stereotaxic frame with atraumatic ear clip. Iliopsoas (Ip), hip flexor; sartorius (Srt), hip flexor and knee extensor; semitendinosus (St), knee flexor and hip extensor; vastus lateralis (VL), knee extensor; gastrocnemius lateralis (GL), ankle extensor and knee flexor; and tibialis anterior (TA), ankle flexor.

A Fentanyl (2.5 mg, 25 µg/h) transdermal patch was applied on the lower back of the cat for continuous analgesia for the first three postoperative days and an antibiotic (Cephatab, 100 mg po) was given prophylactically for 10 days after surgery.

**SPINAL CORD LESIONS.** After obtaining the baseline values of kinematics and EMG activity during locomotion in the intact state, the cats were anesthetized, and a laminectomy was performed at the $T_{13}$ vertebra. The dura was removed carefully and Lidocain (2% xylocaine) was applied on the surface of the cord and ~100 µl injected bilaterally into the spinal cord. The spinal cord was then completely transected using a pair of surgical scissors. The space between the rostral and caudal ends was packed solidly with a sterile absorbable hemostat (Surgicel, oxidized regenerated cellulose). An antibiotic (Aycerciline, 40,000 IU/kg im) and an analgesic (Anafen, 2 mg/kg sc) were administered at the end of the surgery.

The same method was applied for the second spinal cord lesion at lower lumbar levels. The different levels of spinal transections are illustrated in Fig. 1 (vertical lines, B–H). The spinal cord of the cat is composed of 13 thoracic and 7 lumbar segments. We used bony landmarks to guide the sites of laminectomies and identified the roots before making the second spinal lesion. The level of these lesions in relation to the entry level of dorsal roots was always checked postmortem. We always attempted to make spinal lesions as straight as possible so that there would be little variation in the level of the section from the dorsal to the ventral side of the cord.

**POSTOPERATIVE CARE.** Immediately after surgery, the cats were placed in a temperature-controlled incubator. Once the animal regained consciousness, it was returned to its individual cage with full access to food and water. The cage floor was covered with a foam mattress to avoid skin ulceration. Cats were attended daily for general inspection, cleaning of the hindquarters and manual bladder expression (Belanger et al. 1996).

**RECORDING AND ANALYSIS PROCEDURE.** After the EMG implantation and before spinalization, locomotor performance (EMG activity synchronized to the video images) in the intact state was recorded. Cats were placed on a treadmill belt equipped with a Plexiglas enclosure and were trained to walk freely at the various imposed speeds. Spinal cats were trained, without drugs, to walk every day until the animals recovered a well-coordinated locomotor pattern. For spinal

![Fig. 1](https://example.com/fig1.png)
locomotion, the forelimbs were placed on a platform located above the treadmill while the hindlimbs walked on the belt. At the beginning of training, perineal stimulation was used, and the experimenter had to support the weight of the hindquarters of the cat. After 2–3 wk, the cats could walk without stimulation and weight support of the hindquarters while the experimenter held the tail merely to provide lateral stability.

The EMG signal was amplified differentially (bandwidth: 100 Hz to 3 kHz) and digitized at 2 kHz; this is adequate to document the amplitude and to establish proper timing between EMG bursts. EMGs were synchronized to the video images of the hindlimbs using a digital time code, recorded in the computer file and the video tape. Reflective markers were placed on the skin overlaying the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal joint (mtp), and the tip of the fourth toe. Video images of the locomotor movement (left side) were captured by a digital camera and recorded on a video cassette recorder at 30 frames/s.

The bursts of activity of all EMGs were rectified and integrated to quantify their amplitude. Their onset and offset were identified automatically by a homemade software and corrected manually by the experimenter to determine temporal parameters of the EMG for each cycle. The video images were digitized and the x-y coordinates of various joint markers were obtained at the frequency of 60 fields/s, by de-interlacing the video frames taken at 60 Hz. These coordinates were used to calculate angular joint movements and could be displayed as continuous angular displacements or stick diagrams of one-step cycle (see Fig. 2A). The step length was calculated as the distance between two consecutive contacts of the same foot. The time interval between the onset and the offset of each muscle was measured and synchronized to the onset of the St muscle.

FAST PAW SHAKE. Fast paw shake was elicited by holding the cat by the thorax and dipping one of its hindpaws in lukewarm water. Although EMG and video images were recorded during fast paw shakes, only the EMG signals were analyzed.

PHARMACOLOGICAL STIMULATION. Clonidine was injected ip (150 μg/kg) once, 2–3 days after the first spinal transaction (T13), because clonidine has been shown to induce locomotion in spinal cats (Barbeau et al. 1987; Chau et al. 1998b; Forssberg and Grillner 1973). The reason for testing the effect of clonidine at this stage was to serve as a comparison for ulterior lesions after which clonidine was also injected to try and induce locomotion. In cases where cats were unable to walk several days after the second spinal lesion, clonidine was injected once again. It should be made clear, however, that clonidine was not used otherwise to train the cats to walk after spinalization. It should also be stated that the effect of clonidine lasts for a period of only 4–6 h (Chau et al. 1998a), and it is thus unlikely that the injection of the drug only once after spinalization exerts a long-time effect.

Acute experiments

Three chronic cats with the second transection located at L2 (cats B and C) and rostral L3 (cat D) as well as two other cats with only one transaction at T13 were used for acute experiments. These acute experiments aimed at avoiding a third chronic lesion in cats that recovered locomotion after the most rostral second lesion (L2 and rostral L3) and at confirming results obtained in chronic cats with lesions at lower levels.

