Characteristics and Mechanisms of Locomotion Induced by Intraspinal Microstimulation and Dorsal Root Stimulation in Spinal Cats

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Intraspinal microstimulation (ISMS) through a single microelectrode can induce locomotion in cats spinalized at T13 1 wk before (untrained) or after 3–5 wk of treadmill training. Here we study the optimal parameters of ISMS and the characteristics of locomotion evoked. ISMS was applied in the dorsal region of segments L3–S1 at different lateralities (midline to 2.5 mm) and after an intravenous injection of clonidine (noradrenergic agonist). Kinematics and electromyographic recordings were used to characterize locomotion. ISMS could induce a bilateral locomotor pattern similar to that obtained with perineal stimulation, and the characteristics of locomotion varied according to the spinal segment stimulated. Mechanisms by which ISMS could evoke locomotion were then investigated by stimulating, inactivating, or lesioning different spinal structures. Dorsal root stimulation (DRS), just like ISMS, could evoke a variety of ipsi- and bilateral nonlocomotor movements as well as locomotor responses. This suggests that sensory afferent pathways are involved in the production of locomotion by ISMS. Microinjections of yohimbine (noradrenergic antagonist) in L3 and L4 segments or a complete second spinal lesion at L1–L4 abolished all locomotor activity evoked by ISMS applied at more caudal segments. Progressive dorsosential spinal lesions at L3 or L4 and restricted ventral lesions at L4 further suggest that the integrity of the ventral or ventrolateral funiculi as well as the L1–L4 segments are critical for the induction of locomotion by ISMS at L5 to S1 or by DRS at these caudal segments.

I N T R O D U C T I O N

Previous reports have shown that intraspinal microstimulation (ISMS) through a single electrode could generate whole hindlimb movements (flexion, extension, abduction and adduction) in anesthetized cats and that a few microelectrodes could be sufficient to achieve functional hindlimb movements (Tai et al. 2003). Lemay and Grill (2004) reported similar results using endpoint force measurements. ISMS applied through multiple electrodes located in the ventral horn of acutely decerebrated cats, chronically spinalized 2–4 wk before, was shown to successfully induce locomotion (Guevremont et al. 2006; Sai-gal et al. 2004). However, the possibility of triggering full locomotor synergies with only one electrode appears to have distinct advantages especially when envisaging future chronic electrode implantation in a rehabilitation perspective after spinal injury. In that context, we have shown that ISMS through a single electrode applied at various spinal segments from L3 to L7 could evoke a variety of ipsi- and bilateral nonlocomotor flexion or extension responses as well as locomotion in spinal cats (Barthélemy et al. 2006). This second paper aims at studying these locomotor responses in more details first by better defining the parameters to evoke locomotion with a single microelectrode and second by identifying the spinal structures involved.

In the first aim, the ISMS parameters that could more easily induce locomotion (tonic or train stimulation, frequency, and intensity) were established in cats that were also injected with the alpha-2 noradrenergic agonist clonidine (Chau et al. 1998b). The importance of previous locomotor training and chronicity of the spinal lesion as well as the effect of the spinal segment stimulated were assessed. In the second aim, the spinal structures involved in evoking locomotion by ISMS were studied in more detail by directly activating them or by inactivating them pharmacologically or surgically. The preceding paper (Barthélemy et al. 2006) showed that ISMS in the dorsal and dorsolateral funiculi as well as in the dorsal horn was most effective in inducing locomotion. This suggested that the stimulation of those sites probably activated afferent pathways or interneurons normally contacted by these sensory afferents. Here we directly compare ISMS with dorsal root stimulation (DRS) at the same spinal segment, and the characteristics of the locomotion evoked by either means were compared. Moreover, although ISMS or DRS could trigger locomotion when applied in various lumbar segments, inactivation of mid-lumbar segments (L1–L2) by intraspinal injections of yohimbine, a noradrenergic antagonist, or sections at L4 (including ventral quadrants) abolished locomotion evoked by electrical stimulation applied at more caudal segments. This suggests that these mid-lumbar segments are essential to evoke locomotion by electrical stimulation of the spinal cord in agreement with previous work (Langlet et al. 2005; Marcoux and Rossignol 2000). These results are discussed in the light of some current models of spinal generation of locomotion. Part of this work has been published as abstracts (Barthélemy et al. 2002, 2005).

M E T H O D S

Twenty-three cats of either sex (3.1–6.7 kg) were used for this study. Eighteen of these cats were spinalized 5–7 days before the acute experiment and were not trained on the treadmill. The remaining five cats were spinalized at the thoracic 13 level (T13) and trained to...
walk on the treadmill (10 min, 3 times/day, 5 day/wk) until they recovered spontaneous hindlimb locomotion on the treadmill i.e., 3 (n = 2), 4 (n = 1), and 5 wk (n = 2) before the acute experiment. The same cats were used in a previous paper (Barthélémy et al. 2006) in which more methodological details are given. The description of the methods will thus be limited to more specific and relevant aspects. All procedures were conducted according to the Guide for the Care and Use of Experimental Animals (Canada), using protocols approved by the Ethics Committee of Université de Montréal.

Acute experiments

Under general anesthesia, 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 around 38°C by a feedback-controlled heating element using DC and with heating lamps if necessary. The end-expiratory pCO₂ was monitored using a Datex Monitor during normal or assisted ventilation and maintained between 3.5 and 4.5%. Cats maintained their blood pressure within the normal range; in two cases Levophed was given to correct a low pressure. A laminectomy of L₃ to L₆ vertebrae exposed the spinal cord segments from L₃ to S₁, and the cats were then mounted on a spinal contention unit over a motor-driven treadmill. To ensure stability of the spinal cord for subsequent exploration with intraspinal stimulation, the spine was fixed with three pairs of lateral pins: one pair on the L₁ pedicles, one on those of L₄ or L₅, and the other on the iliac crests. Although such a sturdy fixation was necessary to ensure repeatability of stimulation at the same site, it could limit the amplitude of the movement and provide a noxious input that may interfere with the full expression of the locomotor pattern. A precollricular, postmamillary decerebration was performed with a narrow spatula, and the rostral nervous tissue was aspirated. Anesthesia was then discontinued. In some cases, assisted ventilation had to be used after decerebration. The dura was opened, and the spinal cord covered with warm mineral oil. The spinal segments were determined by identifying the most rostral and caudal rootlets of each dorsal root.