Under general anesthesia (see Spinalization for details) and endotracheal intubation through a tracheotomy, one carotid artery was cannulated for monitoring blood pressure and the other one was ligated. One jugular vein was cannulated for the administration of fluid and medication. The temperature was measured with a rectal thermometer and maintained ~38°C by a feedback-controlled heating element using DC and with heating lamps. The end-expiratory pCO2 was monitored using a Datex Monitor during normal or assisted ventilation and maintained between 3.5 and 4.5%. The blood pressure was maintained within physiological limits. A laminectomy was performed, removing T13–L4 vertebrae, and the cats were then mounted to a stereotaxic frame attached to a motor-driven treadmill. The spine was fixed with three pairs of lateral pins: one at the iliac crest, one at L4–5 and the other at L1. A precollicular, postmamillary decerebration was performed with a spatula and the rostral nervous tissues removed. Anesthesia was then discontinued. The dura was opened and the spinal cord covered with warm mineral oil. The spinal segments were determined by identifying the most rostral and the most caudal dorsal rootlets at each segment.

One hour after the cessation of anesthesia, locomotor capacities of the cat were evaluated on a treadmill at a speed of 0.2–0.3 m/s while using perineal and/or abdominal manual stimulation. All cats received an intravenous injection of clonidine (500 μg/kg). To study the importance of each segment, dorsal funiculi lesion, dorsal hemisection and complete cord transections were made sequentially at different levels between L1 and L5. The same recording and analysis procedures as in the chronic experiments were used.

Histology

At the end of the experiments, the cats were killed with an overdose of pentobarbital sodium and lesions were verified postmortem. Each spinal segment was first identified by the location of the dorsal roots, and after visual examination of the lesion, relevant portions of the cord were removed and fixed by immersion in formalin (10%) for routine histology. Inspection of serial Nissl-stained cross sections (30 μm thick) of the spinal cord was then made for all cats to ascertain the completeness and the rostrocaudal extent of the lesions. Signs of obvious cellular damage due to the second spinal transaction was evaluated under the microscope. The rostrocaudal extent of damage to

<table>
<thead>
<tr>
<th>Cat Name</th>
<th>Days Between the 1st and 2nd Spinal Section</th>
<th>Level of 2nd Section</th>
<th>Days After 2nd Section</th>
<th>Total Extent of Damage of the 2nd Section, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>36</td>
<td>Rostral L3</td>
<td>28</td>
<td>NA</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>Caudal L3</td>
<td>27</td>
<td>7.50</td>
</tr>
<tr>
<td>D</td>
<td>95</td>
<td>Rostral L3</td>
<td>17</td>
<td>8.13</td>
</tr>
<tr>
<td>E</td>
<td>36</td>
<td>Caudal L3</td>
<td>24</td>
<td>6.12</td>
</tr>
<tr>
<td>F</td>
<td>27</td>
<td>Mid L3</td>
<td>17</td>
<td>6.43</td>
</tr>
<tr>
<td>G</td>
<td>46</td>
<td>Caudal L4</td>
<td>44</td>
<td>8.64</td>
</tr>
<tr>
<td>H</td>
<td>21</td>
<td>Mid L3</td>
<td>52</td>
<td>5.76</td>
</tr>
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</table>

Each cat is identified by a letter (cat name), the number of days between the first section at T13 and the second section, the level of the second section, the number of days after the second section and the total extent (in mm) of the rostral and caudal damage of the second section evaluated with histological methods.
the cord resulting from the second spinal transection is given for each cat, except cat B, in Table 1. Overall, in these chronic experiments, an average total of 7.1 ± 1.2 (SD) mm of nervous tissue was damaged by the lesion. In contrast, the sections adjacent to the second lesion in all acute experiments did not reveal any evidence of significant damage.

Statistical analysis

Results are presented as means ± SD for cycle periods and amplitude, and the difference between groups was tested by a one-way ANOVA. Bonferroni’s t-test was used as a post hoc test to determine the statistical significance (probability level of <0.05).

RESULTS

Chronic double lesions

EFFECTS OF THE FIRST THORACIC LESION (T13) ON LOCOMOTION (CATS WITH LESIONS AT LEVEL A ON FIG. 1). Figure 2A represents the typical locomotor pattern of a cat (cat B) in the intact condition before the first spinal lesion at T13. The characteristics of locomotion are reflected in the stick diagrams representing one-step cycles (Fig. 2A, left) and normalized angular plots of the hip, knee, ankle and metatarsal joints (Fig. 2A, middle). EMG activity of the corresponding sequence is shown to the right of the diagram.
Fig. 2A. EMG activity illustrates a regular alternation of activity on the left (L) and right (R) sides, as well as between flexors (St, Srt) and extensors (VL and GL) on the same sides, organized reciprocally, with respect to muscle activity on the other side.

Eight days after T13 spinalization, this cat was unable to walk when placed on the moving treadmill, even after attempts at locomotor training with perineal stimulation (Fig. 2B). The EMG activity was not rhythmically organized. Now and then, only slight flexion movements were observed and, of course, there was no weight support.