Electrical stimulation

ISMS using a custom-designed stimulating software and a linear stimulus isolator unit (World Precision Instruments; model A395) was applied monopolarly to the spinal cord with a single tungsten electrode insulated except for the tip (~1–2 μm; impedance: 0.08–0.1 MΩ; in 5 cats, electrodes of 5–20 MΩ were used). The indifferent electrode was inserted into back muscles. Biphasic pulses (10–90 μA, 250–500 μs) were delivered either as single pulses with varying frequencies (1–300 Hz; referred to as tonic ISMS) or as trains of 50–500 ms duration with an intra-train frequency of 70–500 Hz and a rate of 0.3–2 train/s (referred to as train ISMS). Tonic stimulation was used to mimic tonic descending inputs from the mesencephalic locomotor region (MLR) (Shik et al. 1966), whereas train stimulation was used to control and entrain the frequency of the locomotion. The stimulation was applied one site at a time in different parts of the dorsal, lateral, and ventral areas of the L₂ to S₁ segments and from the midline to 2.5 mm on either side. However, we concentrated on segments L₁ to L₄, stimulating both left and right sides of the cord from the first rootlet of L₃ to the last rootlet of L₇, by steps of 0.5 mm. At each stimulating entry point, the electrode mounted on a manipulandum was lowered while the stimulation was continuously on. The descent was stopped every 0.5 mm to assess the effects at that point. Stimulation parameters (location, frequency, intensity) were considered efficient and optimal when a sustained bilateral stepping with adequate kinematics as well as EMG characteristics was obtained.

DRS (10–300 μA) was applied with a bipolar hook electrode in three cats. The dorsal rootlets were cut as distally from the spinal cord as possible and laid on the hook electrodes, which were bathed in warm mineral oil. These experiments aimed at comparing responses triggered by DRS and ISMS at the same spinal segment.

Experimental protocol

At least 1 h elapsed between the cessation of anesthesia and the start of stimulation. First, the locomotor capacity of all cats was evaluated on the treadmill at a speed of 0.2 m/s while using perineal and/or abdominal manual stimulation. Second, the ability of electrical microstimulation to induce locomotion by itself, i.e., without drugs, was tested in five animals. After having tested locomotor capacities before drug injection, clonidine was injected intravenously (500 μg/kg) into those five animals to determine if the ability of electrical stimulation to trigger locomotion could be improved. In all the other cats (n = 13), clonidine was injected before the effects of electrical stimulation were tested. Only the data obtained after clonidine will be presented in the present paper. Clonidine was used because noradrenergic agonists have been found to be the most efficient to initiate locomotion in spinal cats on a treadmill (Barbeau and Rossignol 1991; Chau et al. 1998a; Forssberg and Grillner 1973). In five cats, naloxone was also injected intravenously (700 μg/kg) to potentiate the effects of clonidine (Pearson et al. 1992). Those preparations were used to study the optimal parameters, location of stimulation, and characteristics of the evoked locomotion. Perineal stimulation was used as a reference to determine the efficiency and the quality of the locomotor pattern induced by electrical stimulation. No difference between the results of cats that received clonidine only and those that received clonidine with naloxone was observed.

In this study, the importance of midlumbar segments L₁–L₄ in the initiation of locomotion as well as the spinal pathways activated by the stimulation were investigated. In two cats, yohimbine (an α₂-noradrenergic blocker, 8 mg/ml) (Marcoux and Rossignol 2000) was injected intraperitoneally. The injections of 2.5 μl each were given using a Hamilton syringe (26 gauge needle; Hamilton, Reno, NY) inserted 2 mm deep paramedially (1 mm on each side of the midline) in the segments L₁ and L₄ (n = 1) or only in L₄ (n = 1). Eight to 10 injections per segments were made to cover the whole segment bilaterally. Yohimbine was used to test if inactivating the mid-lumbar segments could block locomotion triggered by electrical stimulation of more caudal segments.

A bilateral rhizotomy at L₁–L₄ was performed in one cat, and successive spinal lesions were performed in 13 cats at L₁, L₃, and L₄. After each spinal lesion, electrical stimulation was applied to more caudal regions. If the portion of the spinal cord left intact was sufficient to produce bilateral locomotion, then another lesion was performed in the next caudal segment. The spinal lesions were applied progressively either dorsventrally or ventrodorsally, and after each partial section, locomotor output was assessed until no locomotion could be triggered by the stimulation of more caudal segments. At the end of the experiments, electrolytic lesions were made in locations that successfully triggered locomotion (n = 5), and all cats were killed by an overdose of intravenous pentobarbital sodium. The spinal cord was then removed for histology.

Recordings and analysis

Kinematics and EMG recordings were important parts of the analysis to determine if bilateral locomotion was induced. A complete step cycle was defined as consisting of a stance and a swing phase. The stance phase begins as soon as the foot contacts the treadmill belt and terminates when the foot starts its forward movement. The swing phase starts at this point and terminates as the foot strikes the treadmill belt again or as the hindlimb initiates a downward and backward movement when a foot drag is present. Foot drag is common after spinalization and consists of an inadequate clearance of the foot during swing. It is defined as the initial period during which the tip of
the toes drags on the surface of the treadmill belt during the forward movement of the foot before it is lifted above the belt. Step cycle duration was defined as the time elapsed between the beginning of the foot lift or the forward movement to the next similar event. Activity was considered to be locomotor only when there was an alternation between flexor and extensor muscles in a hindlimb, an out-of-phase alternation with the other hindlimb and a forward and upward movement during the swing phase of the cycle.

Coupling between the right and left hindlimb (interlimb coupling) was measured from flexor and extensor muscle bursts, and the values are given in Tables 1 and 2. Coupling between the flexor and extensor muscles within each limb (intralimb coupling) was also measured. The values displayed in the tables represent the onset of extensor bursts relative to the onset of the flexor burst activity which is set at 0. A significantly different intralimb coupling between both limbs reflects an asymmetrical gait.

Statistics

Differences between measurements of cycle duration, intralimb, and interlimb coupling were compared with Student’s t-test and were considered to be significant if the probability of α type error was <0.05. This Student’s t-test was also used to determine if the correlation between the segment stimulated and the distance of the foot contact and the foot lift from the hip axis was significant. Differences between proportions of responses evoked by dorsal roots, and between the coefficient of variation in the inter- and intralimb coupling were compared using a χ² test (χ²) with significance at the 5% level.

RESULTS

Locomotion induced by ISMS

PARAMETERS TO INDUCE LOCOMOTION. Data were collected from locomotor sequences induced in spinal cats with clonidine and, in five experiments, clonidine was supplemented by naloxone. Perineal stimulation induced bilateral locomotion in the 23 cats tested and served as an indicator of the ability of the cats to walk in these experimental conditions. The locomotor pattern induced was used as a reference to compare with the pattern evoked later on with ISMS. The kinematics and EMG activity of locomotion at 0.2 m/s induced by perineal stimulation are displayed in Fig. 1A for an untrained cat spinalized 1 wk previously. Note the forward and upward movement during the swing phase and some foot drag at the beginning of the swing phase (see METHODS). The excursion of the four joint angles is averaged over seven consecutive step cycles and shown below the EMG traces. The total excursion for the hip, knee, ankle, and MTP is 20 ± 1, 15 ± 1, 32 ± 2, and 32 ± 3° (±1 SD), respectively. The corresponding EMG activity shows clear alternation between flexor and extensor muscles of each limb as well as between left and right hindlimbs.