It was important to document the locomotor performance with clonidine at this early stage after a T13 lesion because, in the subsequent phases of the experiment, we intended to use pharmacological stimulation (clonidine) to try and induce locomotion after lesions at lower levels. Within 20 min of an intraperitoneal injection of clonidine (150 μg/kg), a robust locomotor pattern (rhythmic alternation of flexor and extensors muscles) was obtained with perineal stimulation. There was some foot drag at the onset of the swing (Fig. 2C, left) and only partial weight support because the foot was often placed behind the hip. But the cat could walk at imposed speed, ranging from 0.2 to 0.6 m/s, with placement of the foot. However, the duration of the flexor and the extensor bursts was comparable to the intact values obtained before spinalization (St: 283.8 ± 6.9 vs. 295 ± 48 ms; Srt: 349 ± 50 vs. 355.8 ± 100 ms, VL: 787.5 ± 112.5 vs. 604.33 ± 46.7 ms, GL: 786.1 ± 114.0 vs. 544.5 ± 62.7 ms, NS for all previous values at P < 0.05).

Such drug injection was performed only once between the first and second spinal lesions and no training was performed under clonidine. However, this cat was trained on the treadmill for a period of 31 days (3–4 training sessions per day). Figure 2D shows that this cat had recovered spontaneous locomotion by day 21 with proper weight support but that the pattern was somewhat more irregular than in the intact condition. The step length was decreased compared with intact conditions (intact: 453 ± 16 mm vs. T13; 204 ± 37 mm, P < 0.05) or compared with values obtained after the first injection of clonidine, when the cat had not been trained (clonidine: 332 ± 32 mm vs. T13: 204 ± 37 mm, P < 0.05). The cycle duration was correspondingly significantly decreased (intact: 1,253.8 ± 173.9 ms, clonidine: 1,045.3 ± 116.2 ms, T13: 669.9 ± 79.9 ms, P < 0.05). The foot was often placed slightly behind the hip during stepping. This locomotor behavior is consistent with our previous reports (Barbeau and Rossignol 1987; Bélanger et al. 1996; Chau et al. 1998a,b; Rossignol et al. 2000).

**TABLE 2. Comparison of values before and after second lesion**

<table>
<thead>
<tr>
<th></th>
<th>Cycle</th>
<th>Semitendinosus</th>
<th>Sartorius</th>
<th>Vastus Lateralis</th>
<th>Gastrocnemius Lateralis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Burst Amplitude</td>
<td>Burst Amplitude</td>
<td>Burst Amplitude</td>
<td>Burst Amplitude</td>
</tr>
<tr>
<td>Cat B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>112</td>
<td>1253.8 ± 173.9</td>
<td>283.8 ± 6.9</td>
<td>6.9 ± 1.2</td>
<td>348.4 ± 49.1</td>
</tr>
<tr>
<td>T13 (3 d)</td>
<td>42</td>
<td>666.9 ± 79.9*</td>
<td>148.1 ± 13.2*</td>
<td>13.2 ± 2.1*</td>
<td>193.5 ± 49.9*</td>
</tr>
<tr>
<td>L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral (2 d)</td>
<td>20</td>
<td>616.1 ± 104.3*</td>
<td>117.6 ± 33.2*</td>
<td>10.2 ± 2.5</td>
<td>148.8 ± 66.1*</td>
</tr>
<tr>
<td>Rostral (27 d)</td>
<td>33</td>
<td>682.3 ± 71.4*</td>
<td>154.5 ± 9.1*</td>
<td>9.1 ± 2.4</td>
<td>99.0 ± 45.6*</td>
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<td>Cat C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>15</td>
<td>921.9 ± 89.5</td>
<td>135.5 ± 39.3</td>
<td>10.3 ± 2.7</td>
<td>271.1 ± 42.9</td>
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<tr>
<td>T13 (27 d)</td>
<td>30</td>
<td>873.5 ± 149.4</td>
<td>230.0 ± 55.4*</td>
<td>12.1 ± 3.2</td>
<td>276.8 ± 133.1</td>
</tr>
<tr>
<td>L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudal (2 d)</td>
<td>22</td>
<td>694.1 ± 93.1*</td>
<td>180.1 ± 43.4*</td>
<td>9.1 ± 2.7</td>
<td>155.1 ± 34.8</td>
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<tr>
<td>Caudal (22 d)</td>
<td>38</td>
<td>677.4 ± 102.0*</td>
<td>244.2 ± 64.9*</td>
<td>14.4 ± 2.0</td>
<td>215.6 ± 103.7*</td>
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</table>

Evolution of the cycle duration, burst duration and amplitude of 4 muscles in intact condition, after the first lesion (T13) and after the second lesion (early and late) in the L2 spinal cats at 0.3 m/s. Values are means ± SD, *significant difference from intact condition, †significant difference from T13, ‡significant difference before versus after training (P < 0.05).

**EFFECTS OF A SECOND LESION AT THE L2 LUMBAR LEVEL (CATS B AND C).** Several weeks after the first transaction at T13 (see Table 1), after cats had regained the ability to walk on the treadmill with adequate weight support, a second lesion was performed at L2 and recovery of locomotion was studied. In one cat (cat B in Fig. 1), the transaction was made at the rostral L2 level and, in the other cat (cat C in Fig. 1), at the caudal L2 level. Postmortem histological verification confirmed that the spinal lesions were indeed complete and that the primary and secondary damage that had taken place after spinal injury affected the nervous tissue in the proximity of the aforementioned regions (not shown). For example, necrosis and scar tissues could be identified at the site of the lesion of cat C for a total length of 7.5 mm. Assuming an equal distribution of damage on each side of the lesion, it is likely that ~3.75 mm of tissue is affected caudally to the lesion. Given the length of the spinal segment, it is possible that the lesion reached a small portion of rostral L3. Because these cats regained locomotion, their recovery is described in two stages: early and late periods.

**Early period.** Two days after the second lesion, both cats B and C were able to walk on the treadmill at speeds ranging from 0.2 to 0.6 m/s with perineal stimulation. No important changes were observed in hip excursion before or after the second lesion, but some decreases were seen in knee excursion as well as in ankle and mtp extension. For cat B, the St burst duration was decreased (~20.5%, P < 0.05) in comparison to the value obtained 31 days after the T13 lesion, i.e., the day before the second lesion. No changes in cycle duration, burst duration of the other muscles, or amplitude were observed after this second lesion when comparing values obtained before (Fig. 2) and after the second lesion (Table 2). There was no change in amplitude right after the lesion, except for the VL (+54.4%, P < 0.05).

Cat C, which had a caudal L2 lesion, showed no change of the step length values, when compared with the values obtained 17 days after the first lesion at T13 (compare Fig. 3, A and B). However, in cat C, a 22.4% (P < 0.05) decrease in cycle duration was observed as well as some modifications of the EMGs (compare Fig. 3, A and B, right). The extensor burst
duration was decreased (VL, −10.9% and GL, 21.8%, *P* < 0.05), which is congruent with the decrease in cycle duration. The Srt burst duration was also decreased (−44.5%, *P* < 0.05). No change in amplitude or time interval (see Fig. 4) was observed.

In *cat C*, clonidine increased flexion at all joints (Fig. 3C), and the EMG was altogether more robust as illustrated by an increase in the amplitude of all muscles (5–170%, *P* < 0.05) as well as in cycle and burst duration (flexors and extensors, 11–61%, *P* < 0.05). No change was observed between the step length values before or after the injection of clonidine. This was also the case with *cat B*.

Late period. *Cats B* and *C* were trained to walk on the treadmill and, by the end of the 22nd day, these two cats were able to walk at various speeds on the treadmill, without perineal or pharmacological stimulation. The cats showed the same pattern of locomotion as that obtained at T13 at 21 days, except for the knee flexion (Fig. 3C). Nevertheless, we have noticed some tendency to abduct the hindlimbs after the second transection (not shown).

**FIG. 3.** Comparison of recovery of locomotion after a second spinal lesion at the caudal part of L2 segment in *cat C*. Stick diagrams, angular displacement and raw EMG traces of hindlimb flexor and extensor muscles are displayed as in Fig. 2. A: recovery of locomotion after a 1st transection at T13 after 17 days of training. B and C: locomotor performance 2 days after the 2nd lesion at the caudal L2 level in the same cat before the injection of clonidine (B) and after an injection of clonidine (C; 150 μg/kg ip). D: locomotion of the caudal L2 spinalized cat after 22 days of training.
For cat B, when comparing the values obtained after 31 days of recovery after the T13 lesion to those after 27 days of training after the second lesion at L2, we found that the cycle duration remained unchanged (see Table 2), whereas there was a decrease of the Srt burst duration and amplitude (–48 and –27%, P < 0.05). However, a significant increase was observed for the burst duration and amplitude of the VL (14 and 83%, P < 0.05).

Comparisons of “before and after training” values showed a tendency for the cycle duration (10.7%, NS) and for the flexors and GL burst duration (3–31%, NS) to increase. Only the VL burst duration was significantly higher after training than it was before (+30%, P < 0.05). There was no change of the step length.

In cat C, spinalized at caudal L2, there was a significant decrease of the cycle and burst durations (22–26%, P < 0.05) when comparing values gathered 27 days after the T13 lesion with those obtained 22 days after the second lesion. No significant changes were observed for the amplitude. The St burst duration was higher (+35%, P < 0.05) after training than before whereas the VL burst duration was shorter (–15%, P < 0.05). However, we noted no change in the step length and the time interval (Fig. 4) between the onset and the offset of each muscle (Srt, VL, and GL) between the values obtained 27 days after the T13 lesion with those obtained 22 days after the second lesion.

In conclusion, the animals that had a previous lesion at T13 and had recovered locomotion could still walk early after a second spinal lesion at L2. However, there were some changes in the locomotor characteristics (cycle and EMG burst duration) after 2 wk of training on the treadmill, which suggests that these higher lumbar segments do exert some role in the expression of the spinal locomotor pattern. Injection of clonidine also modulated the spinal locomotor pattern, by increasing flexion movement and yielding more robust EMG activity.

**FIG. 4.** Evolution of phase of the burst of activity in the iSrt, iVL and iGL muscles of the L2 spinal cats (A, cat B; B, cat C) in intact condition, after the 1st (T13) and the 2nd lesion (early 2 days in both cats and late, 27 and 22 days, respectively, for both cats). Muscles are synchronized on the iSt muscle. The numbers in the St rectangle represent the number of cycles utilized for the analysis.
second transaction, strong perineal stimulation elicited bilateral tonic flexion of the hindlimbs and some small movements but no rhythmic movements (Fig. 5B). Neither could locomotion be triggered with clonidine; in fact, clonidine evoked a strong and maintained bilateral hyperextension that was exacerbated by perineal stimulation. At day 20 after the second spinal transaction, another injection of clonidine also produced only bilateral hindlimb extensions. These hyperextensions are illustrated by the tonic EMG activity obtained in extensor muscles and by the stick figures (Fig. 5C). We were unable to induce locomotion despite 3 wk of intensive training. However, vigorous muscle activity was observed during reflex movements.
by pinching the skin at various locations or during fast paw shake responses (FPS in Fig. 5D and see later).