INTENSITY OF THE STIMULATION. Electrical stimulation could evoke bilateral locomotion in 21 of the cats. For both tonic and trains of ISMS, bilateral locomotion was achieved at low intensity (20–90 μA). However, this intensity was high compared with the motor threshold determined by muscle twitches observed visually in the hindlimb, typically at around 5–15 μA, depending on the region stimulated and the state of the spinal cord. For example, when stimulation was applied in the dorsal part of mid-L₆, 1.5 mm from the midline in a 1-wk spinal cat, motor response in the ipsilateral hindlimb was detected at 5 μA. From 5 to 19 μA, the ipsilateral response became stronger with increasing intensity. At 20 μA, a motor response was observed in both hindlimbs simultaneously and in synchrony with the stimulation. At 40 μA, contralateral locomotion was recorded and bilateral locomotion was triggered from 40 to 50 μA. Stimulating at higher intensities than 50 μA did not induce locomotion but only ipsilateral flexion. Those intensity values were not absolute and varied from one cat to another but this gradient was observed in all cats.

TONIC ISMS. Sustained locomotor activity on the treadmill (0.2 m/s) was obtained by tonic stimulation when applied in the dorsal area of the spinal cord and at all lateralties tested from the midline to 2.5 mm laterally. Low intensity (20–90 μA) and low frequencies (2–6 Hz) were optimal to induce long-lasting locomotion, resembling the pattern evoked with perineal stimulation. Tonic ISMS induced locomotor activity in 9 of the 16 cats in which it was tested. In Fig. 1B, the movements are smaller than those observed with perineal stimulation and the

<table>
<thead>
<tr>
<th>Cats</th>
<th>n</th>
<th>Interlimb Coupling</th>
<th>Intralimb Coupling</th>
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<tr>
<td></td>
<td></td>
<td>St VL or GL</td>
<td>Right Left</td>
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<tr>
<td></td>
<td></td>
<td>St</td>
<td>VL or GL</td>
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<td>A. Perineural stimulation</td>
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<tr>
<td>1</td>
<td>27</td>
<td>0.47 ± 0.15 (32)</td>
<td>0.58 ± 0.11 (17)</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>0.52 ± 0.06 (12)</td>
<td>0.49 ± 0.06 (12)</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>0.66 ± 0.12 (18)</td>
<td>0.45 ± 0.15 (33)</td>
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<tr>
<td>B. ISMS</td>
<td></td>
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<tr>
<td>1</td>
<td>54</td>
<td>0.45 ± 0.13 (28)</td>
<td>0.5 ± 0.14 (28)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.47 ± 0.15 (32)</td>
<td>0.39 ± 0.17 (44)</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>0.51 ± 0.14 (27)</td>
<td>0.56 ± 0.14 (25)</td>
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</table>

This table displays the values for interlimb coupling between right and left flexor muscles [semitendinosus (St)] as well as between right and left extensor muscles [vastus lateralis (VL) or gastrocnemius lateralis (GL)] during locomotion induced by perineural stimulation or intraspinal microstimulation (ISMS). Interlimb coupling compares the onset of electromyographic (EMG) burst of the same muscle in the left and the right hindlimb. Interlimb coupling based on St, VL, or GL compares the onset of St, VL, or GL EMG bursts, respectively, in the right and left hindlimb. A coupling of 0.5 means that there is a perfect reciprocity between the two muscles studied. Intralimb coupling compares the onset of a flexor (St) and an extensor (VL or GL) muscle in the left or the right hindlimb. In other words, it displays values of coupling between flexor and extensor muscles of the same limb (right or left), and the values in this table represent the onset of extensor burst relative to the onset of flexor burst activity, which is set at 0. The coefficient of variation is indicated as a percentage in parentheses. The data of 3 cats are represented in this table (cats 1, 2, and 3) and the number of cycles analysed is indicated in the n column. Values are means ± SD.
Paw drag is more pronounced (as also seen in the joint excursion). The angular excursions of hip, knee, ankle, and MTP were 11°/0.6, 13°/1, 29°/2, and 40°/5°, respectively. Therefore the decrease in angular excursion here is mainly at the hip joint with some increase in the MTP joint excursion. EMG bursts reflect a locomotion pattern that is well defined and organized. Although low frequencies were found to be optimal, few cycles of bilateral locomotion were also triggered at 30, 70, and 100 Hz. Stimulation at higher frequencies than 100 Hz (150, 200, and 300 Hz) could not induce sustained bilateral locomotion in this preparation.

Train ISMS. Trains of stimulation could also evoke a regular bilateral and alternating locomotor pattern when applied in dorsal areas of all segments tested, from L2 to S1 (Barthélemy et al. 2006). In Fig. 1C, the amplitude of movements is diminished compared with those induced by both perineal and tonic stimulation in this cat. Indeed, the total excursion for the hip, knee, ankles, and MTP is 7 ± 1, 14 ± 2, 26 ± 4, and 17 ± 3°, respectively. Note that although the frequency of the trains and the frequency of the step cycle are very close, the stimulus train does not clearly trigger a burst in any of the recorded muscles. The optimal train duration was found to be between 150 and 300 ms. Different intra-train frequencies were tested (50–500 Hz), but 50 Hz was used more commonly. Inter-train frequency from 0.3 to 2 train/s were tested, and the window between 0.65 and 1 train/s could induce bilateral locomotion with a treadmill speed of 0.2 m/s. At higher frequencies, locomotion could be induced in the contralateral hindlimb only, and short-latency responses (mainly flexion of the hindlimb) would be observed in the ipsilateral leg. At lower frequencies, short-latency responses (mainly flexion) were induced in the ipsilateral hindlimb only.

Thus tonic and train ISMS could induce locomotor pattern that are robust, sustained, and long-lasting. Although the locomotor pattern and the easiness with which locomotion was induced varied from cat to cat, train ISMS triggered locomotor sequences more frequently (21 of 23) than tonic stimulation (9 of 16) and was therefore used predominantly in the present study.