In conclusion, two patterns were observed after a second transection at the L3 level. First when the transection was made at the rostral level of L3, the cat was able to walk, but this locomotor capability was not maintained for any significant length of time. Second, when the transection was performed at the caudal level of L3, locomotion was abolished, but other rhythmic activities, such as fast paw shake, could still be evoked.

EFFECTS OF A SECOND LESION AT THE L4 LUMBAR LEVEL (CATS F AND G). The second spinal transection at middle L4 (cat F; Fig. 1) and caudal L4 (cat G; Fig. 1) also abolished locomotion completely and permanently. When compared with cats lesioned at caudal L3 level transection, there was even less tonic flexion with perineal stimulation or faint locomotor movements of the hindlimbs.

Again, we were interested in finding out if clonidine could induce locomotion as it did after the first lesion at T13. Figure 6 illustrates the effect of clonidine in cat F 6 days after the lesion at T13 (Fig. 6A), then 16 days after the second transection at L4. Whereas it was clear that stepping movements could be enhanced by clonidine after the T13 lesion (Fig. 6A), clonidine only induced a bilateral and sustained hyperextension of the hindlimbs (Fig. 6B), much as what had been seen in cat E after a lesion at caudal L3 (Fig. 5C). Perineal stimulation indeed induced tonic hip extension in both legs. The amplitude of the knee extensor VL was as high (LVL) or higher (RVL) during these hyperextensions after the second lesion as during locomotion evoked by clonidine after the first lesion at T13. This pattern of clonidine-induced bilateral hyperextension was consistent in cats F and G for injections made at 2 and 16 days after the second spinalization. The abolition of locomotor activity persisted despite intensive attempts at locomotor training on the treadmill for several days (see Table 1). Occasionally, we observed some rapid and clonic movements of the hindlimbs with perineal stimulation and weak alternation of the hindlimbs but no locomotor movement.

EFFECTS OF A SECOND LESION AT THE L5 LEVEL (CAT H). In the first few days after the L5 transection (middle, cat H on Fig. 1), there were no spontaneous hindlimb movements, and clonidine produced the same hyperextension effects as in cats with the L4 lesions. Two weeks after this second transection at middle L5, we were able to induce a slow rhythmic activity in the proximal joints of the left leg only by applying strong tonic pinches of the abdominal skin. These pinches induced only some movements of the hip and the knee (see Fig. 7A). No rhythmic activity was observed in the distal joints. A strong EMG activity was obtained in the Ip, the Srt, and the VL (Fig. 7B) but not in distal muscles such as GL. This pattern of activity has been observed in each experimental session for a period of 1 mo.

Another effect of the second lesion at L5 was a marked spasticity of the right leg observed 2 wk after the transection. No movement was ever noted in that limb, only tonic EMG activity (not shown). No FPS could be elicited in this leg, although it was observed in the left leg.
FAST PAW SHAKE. We wanted to know if the fact that the spinal cat could not express locomotion after the second transection at L3, L4, or L5 meant that motoneurons were not functional or that the segments below the second lesion had lost all their potential for rhythmogenesis. To that end, we tested another rhythmic activity, the fast paw shake (FPS) response that was elicited in each cat in the intact condition, after the first thoracic lesion and after the second transection by dipping the paw in water (Fig. 8).

In the intact condition, FPS was not elicited in all cats. The FPS was characterized by the typical TA-VL synergy (Smith et al. 1985), and this synergy was still present after the transection at T13 and after lumbar lesions (see Fig. 8, A–C, top). The frequency of the FPS was 9.92 ± 1.07 Hz in the intact cats. Thirty-five days after the first spinal transection at T13, the frequency was similar to that obtained in the intact condition (9.07 ± 1.41 Hz, mean on 10 cycles/record session). Whatever the level of the second transection at the lumbar level, a strong sequence of FPS was observed along with a tendency to increase its frequency compared with the intact condition or to the period after the first T13 lesion. The frequency of the FPS obtained for the cat spinalized at L3 or L4 level tended to be higher (11.39 ± 1.23 Hz, mean on 20 cycles/test session) than in cats spinalized at L2 (10.98 ± 1.73 Hz, mean on 20 cycles/record session), but this difference was not significant. The total duration of FPS episodes also tended to be somewhat longer (but not statistically significant) after a second lesion at L1–L4 (1,124 ± 628 ms) compared with after the first lesion at T13 (963.74 ± 240 ms) or the intact condition (926.58 ± 307 ms). Administration of clonidine abolished the FPS responses, as reported before (Barbeau and Rossignol 1991).

In conclusion, after a second transection at the caudal L3, L4, or L5 level, all locomotor activity was abolished, but the cat was able to produce other rhythmic activities such as FPS, which even became somewhat more vigorous.

Serial spinal transections in acute experiments

Because it was not deemed justifiable to perform a third chronic spinal lesion, acute experiments were carried out in three cats that continued to walk after the second lesion, i.e., the two cats spinalized at L2 (cats B and C) and rostral L3 (cat D). These acute experiments also allowed us to corroborate the results obtained in the chronic state. Furthermore, it was possible to perform progressive dorsoventral lesions at different lumbar levels; this gave us an indication of the importance of dorsal and ventral pathways. In two other cats spinalized only at T13, we also investigated the effect of serial and progressive spinal transections.