### TABLE 2. Locomotor coupling in trained spinal cat

<table>
<thead>
<tr>
<th>Cats</th>
<th>n</th>
<th>Interlimb Coupling</th>
<th>Intralimb Coupling</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>St</td>
<td>Gl</td>
</tr>
<tr>
<td>A. Perineal stimulation</td>
<td></td>
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<tr>
<td>4</td>
<td>23</td>
<td>0.4 ± 0.08(20)</td>
<td>0.59 ± 0.1 (17)</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>0.49 ± 0.05(10)</td>
<td>0.51 ± 0.06(12)</td>
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<tr>
<td>B. Electrical stimulation</td>
<td></td>
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<tr>
<td>4</td>
<td>15</td>
<td>0.47 ± 0.1 (21)</td>
<td>0.45 ± 0.07(15)</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>0.63 ± 0.13(21)</td>
<td>0.43 ± 0.06(14)</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>0.31 ± 0.08(26)</td>
<td>0.59 ± 0.07(12)</td>
</tr>
</tbody>
</table>

This table displays the values for interlimb coupling between right and left flexor muscles (St) as well as between right and left extensor muscles (VL or GL) during locomotion induced by perineal stimulation or ISMS. It also displays the values of intralimb coupling, between flexor and extensor muscles of the same limb (right or left). The data of 3 cats are represented in this table (cats 4, 5, and 6), but the data of only two of those cats are presented during perineal stimulation. The number of cycles analysed is indicated in the n column. The percentage of variation is indicated in parentheses.
Characteristics of locomotor movements

PERINEAL VERSUS ISMS-EVOKED LOCOMOTION. Locomotor pattern induced by perineal stimulation and ISMS were compared, by calculating the interlimb and intralimb coupling (see Methods) in untrained 1-wk spinal cats (Table 1). Interlimb coupling was similar during locomotion induced by ISMS and perineal stimulation, and no statistical difference was observed in two of the three cats tested (cats 1 and 2). Intralimb coupling showed differences between the left and right hindlimb for each cat during locomotion triggered by perineal or ISMS stimulation. This led to an asymmetrical gait that seemed more prominent with ISMS than with perineal stimulation. However, this was not shown to be statistically significant for those three cats. Furthermore, the variability of the coupling from step to step during a given locomotor sequence, referred to as coefficient of variability, was not different between locomotion evoked by either mean (χ² not significant). It can thus be concluded that the quality of the locomotor pattern evoked by ISMS is similar to that evoked by perineal stimulation, which was our point of reference in these experimental conditions.

The effect of locomotor training and chronicity were determined by comparing the locomotor pattern induced in untrained 1-wk spinal cats with the one induced in chronic, trained spinal cats (Table 2). Interlimb coupling was similar in both preparations whether the locomotor sequence was induced by perineal stimulation or ISMS (see cats 4 and 5). The intralimb coupling values reveal asymmetrical gait for all cats with ISMS but only for cat 4 with perineal stimulation. Thus inter- and intralimb values were similar in both untrained and chronic, trained spinal cats. However, the coefficients of variation observed in the three untrained spinal cats with ISMS and shown in Table 1 were significantly higher than those observed in the trained spinal cats (χ²; P < 0.05). Thus in these experimental conditions, the locomotor pattern does not differ between both preparations, but locomotion in chronic, trained spinal cats is less variable and more regular from step to step.

ONSET OF LOCOMOTION. The majority of bilateral locomotor bouts induced by tonic and trains of electrical stimuli followed two main patterns of onset. In the first pattern (Fig. 2A), alternation between flexion and extension was only observed in the contralateral side, and the ipsilateral flexor muscle (LSrt) was firing rhythmically and out of phase with the contralateral flexor muscles. Bilateral locomotion began when the ipsilateral extensor muscles bursted rhythmically and out of phase with ipsilateral flexor muscles. The delay between the appearance of alternation in the contralateral hindlimb only, and bilateral locomotion varied from a few milliseconds to several seconds and moving the electrode up or down slightly in the dorsal area was often necessary to trigger a bilateral locomotor sequence. The second most frequent pattern of induction started with a burst in the contralateral extensor and ipsilateral flexor muscles simultaneously (Fig. 2B). Then contralateral flexor and ipsilateral extensor muscles fired simultaneously to produce bilateral locomotion. Thus locomotion can start in the contralateral hindlimb first or in both limbs simultaneously. When a successful site was found, it was possible to return to that site repeatedly even if adjoining sites were sometimes ineffective.

OFFSET OF LOCOMOTION. Locomotion could persist after the termination of either tonic or train ISMS. Indeed, in Fig. 3A, train ISMS evoked bilateral locomotion that persisted for three more cycles after the cessation of the ISMS. The cycle duration progressively lengthened, and locomotion eventually faded out although the treadmill was still moving. Thus ISMS clearly activated a locomotor network the activity of which could persist temporarily after cessation of ISMS but needed ISMS to be maintained. Figure 3B illustrates that although the rhythm is contingent on the presence of ISMS it is not triggered cycle by cycle. Indeed, at the beginning of this sequence on the left side (shaded area), an independent extensor burst in LVL and LGL was observed just before the stimulation induced other EMG bursts in those muscles. Toward the middle of this sequence, the independent extensor burst is in synchrony with the one evoked by the stimulation. At the end, the independent extensor bursts emerged and followed the activity triggered by the stimulation (second shaded area). This phenomenon was also observed for Srt muscle. Therefore a sustained stimulation is needed to maintain the locomotor rhythm but each stimulus train does not evoke a step.

ENTRAINMENT. The frequency of the locomotor cycle induced was related to the frequency of tonic stimulation and increasing the frequency of the stimulation from 2 to 6 Hz, increased the locomotor frequency proportionally. This influence is more evident with train ISMS in which the locomotor frequency often matched the stimulation rate. The possibility of entraining the locomotor cycle was studied in further detail in 11 cats. In Fig. 4A, each train ISMS occurs at the same point in the step cycle of the ipsilateral hindlimb, although there may be a slight
variation in the step cycle of the contralateral hindlimb. Entrainment in this cat could be obtained with stimulation at 0.7, 0.8, and 1 train/s for a treadmill speed of 0.2 m/s (Fig. 4A). Outside this range and with the same treadmill speed, bilateral locomotion was no longer evoked although contralateral locomotion could still be induced. In Fig. 4C, the locomotor rhythm triggered by perineal stimulation and ISMS at a treadmill speed of 0.2 m/s is illustrated. With perineal stimulation, the frequency of bilateral locomotion is at 0.75 cycle/s. In Fig. 4, D and E, locomotion is evoked with ISMS at rates of 0.8 and 1 train/s while the treadmill is kept at the same speed. Ipsilateral extensor muscles and contralateral flexor and extensor muscles exhibited shorter bursts with increased frequencies. In 7 of the 11 cats in which it was assessed, a clear entrainment by the stimulation (where the locomotor rhythm was identical to or had a difference of \( \pm 0.01 \) cycle/s with the stimulation rate) was observed for train rates of 0.65 to 1 train/s.

**ROLE OF SEGMENTAL LEVEL OF STIMULATION.** Locomotion could be induced at all segments (L2–S1), from midline to 2.5 cm lateral. Figure 3B illustrates nonentrainment of the locomotor cycle. A: locomotor activity induced by the stimulation (rostral L4, 0.5 mm deep, 1 mm laterally; stimulation: 50 \( \mu \)A, 300 Hz, 166 ms, 1 train/s) continues beyond the end of its application in an untrained spinal cat. B: frequency of locomotion does not follow the frequency of stimulation. Ipsilateral VL discharge spontaneously before the stimulation-induced burst at the beginning of this sequence. It then discharges in phase with the stimulation bursts toward the middle of the sequence, but after 6 trains, the VL burst is observed after the stimulation induced-train.
mm laterally, and with a higher occurrence when stimulation was applied medially (Barthély et al. 2006). However, differences in the pattern of locomotion varied depending on the localization of the stimulation. In Fig. 5, stimulation is given in segments L₃–L₇, at the same laterality on the left side of the cord. In Fig. 5A, the position at which the foot contacts the ground (●) and lifts from the ground (■) is displayed for each segment stimulated as well as for perineal stimulation. At L₄, the toe contacts the treadmill at ~33 mm in front of the hip, and the toe lifts up from the treadmill at ~50 mm behind the hip axis. Stimulation of more caudal segments decreases the amplitude of the excursion in front of the hip during swing, while increasing the excursion of the leg behind the hip during stance. On average, the foot contacts the treadmill belt significantly more in front of the hip axis during locomotion induced by ISMS at L₃–L₄ (26.6 mm), compared with when ISMS is applied at L₆–L₇ (−1.8 mm; Student’s t-test P < 0.001). Conversely, the foot would end the stance and lift from the treadmill at a point that is significantly behind the hip axis when stimulation was applied at L₆–L₇ (−81.8 mm) than at L₃–L₄ (−57.9 mm; P < 0.01). A similar observation also applied to the contralateral leg. In the cat shown in Fig. 5, the foot contacts the treadmill belt at a more forward point during locomotion induced by ISMS at L₄ and L₅ (17.2 mm) compared with ISMS at L₆–L₇ (−24.7 mm; Student’s t-test P < 0.001). However, there was no statistical difference in the distance at which the leg lifted from the treadmill after stance between locomotion induced by rostral or caudal stimulation. Furthermore, the trajectory analysis reveals a higher excursion of the leg during the swing with a stimulation applied more rostrally at L₃ compared with a stimulation applied at L₇ (Fig. 5B). The duration of the step cycle as well as its sub-phases varied slightly for the stimulation of various segments as well as with perineal stimulation (Fig. 5C), but this was not statistically significant. Similarly, the total step length as well as each sub-phases remained stable during locomotion induced by ISMS at all segments and by perineal stimulation (Fig. 5D). This analysis was done for the three cats exhibiting the best bilateral locomotion when stimulated at all segments, and this stability was observed in two of them. In the same cats, different lateralities were compared within a given segment but no statistical difference was found.

Spinal structures involved in the induction of locomotion by ISMS

To better understand how ISMS evokes locomotion, we compared first ISMS-evoked locomotion with the DRS at different levels because the preceding findings suggested that ISMS applied to the dorsal quadrants were particularly efficient in triggering locomotion. Then using either ISMS or DRS, we studied the effect on evoking locomotion after inactivating
midlumbar segments pharmacologically or disconnecting these segments with progressive spinal lesions of the same midlumbar levels.

**DRS.** DRS from L$_3$ to S$_2$ was tested in three cats and could trigger ipsi- and bilateral nonlocomotor responses as well as locomotor responses (Barthélémy et al. 2006). Ipsilateral flexion (19% of all responses) and bilateral flexion (14% of all responses) could be elicited by stimulation of all roots. Ipsilateral extension (5%) was mainly induced by stimulation of caudal roots. Its distribution was significantly different from that of the ipsilateral flexion or of the bilateral flexion ($\chi^2 P < 0.01$). Contralateral locomotion represented 40% of all responses triggered by DRS and was accompanied on the ipsilateral side by flexion (31%) when stimulating dorsal roots at all levels or by extension (7%) when stimulating dorsal roots of more caudal segments L$_5$ to S$_2$. The distribution of those two responses was also found to be statistically different ($\chi^2 P < 0.01$). Bilateral locomotion represented 12% of the responses evoked. Increasing the intensity of stimulation generated movements of the contralateral hindlimb, leading to bilateral flexion, bilateral extension, or ipsilateral flexion with contralateral extension. Further increase of the intensity would induce locomotor responses. Locomotion of the contralateral hindlimb was the most frequent response induced, and increasing or decreasing the intensity could trigger bilateral locomotion. However, further increase of the intensity would stop bilateral locomotion and induce a strong ipsilateral movement with or without contralateral locomotion. Such an evolution of the responses triggered with variation of the intensity was observed with stimulation of all roots.

Figure 6 displays the responses evoked by ISMS and DRS at the same level (L$_3$) in the same cat. In Fig. 6A, electrical stimulation was applied slightly to the right of the midline along the dorsoventral axis. Alternation between St and VL/GL is observed in both limbs. Descending the electrode to 2.5 mm deep induced ipsilateral extension (RVL and RGL) and contralateral flexion. At 3 mm below the dorsal surface, contralateral locomotion was induced in the left hindlimb, whereas extension was evoked in the ipsilateral hindlimb. The small discharge observed in RSt was not large enough to induce a flexion movement. The last site stimulated in this track at 5.3 mm deep induced ipsilateral extension.

In Fig. 6B, stimulation of the right L$_7$ dorsal root induced different responses depending on the intensity applied. In this example, stimulation near threshold induces bilateral locomotion. It also triggers short-latency responses superimposed on the locomotor rhythm of all ipsilateral muscles (right). At 15 $\mu$A, only contralateral locomotion is observed and flexion in the ipsilateral hindlimb is evoked. There is still an activity in the ipsilateral extensor, but it is barely noticeable and not large enough to induce an extension movement. At 30 $\mu$A, ipsilateral extension with contralateral locomotion is evoked, and at 40 $\mu$A, contralateral locomotion is still present but accompanied by ipsilateral flexion. Thus increasing the intensity evokes a variety of responses that are similar to those evoked with ISMS at different depths.

The efficient intensities to induce locomotion could go as high as 300 $\mu$A for DRS, which is a larger range than for ISMS. In contrast to what is observed in Fig. 6B, higher intensities were usually more efficient to induce bilateral locomotion with DRS. On the three cats where it was systematically tested, DRS either as tonic stimulation (2 cats) or with trains of pulses (3 cats) was effective to induce sustained bilateral locomotor activity. Contralateral locomotion was induced in all cats with both tonic and train stimulation. As for ISMS, the locomotor pattern of the ipsilateral hindlimb was more perturbed by train stimulation than with tonic stimulation, which resulted in a more pronounced asymmetrical gait. Optimal parameters to induce bilateral locomotion with DRS were similar to those found with ISMS, for both tonic and train stimulation.

Induction sequence of locomotor bouts induced by DRS was similar to that described for ISMS. The frequency of trains applied by DRS also had an effect on locomotor frequency and an entrainment was obtained in two cats of the three tested. Furthermore, cycle duration could also be influenced by the intensity of the stimulation. At high intensities, rapid locomotion was induced, whereas lower intensities lengthened the cycle and slowed down the locomotor rhythm.