After the decerebration and removal of the anesthesia, none of the cats displayed spontaneous locomotor activity, but reflexes could be elicited by pinching the skin of the hindlimbs. It should be remembered that, in this protocol, the cats are

FIG. 7. Rhythmic pattern obtained by continuous stimulation of the abdominal skin in cat H with a lesion at L5. A: the stick figure represents two consecutive cycles obtained with a tonic pinch of the abdominal skin. B: left limb EMG corresponding to 5 cycles of movement. Note that rhythmic activity was obtained in the Ip (iliopsoas, hip flexor) and in the VL (Vastus Lateralis, knee extensor) but not in distal muscles such as LGL.

FIG. 8. EMG of flexor and extensor muscles recording during fast paw shakes evoked by dipping the paw in water. Top: averaged data of 5–10 cycles in that sequence. Same animal as Fig. 5. Intact state (A), after T13 section (B), and after the 2nd section at L3 (C). A vigorous activity was obtained after the 2nd spinalization at L3 level with a frequency higher than in intact cat. Note that the TA-VL synergy is still present after the 1st and 2nd transection.
pinned firmly to a stereotaxic frame (see METHODS), which can prevent spontaneous locomotion. Administration of a higher dose of clonidine (500 μg/kg iv) was thus required, in these acute experiments, to generate locomotion in these cats.

After the establishment of the locomotor rhythm with clonidine, successive cuts were made to the spinal cord caudally to the last chronic lesion. In cats that had been chronically spinalized at L2, successive lesions were made at rostral L3, mid L3, caudal L3, rostral L4, mid-L4, etc. At each level, the following sequence of lesion was strictly observed: removal of the dorsal funiculi, dorsal hemisection, and complete transection of the cord. After each lesion, the locomotor capacity of the animal was assessed for 20–30 min before performing the subsequent lesion and until locomotion was totally abolished. Further injections of clonidine were always made when needed to make sure that the absence of locomotion was not due to a lack of central stimulation. For the cat spinalized at rostral L3, the same paradigm was observed but the lesions were initiated at caudal L4 and at rostral L2 for the two cats spinalized at T13.

SPINAL T13–L2/L3 CATS. Figure 9 illustrates the effect of progressive dorsoventral lesions at different segmental levels of cat C that was previously chronically spinalized at caudal L2. Figure 9A illustrates the locomotor activity obtained 30 min after decerebration and injection of clonidine. B: effect of locomotor activity of a complete transection at mid-L4. C: dorsal hemisection at the caudal L4 level. The locomotion was impaired. D: locomotor activity after a complete transection at caudal L4. This transection completely abolished all rhythmic activity of the hindlimbs.

FIG. 9. Effects of multiple spinal transections in the acute state in one cat previously spinalized at T13 and a chronic secondary lesion at caudal L2. A: locomotor pattern obtained in the same cat as Fig. 3 after decerebration and injection of clonidine. B: effect of locomotor activity of a complete transection at mid-L4. C: dorsal hemisection at the caudal L4 level. The locomotion was impaired. D: locomotor activity after a complete transection at caudal L4. This transection completely abolished all rhythmic activity of the hindlimbs.
injection of clonidine and before the additional lesions. Normal amplitude of flexion of the hip, knee, and ankle, plantar foot placement (Fig. 9A, right) as well as a robust EMG activity with a left-right alternation (Fig. 9A, left) was obtained. This locomotion can be compared with the one obtained in this cat during the chronic experiments before decerebration (compare with Fig. 3D).

Hindlimb locomotion was still present in this cat after progressive lesions at caudal L2, L3, and part of L4. An illustrative example is shown in Fig. 9B after a complete lesion at mid-L4. Locomotion was elicited by clonidine and perineal stimulation, which could at times produce hyperflexion in swing as seen on the far right panel of Fig. 9B. After a dorsal hemisection of caudal L4 (Fig. 9C), the locomotor pattern was still present with large amplitude EMG bursts (Fig. 9C, left), and the steps were of shorter duration (Fig. 9C, right). Locomotion was totally abolished after the caudal L4 transection was completed to include ventral and ventrolateral pathways, and this even after a further injection of clonidine (Fig. 9D).

In the cat that was chronically spinalized at rostral L2 (cat B), locomotion was obtained until a complete transection at rostral L3 was performed. After this transection, all rhythmic activity was abolished (not shown). For the cat chronically spinalized at L3 (cat D), abolition of locomotion was obtained after a complete lesion at mid-L4.

**SPINAL T13 CATS.** The two cats spinalized at T13 were trained with a left-right alternation (Fig. 9A) as well as a robust EMG activity. Locomotor activity persisted after transection at a speed of 0.3 m/s with alternation of the left and right side and with flexion and extension of the knee and the ankle. The amplitude of the swing and of the stance phases was decreased compared with that obtained before the injection. When an adequate locomotor pattern was obtained, a second transection was made. For the two cats, the first second lesion was done at rostral L3. This transection did not affect the locomotor performance. The cats were still able to walk on the treadmill with a proper alternation of the hindlimbs and a robust EMG activity. Locomotor activity persisted after transections at the rostral L3 level and at mid-L3 level, but there was no foot placement and the foot still landed behind the hip. But in one cat, we removed the vertebral pins used for fixation, and a well-organized locomotor pattern was obtained with amplitude of the swing and the stance phase comparable to those observed in the chronic state (flexion and extension of all joints).