The remarkable similarities between the locomotor patterns obtained by DRS and ISMS at the same levels suggest that activation of the afferents plays a critical role in ISMS-evoked locomotion.

**INTRASPINAL MICROINJECTIONS OF YOHIMBINE.** To study the role played by certain midlumbar segments in generating locomotion by ISMS or DRS, we injected yohimbine, an alpha-2 noradrenergic antagonist, in the spinal cord at various spinal segments. Intraspinal injections of yohimbine restricted to both L$_3$ and L$_4$ segments or only to the L$_4$ segment abolished locomotion that could previously be evoked by electrical stimulation of segments L$_3$ to S$_1$ (see Table 3). The locomotor pattern could later be reinstated after injection of clonidine intraspinally (is) or intravenously (iv). Figure 7A illustrates an example in which locomotion was evoked by stimulation of the L$_6$ segment in an untrained spinal cat. The EMG averages clearly show an alternation between flexor (St) and extensor muscles (VL, GL) in both hindlimbs. Ten minutes after microinjection of yohimbine at L$_3$ and L$_4$ (Fig. 7B), locomotion could no longer be elicited by perineal stimulation or electrical stimulation. Instead a flexion of all the joints of the ipsilateral hindlimb is evoked by each train of pulses, and EMG of both hindlimb muscles synchronously discharge with the stimulation. Two hours after intraspinal injection of clonidine in L$_3$ and L$_4$ supplemented by an intravenous dose, locomotion was re-instated with perineal stimulation (Fig. 7C) and ISMS (Fig. 7D) as can be seen by the alternation between flexor (St) and extensor (VL and GL) bursts. Therefore it appeared that pharmacological inactivation the mid-lumbar segments could prevent ISMS-evoked locomotion.

**SPINAL LESIONS.** Progressive spinal cord lesions were made at different locations of segments L$_2$–L$_4$ (see Table 3), and electrical stimulation was applied caudal to these lesions. In the 1-wk spinal cat shown in Fig. 8, ISMS applied at caudal L$_6$ could induce bilateral locomotion. After a complete spinal section at the junction of L$_2$ and L$_3$, ISMS could still induce locomotion. Thus segment L$_2$ is not critical for ISMS-evoked locomotion by caudal segments, but an analysis of EMG activity after the lesion shows an increase in the flexor bursts especially ipsilaterally (Fig. 8, $\rightarrow$). Also extensor bursts were decreased in the ipsilateral hindlimb (LVL). In the two 1-wk
untrained spinal cats tested, a complete section at the junction of L3 and L4 abolished the locomotion that was present prior to the lesion.

In Fig. 9, electrical stimulation at mid-L6 triggers a bilateral locomotor pattern in a trained spinal cat with clonidine. A complete section at the L3–L4 junction abolishes all locomotor rhythm elicited by stimulation of mid-L6 segment as was the case in the 1-wk untrained spinal cats. However, electrical stimulation at higher intensity (90 μA instead of 40 μA) applied at segment L7 evokes a bilateral locomotor rhythm 30 min after the acute spinalization at L3–L4, but the EMG amplitude of extensor muscles were diminished (LVL and LGL) or abolished (Fig. 9B). The locomotor pattern is still triggered by electrical stimulation of L7 segment after a dorsal rhizotomy.

TABLE 3. Summary of pharmacological inactivation and lesions

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>Level</th>
<th>No. of Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>L3–L4</td>
<td>1</td>
</tr>
<tr>
<td>Spinal lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>L2</td>
<td>3</td>
</tr>
<tr>
<td>Dorsoventral</td>
<td>L3</td>
<td>3</td>
</tr>
<tr>
<td>Ventral</td>
<td>L4</td>
<td>5</td>
</tr>
<tr>
<td>Rhizotomy</td>
<td>L1–L2</td>
<td>1</td>
</tr>
</tbody>
</table>

This table summarizes the surgical lesions or yohimbine blocks performed at different levels. In some cats, more than one lesion was made when locomotor rhythm could still be evoked from segments caudal to that lesion.

In Fig. 9A, electrical stimulation at mid-L6 triggers a bilateral locomotor pattern in a trained spinal cat with clonidine. A complete section at the L3–L4 junction abolishes all locomotor rhythm elicited by stimulation of mid-L6 segment as was the case in the 1-wk untrained spinal cats. However, electrical stimulation at higher intensity (90 μA instead of 40 μA) applied at segment L7 evokes a bilateral locomotor rhythm 30 min after the acute spinalization at L3–L4, but the EMG amplitude of extensor muscles were diminished (LVL and LGL) or abolished (Fig. 9B). The locomotor pattern is still triggered by electrical stimulation of L7 segment after a dorsal rhizotomy.
hemisection at mid-L4 (Fig. 9C) although a higher intensity is required (200 μA). In the eight cats tested, dorsal hemisection at L3 or L4 did not prevent evoking locomotion from stimulation of more caudal or rostral segments although the intensity of stimulation required was higher. In Fig. 9D, locomotion was abolished after a complete section at mid-L4 although short-latency responses were present in both hindlimbs synchronously. ISMS at more caudal levels or with higher intensities 2 h after this third spinal section did not evoke locomotion. Similar results were also obtained with DRS caudal to the lesion level.

To evaluate further the contribution of ventral spinal pathways, a section restricted to the ventral and ventrolateral funiculi was performed at caudal L4. In one of those cats, a bilateral rhizotomy was done at segments L3–L4 prior to the ventral lesion. A reconstruction of the lesion based on histological slides is shown in Fig. 10. ISMS at L5 as well as stimulation of dorsal root L7 could induce bilateral locomotion after the bilateral rhizotomy at L3–L4. However, after the ventral hemisection, bilateral locomotion could no longer be induced with perineal stimulation, ISMS or DRS, even at the highest intensity used. Short-latency responses were still present, however, as dorsal root stimulation could evoke ipsilateral extension and intraspinal stimulation induced bilateral extension.

DISCUSSION

The results of this study clearly show that ISMS applied in the lumbar cord of cats signalized at T13, after having received clonidine intravenously, can elicit bilateral and alternate locomotor movements of the hindlimbs. The locomotion induced by unilateral ISMS (tonic at 2–6 Hz and train at 0.65 to 1 train/s, 20–90 μA) resembled that evoked by perineal stimulation, and the individual characteristics displayed by each cat (e.g., cycle duration, angle excursion) account for most of the differences observed between locomotion induced by each type of stimulation. However, train stimulation would evoke locomotor sequences more easily than tonic stimulation, and pre-
Previous locomotor training had a positive effect on the regularity of cycles. Nonlocomotor responses as well as characteristics of locomotion evoked by DRS, such as entrainment and induction pattern, were similar to those observed with ISMS, suggesting the activation of common elements with either type of stimulation. Furthermore, locomotion induced by segments L5 to S1 could no longer be evoked when the mid-lumbar segments L3–L4 were either inactivated pharmacologically by restricted intraspinal injections of yohimbine or the ventral pathways were disconnected surgically at L4 from lower lumbar segments. Taken together, these results strongly suggest that stimulation of dorsal pathways and dorsal roots is involved in inducing bilateral locomotion by ISMS but that the ventral pathways connecting midlumbar segments to more caudal lumbar segments are critical to evoke locomotion by ISMS.

It is realized that stimuli delivered through ISMS or DRS will activate numerous and different spinal elements in the cord such as afferents, interneurons, and propriospinal tracts. Some aspects of the results will be discussed in this light before speculating on some of the intraspinal mechanisms that could participate in the generation of spinal locomotion.

**Structures activated by electrical stimulation of the cord**

**DORSAL ROOT AFFERENT.** ISMS applied dorsally and DRS evoked bilateral locomotor patterns that share the same characteristics, suggesting that afferent pathways were central to this locomotion. This is in agreement with previous studies showing that DRS could induce bilateral locomotion on a moving treadmill in decerebrate and acute spinal cats with L-Dopa (Budakova 1971) as well as bilateral fictive locomotion.
Moreover, a mechanism of action was proposed where the activation of localized terminal branches of afferents would spread antidromically to all terminal branches of the afferents (±17 mm rostral or caudal to the stimulation point) and transsynaptically excite motoneurons and interneurons far removed from the stimulation site.

MOTONEURONS. ISMS or DRS at various levels can evoke locomotion that may have somewhat different characteristics depending on the spinal level stimulated. With stimulation in more rostral lumbar segments, the ipsilateral hindlimb made more pronounced forward movements so that the foot was placed well in front of the hip joint. When the stimulation was applied at more caudal segments, the hindlimb reached further caudally. These results could be explained in part by the activation of more hip flexor motoneurons in rostral segments and more extensor motoneurons in caudal segments; this is in general agreement with the distribution of motoneuron pools in the cat (Vanderhorst and Holstege 1997).

The movement induced by the perineal stimulation is closer to that evoked by ISMS at caudal segments. This could be explained by the preparation used. During the recovery of hindlimb movement after spinal lesion, the cats performed small movements with the hindlimbs mainly in extension (Barbeau and Rossignol 1987), and the foot contact was made on the dorsum. This is similar to the kinematics observed in the present study with perineal stimulation as well as with ISMS applied in more caudal segments. After a few weeks of training, the cat can make plantar foot contact and performs larger forward movements during swing, contacting the floor in front of a vertical axis passing through the hip. This kinematic pattern was seen with more rostral ISMS. This suggests an activation of both rostral and caudal elements of the CPG when the cats recover the full range of locomotion after training.

REFLEX AND INTERNEURONAL PATHWAYS. ISMS or DRS activate simultaneously several spinal pathways. Nevertheless, some predominant and distinct response patterns emerged from the analysis. Bilateral locomotion sequence would often start by locomotor movements restricted to the contralateral side only or by EMG activity in the contralateral extensor muscles and ipsilateral flexor muscles simultaneously, akin to the crossed extension reflex (Matsuyama et al. 2004; Schouenborg 2002; Sherrington 1910). These observations suggest the importance of crossed connections in the induction of locomotion by ISMS. A group of interneurons mediating crossed reflexes reside in lamina VIII, and their axons contact motoneurons of the contralateral side in segments L_{4} to S_{1} (Harrison et al. 1986; Jankowska and Skoog 1986). Those neurons are most densely found at the border of L_{3}–L_{4} and L_{4}–L_{5} segments as well as within segments L_{6}–L_{7}. The main polysynaptic input to those lamina VIII interneurons (commissural interneurons) is via shared pathways, namely the flexor reflex afferent pathways (FRA) (Jankowska et al. 1967a,b; Lundberg 1979), which subserve ipsilateral flexion and crossed extension responses and are a probable target of electrical stimulation because of the intensities used. Indeed, though the intensity used was relatively low, locomotion would not appear until the stimulation reached four or five times the motor threshold observed visually by muscle twitches. This is in agreement with the general scheme put forward by the group of McCrea (Rybak et al. 2006b) whereby afferents may exert restricted effects through specific reflex pathways but also more general
effects through higher level structures in the organization of locomotion which involve widespread effects over several muscle groups.

**SPINAL SEGMENTS.** Our results suggest that segments L₃–L₄, which are rostral to segments containing the main motoneuronal pools of the hindlimb (Vanderhorst and Holstege 1997), play a crucial role for the electrical activation of locomotion in spinal cats, be it by ISMS or DRS.

Studies in the neonatal rat have suggested that the locomotor network is distributed along the spinal cord although rostral segments are more excitable (as reviewed in Kiehn 2006). In other work, it was suggested that rostral segments L₁–L₂ could provide the necessary command for the generation of the hindlimb locomotor pattern in the neonatal rat (Cazalets and Bertrand 2000). Such an organization has also been suggested in the adult rat because kainic acid lesions destroying the gray matter in rostral segments abolish locomotion (Magnuson et al. 1999). In spinal rats that received a graft of 5-HT mesencephalic embryonic cells, (Gimenez y Ribotta et al. 2000) reported a recovery of locomotion only in rats in which the rostral L₁–L₂ levels were reinnervated by serotoninergic fibers. The importance of rostral segments were also observed in the turtle (Mortin and Stein 1989) and the mudden puppy (Wheatley et al. 1994). Even in spinal-cord-injured patients, epidural stimulation of the dorsal surface of spinal segments L₂ was shown to be optimal to induce locomotor movements of the legs (Gerasimenko et al. 2002).

In the cat, previous studies of this laboratory have also demonstrated the importance of more rostral segments in the generation of spinal locomotion. In acute experiments on 1-wk spinal cats (T₁₃), microinjections of clonidine restricted to the midlumbar segment L₃ or L₃ and L₄ were sufficient to induce bilateral locomotor rhythms on a treadmill, and microinjections of alpha₂-noradrenergic blocker yohimbine at L₃–L₄ could block locomotion induced by clonidine intravenously (Marcoux and Rossignol 2000). Microinjections of only L₀ segment or L₄ and L₇ segments did not evoke measurable steps. These results were reproduced in conditions of fictive spinal locomotion (Leblond et al. 2001). In cats previously spinalized at T₁₃ and having recovered locomotion, a second spinal lesion (Langlet et al. 2005) at caudal L₃ or L₄ abolished all locomotion even after several weeks of attempted training. However, alternating rhythmic activity typical of fast paw shake could still be observed. Similarly, it was also shown (Deligianna et al. 1983) that although rhythmic activities are distributed throughout the lumbar spinal cord, segment L₄ was essential for the expression of scratching. In a paralyzed spinal cat injected with L-Dopa (fictive locomotion preparation), alternating activity between TA and GL nerves could be observed after an acute spinal transection at L₅, further indicating that a potential for rhythogenesis is present at lower spinal levels (Grillner and Zangger 1979). Taken together, these results suggest that there is a rhythmonic potential throughout the lumbo-sacral cord, but the integrity of segments L₃–L₄ is needed for full hindlimb locomotion on the treadmill.