All rhythmic activity was abolished when the transection was performed at the rostral L1 level in one cat and at the mid-L4 level for the other cat. At this time, only strong movements of the hindlimbs and hyperflexion, but no locomotor movement was observed. A last injection of clonidine was applied to ensure that the absence of locomotion was not due to a lack of central stimulation. In these cases, clonidine could not be obtained because it is abolished by the injection of clonidine as mentioned earlier. Therefore the acute experiments confirm the results obtained with the chronic lesions. Spinal locomotion can be obtained in cats previously spinalized at T13 until the L3–L4 segments are disconnected from the lower lumbar levels.

**DISCUSSION**

**Importance of the spinal level of transection for spinal locomotion**

**TREADMILL LOCOMOTION.** The main conclusion of the present study is that spinal locomotion of the cat on a treadmill relies critically on the integrity of the L3–L4 spinal segments of the spinal cord. It is well known that a few weeks after spinalization at T13, kittens and adult cats can walk on a treadmill with plantar foot contact during stance and good weight support of the hindquarters (Barbeau and Rossignol 1987; Bélanger et al. 1996; Chau et al. 1998a; de Leon et al. 1998; Forsberg et al. 1980; Lovely et al. 1990; Rossignol 2000). Lesions at other spinal levels have rarely been reported. In kittens, one study (Shurrager and Dykman 1951) mentions a cat spinalized at L3 that could walk, but unfortunately no details were given. A study using chronic double lesions of the cord (Afzel 1970) concluded that there is no locomotion with a spinalization below L4, although other simpler reflex behaviors were seen (rhythmic patterns such as FPS were not reported). The present work shows that a second spinal transection that disconnects the segments above L4 from the more caudal lumbar segments abolishes spinal locomotion, even after 2–4 wk of sustained attempts at locomotor training on the treadmill. Our previous results had reported that a spinal transection at L4 abolished all locomotion in acute preparations (Marcoux and Rossignol 2000). This condition cannot be interpreted as being due to a secondary spinal shock after the second spinal lesion (which should have subsided after a week or so) but is due instead to the disconnection of the mid-lumbar segments with the more caudally located motoneurons. Because in the present report, spinal locomotion was still possible after the second lesions were performed at L2 or rostral L3, it can be concluded that merely performing a second lesion caudal to T13 is not sufficient to abolish locomotion but that the specific inactivation of caudal L3 and L4 is necessary.

**FICTIVE RHYTHMIC PATTERNS.** A study in paralyzed cats, using a combination of lesions and a cooling probe to inactivate specific spinal segments, has demonstrated that “fictive scratching” was strongly dependent on mid-lumbar segments L3–L4 (Deligianna et al. 1983), although rhythmic oscillations could be obtained in the isolated L4 segment. In acute curarized spinal cats (T12–13) injected with nialamide and L-DOPA, rhythmic alternating activity in the TA and GL nerves remained, even after a lesion at rostral L5, when only segments L6, L7, and S1 were intact (Grillner and Zangger 1979). This shows clearly that the L5–S1 segments have the necessary interneuronal circuitry to produce spinal rhythmicity. In this acute experimental condition, it is probable that the chemical stimulation produced by DOPA through the still-functional monoamine terminals may indeed mimic the inputs normally received by supraspinal pathways.

**PHARMACOLOGICAL STIMULATION.** We have not studied DOPA in our chronically spinalized preparations because, in principle, the noradrenergic terminals have degenerated, and, therefore
DOPA, the precursor of NA that needs to be taken up by the terminals to release NA, would be inefficient. Accordingly, we have studied only the effect of an alpha-2 receptor agonist, clonidine, which has been shown to induce locomotion in spinal cats in many previous studies (Barbeau et al. 1987; Chau et al. 1998b; Forssberg and Grillner 1973; Giroux et al. 2001). In the present study, clonidine injected in cats with a single chronic lesion at T13 also induced robust locomotion with marked effects on the overall step cycle but especially on flexor burst duration.

It was striking and unexpected to observe that clonidine injected after a second lesion at L3, L4, or L5 induced a tonic bilateral hyperextension on the treadmill only when combined with perineal stimulation. These puzzling effects suggest that segmental distribution of receptors may account for this finding. Indeed, it can be postulated that, if receptors for various transmitters are unevenly distributed along the length of the spinal cord (Rossignol et al. 2002), transmitter agonists may induce different pharmacological effects depending on the level of the transection. Such a regional difference in the activation of locomotion by 5-HT was shown in the neonatal rat (Cowley and Schmidt 1997). It is possible, therefore, that the net effect of clonidine on the low lumbar spinal cord may favor extensor hyperexcitability, while the effect on the upper lumbar cord may have a net effect that is biased toward the flexors. Because various types of spinal interneurons are sensitive to adrenergic stimulation (Hammar and Jankowska 2003), it is indeed tempting to speculate that the effects of pharmacological stimulation may depend on the segmental distribution of receptors for a given neurotransmitter. Clearly, however, the effect of clonidine on segments L5–S1 seems to be predominant in the extensor muscles and are nonlocomotor in the chronic spinal cat.

**Mechanisms of locomotor abolition after L4 lesions**

**Damage to afferent inputs.** One mechanism that could be implicated with the L3–L4 transection is a partial deafferentation from proximal joints, muscles, or skin. After a unilateral hindlimb deafferentation (caudal to L2 or L3 in decerebrate but not spinal cats), it was reported that hindlimb locomotion could be abolished (Orlovsky and Feldman 1972), although lumbar interneurons still discharged rhythmically in the absence of forelimb movements. In decerebrate cats with mesencephalic stimulation (MLR), locomotion could be induced after unilateral deafferentation even though the pattern was more labile (Grillner and Zangger 1984). Here again, inputs from the forelimbs could have played a role in providing timing cues to the deafferented hindlimb. However, deafferentation alone, involving L1–L4, does not seem to be sufficient to abolish locomotion in decerebrate cats.

Some studies have looked at deafferentation in spinal cats. In one study, unilateral lumbar deafferentation was done first in otherwise intact cats, and they recovered locomotion. After performing a hemisection on the deafferented side, the cats stopped walking (Goldberger 1977). On the other hand, complete spinal cats that have recovered locomotion will still be able to perform some stepping movements on the treadmill after a unilateral deafferentation (Giuliani and Smith 1987).

Although we cannot discard categorically that our lesions at caudal L3 and L4 abolish critical sensory inputs (for instance from hip muscle, joint, or skin afferents), the above deafferentation experiments involving L1–L4 dorsal roots lead us to believe that this cannot be the main mechanism, suggesting that deafferentation alone of the L3–L4 segments might not be sufficient to explain the total absence of locomotion reported here, after a chronic lesion at caudal L3 or L4.

**Damage to motoneurons**

One obvious concern in such experiments is whether the second lesion could induce damage to motoneurons, thus preventing any motor pattern. First our histological assessment of the second lesion clearly indicated damage of ~7 mm, including the lesion itself and damage above and below the lesion. This could hardly explain how a lesion at L4 could abolish activity in motoneurons of L2–S1. However, interestingly, it might explain that lesions close to the L3–L4 border may have various effects that may evolve with time and the progressive extent of the damage that could gradually affect L4. Furthermore, when comparing the levels of transection necessary to abolish locomotion in acute and chronic spinal cats, it was observed that a slightly higher number of caudal lesions in the acute experiments was needed to abolish locomotion. This may suggest that, in the chronic cats, more rostral lesions induce damage lower down in the L4 segments.

However, the following results are even more compelling. Although there was no spinal locomotion after the second spinal lesions below caudal L3, vigorous fast paw shakes could still be elicited and tend to be even more vigorous. The characteristic synergy of TA and VL co-activation (Pearson and Rossignol 1991; Smith et al. 1985) was preserved after the first and the second spinal transections. This shows that most motoneurons controlling hindlimbs muscles of the cat, located in L5–S1 segments (Sherrington 1892; Vanderhorst and Holstege 1997) were not significantly damaged by the second lesion, except in cat H, lesioned at L5. Although stimulation of L4 leads to abdominal contractions and hip flexion (Sherrington 1892), hip flexor muscles such as Ip or Srt also receive an innervation through the L5 ventral root, so that a transection at caudal L3 or L4 should not denervate these proximal muscles. The abolition of spinal locomotion reported here, therefore is not a consequence of damage to motoneurons but to a lack of their recruitment into a locomotor pattern. This finding also establishes that, despite the absence of locomotion, the spinal cord still has a strong rhythmicogenic potential in segments below L4 and that the circuitry responsible for FPS is largely distinct from that of the locomotor CPG. Because we are looking at actual behaviors, we can be certain that the rhythms observed correspond to distinct behaviors; this might not always be the case when looking at recordings in paralyzed preparations.

**Damage to interneurons**

The most likely cause of the abolition of locomotion after our secondary lesion is a disconnection of neural circuits in the midlumbar segments that are important for spinal locomotion. The spinal cord is endowed with a rich network of propriospinal interneurons (Kostyuk and Vasilenko 1979), which make excitatory and inhibitory connections with motoneurons through the DLF (Kostyuk et al. 1971). In the cat, some
interneurons, in the intermediate zone and in the ventral horn of mid-lumbar segments (mainly L₃) of the spinal cord, receive dominant inputs from group II muscle spindle afferents (especially quadriceps and Srt) and project to motoneurons in more caudal segments through the lateral funiculi (Edgley and Jankowska 1987). It was proposed that these mid-lumbar interneurons (especially the ventral group) might play a role in locomotion (Edgley et al. 1988) and were shown directly to be activated during MLR-evoked fictive locomotion in decerebrate cats (Shefchyk et al. 1990). Other commissural interneurons located at mid-lumbar levels and that normally receive supraspinal inputs (such as reticular formation) and project to contralateral motoneurons (Jankowska et al. 2003) could also be implicated. It is thus possible that all these groups of interneurons located rostrally in the lumbar cord may play a role in locomotion and that after spinalization some may actually become even more critical for the expression of locomotion. This is in keeping with suggestions made by others (Jordan and Schmidt 2002; Shik 1983) that chains of spinal propriospinal interneurons may play a key role in organizing locomotion. Our present work would suggest that the L₃–L₄ interneurons are critical in that chain and may actually be the last representative of that chain after chronic spinal transection at T₁₃. We do not infer here that the CPG for locomotion is located at L₃–L₄ but rather that these segments re-innervated the rostral L₁–L₂ segments (Gimenez y Ribotta et al. 2000).

It can thus be concluded that the upper lumbar segments in rats (L₁–L₂) and mid-lumbar segments in cats (L₃–L₄) play a crucial role in the organization of the spinal locomotor pattern. An appealing consequence of this work is the possibility to target circumscribed critical spinal segments for pharmacological stimulation (Marcoux and Rossignol 2000), electrical stimulation (Barthélemy et al. 2002), or cell grafts (Gimenez y Ribotta et al. 2000), to induce locomotion after spinal cord injury.

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REFERENCES