**PROPRIOSPINAL PATHWAYS.** Propriospinal neurons located in the preenlargement segment L₃, L₄, and rostral L₅ receive signals from the supraspinal system as well as peripheral afferents and relay these inputs to more caudal segments in the cat (Kostyuk et al. 1971). Hence it is possible that the integration of both inputs for locomotion takes place at that level. Candidate interneurons might include mid-lumbar interneurons located in L₃–L₄ segments receiving peripheral inputs mainly from group II afferents of quadriceps and sartorius and projecting mainly in the ventrolateral funiculus to make contact with motoneurons in L₄ to S₁ (Edgley and Jankowska 1987a,b; Lundberg et al. 1987a–c). Those group II interneurons located in L₄ were shown to be active during MLR-evoked fictive locomotion in decerebrate cats (Shefchyk et al. 1990). Another target might be commissural interneurons located in lamina VIII of mid-lumbar segments L₁–L₄ as discussed earlier. They receive input from reticulo and/or vestibulospinal neurons and were shown to influence the activity of contralateral muscles (Krutki et al. 2003). Based on their rostral location and on their ventral projections, these groups of interneurons might be central in the initiation of locomotion by electrical stimulation. The stimulation of caudal segments might activate those mid-lumbar neurons by antidromic activation of their axons or by activation of ascending propriospinal pathways, such as axons sent by another class of group II interneurons located in L₅–L₇ segments (Riddell and Hadian 2000). Almost half of these interneurons project ipsilaterally within the lateral funiculus to the L₂ segment, whereas some of them contact motoneurons directly. In the present study, ventral and ventrolateral pathways were found to be crucial to elicit locomotion by intraspinal stimulation of the dorsal regions because dorsal hemisection and bilateral rhizotomy of L₃ and L₄ did not prevent it. Based on the pathways used by the propriospinal neurons described in the preceding text, the section of ventral quadrants might not only prevent the output of the mid-lumbar interneurons but also some of their activation input. Although the involvement of the above-mentioned interneuronal pathways might be more important, other described propriospinal pathways are also involved, such as pathways linking middle lumbar segments with lower lumbar and sacral segments that have been described (Kostyuk et al. 1971).

Therefore it is believed that the rostral lumbar segments play a critical role in the expression of spinal locomotion, and it is probable that afferent pathways project to these midlumbar levels directly or through propriospinal pathways which in turn provide a critical signal to more caudal levels to produce locomotion. This view does not preclude that the lower lumbar levels have a rhythmonic capability that may be activated by midlumbar segments. Whatever the mechanisms involved by which these upper lumbar segments exert their effects, it is clear that their inactivation has a major impact on locomotion in the acute or chronic spinal cat. Further studies are required to understand if these segments exert their effects mainly on the proximal hip joint or have a more distributed influence.

**ISMS and CPG**

On the basis of experiments showing that stimulation of afferents can modify the ongoing step cycle without affecting the following step cycle (Stecina et al. 2005) and on other experiments showing that nonresetting deletions can occur spontaneously during fictive locomotion evoked by MLR stimulation (Lafreniere-Roula and McCrea 2005), it was suggested that the spinal central pattern generator in cats is organized in at least two layers. The first layer would be a rhythm generation (RG) with mutual inhibitory connections.
between flexors and extensors and that would determine the overall cycle duration as well as the respective duration of each locomotor subphases (swing and stance). The second layer, named the pattern formation layer is subdivided in various subunits [unit CPGs as suggested by Grillner (1981)] that could activate flexor and extensor muscles as groups (not necessarily related to a single joint). Other sublayers involving various types of local interneurons would ensure reciprocal inhibition and other local interactions. This model whose formalism was introduced recently (Rybak et al. 2006a,b) is meant to account for phenomena encountered during fictive locomotion such as deletions of all flexors or extensors with or without a concomitant tonic (or phasic) excitation of the antagonists and a resumption of the rhythm at the time corresponding to the expected burst of activity if there had been no deletion. This presupposes the existence of some timing device in the CPG. We agree that the spinal mechanisms for the generation of locomotion are complex as also evidenced by the present results as well as those reported before (Barthélemé et al. 2006). We described that ISMS could generate nonlocomotor as well as locomotor responses in different sites of the spinal cord. Thus simple flexion followed or not by extension as well as simple extension could be evoke. Although we could observe movements at a single joint, the responses usually implicated movements of varying amplitude in all joints of the limb. These multiple joint synergies resemble a single step in many respects.

It is hard to say at this stage whether single complex limb movements evoked by such electrical stimuli could represent a discrete activation of a RG circuit for one cycle or a pattern formation circuit with different participation of unit burst generators. When rhythmic trains of stimulation are applied, the locomotor rhythm may at times be entrained (in a rather narrow range) or be independent of the stimulation frequency itself although it will be dependent on the presence of the stimulation because it will die out after only a few cycles after the cessation of the stimulation. The entrainment of the rhythm would suggest some form of entrainment of the rhythm generator layer (Rybak et al. 2006b). However, the numerous cases of dissociation between the output rhythm and the imposed stimulation rhythm rather suggest that the stimulation raises the intrinsic excitability of circuits that are brought to oscillate at their own intrinsic frequency and die out when the excitability is reduced on stimulation cessation. Much more work will be needed to understand the relationship between the location of ISMS and spinal locomotor pattern generation.

Implication for neuroprosthetics

These results might also have implications for the artificial control of locomotion in humans. Already electrical epidural stimulation in spinal-cord-injured patients (Dimitrijevic et al. 1998; Herman et al. 2002; Minassian et al. 2004; Shapkova and Schomburg 2001; Struijk et al. 1993) show promising results and an approach similar to the one used here, with a combination of pharmacological and electrical stimulation, might be adaptable for patients. Electrical stimulation could induce locomotion either intraspinally or by dorsal root stimulation. Indeed a small number of electrodes could be implanted chronically, each one being able to produce the whole locomotor synergy. Either tonic or train stimulation was used and showed to be successful. However, tonic stimulation caused less disruption of the locomotor cycle. This was expected, but the possible benefits from train stimulation are not to be overlooked. Indeed, with a signal from the forelimb EMG or from another supraspinal source, hindlimb locomotion evoked by ISMS or dorsal root stimulation could be entrained at a frequency determined by a signal derived from supraspinal levels. Thus combination of locomotor training (Barbeau and Rossignol 1987), pharmacology (Chau et al. 1998a), and electrical stimulation (ISMS or dorsal root) might be beneficial to enhance the spinal capacity to express any inherent spinal locomotor circuitry after spinal injury.

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