Nonlocomotor and Locomotor Hindlimb Responses Evoked by Electrical Microstimulation of the Lumbar Cord in Spinalized Cats

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Barthélemy, Dorothy, Hugues Leblond, Janyne Provencher, and Serge Rossignol. Nonlocomotor and locomotor hindlimb responses evoked by electrical microstimulation of the lumbar cord in spinalized cats. J Neurophysiol 96: 3273–3292, 2006. First published August 30, 2006; doi:10.1152/jn.00203.2006. As a preliminary step to using intraspinal microstimulation (ISMS) for rehabilitation purposes, the distribution of various types of hindlimb responses evoked by ISMS in spinal cats (TnL4) is described. The responses to ISMS applied through a single electrode was assessed, before and after an intravenous injection of clonidine (noradrenergic agonist), using kinematics and electromyographic recordings in subacute (5–7 days, untrained) or chronic (3–5 wk trained on a treadmill) spinal cats. ISMS was applied in the dorsal, intermediate, and ventral areas of segments L2–L7 from midline to 3 mm laterally. Uni- and bilateral nonlocomotor responses as well as rhythmic locomotor responses were evoked. In the subacute cats, ipsilateral flexion was elicited in the dorsal region of L3–L5, whereas ipsilateral extension was evoked more ventrally and mainly in the caudal segments. Dorsal stimuli could induce ipsilateral flexion followed by ipsilateral extension. Sites inducing bilateral flexion and bilateral extension were similarly distributed to those evoking ipsilateral flexion and extension in the rostrocaudal axis but were evoked from more medial sites. Ipsilateral flexion with crossed extension was evoked from intermediate and ventral zones of all segments and lateralities. Unilateral ipsilateral locomotion was rarely observed. Contralateral locomotion was more frequent and mainly evoked medially, whereas bilateral locomotion was evoked exclusively from dorsal regions. With some exceptions, those distribution gradients were similar in the four conditions (subacute, chronic, pre- and postclonidine), but the proportion of each response could vary. The distribution of ISMS-evoked responses is discussed as a function of known localization of interneurons and motoneurons.

INTRODUCTION

Studies in the spinal paralyzed cat, injected with the noradrenergic precursor L-DOPA, have shown that there is an autonomous spinal network in the lumbosacral cord capable of generating a bilateral and alternating hindlimb locomotor pattern, independent of supraspinal centers or peripheral afferents (Grillner and Zangger 1979). This was also shown using α2-noradrenergic agonists such as clonidine that could trigger locomotion on a treadmill in the acutely spinalized cat when perineural stimulation was used (Barbeau and Rossignol 1987; Chau et al. 1998; Forssberg and Grillner 1973). Without pharmacological stimulation, hindlimb locomotion on the treadmill can be expressed several weeks after the complete spinal section with daily locomotor training (Barbeau and Rossignol 1987; de Leon et al. 1998), but recovery of spontaneous spinal locomotion can be accelerated by noradrenergic stimulation (Chau et al. 1998).

Could this spinal locomotor synergy be activated by electrical stimulation? Various studies suggest that it might be possible to use electrical stimulation of the spinal cord to evoke complex motor patterns such as locomotion. This might constitute an interesting approach in the context of rehabilitation after spinal cord injury. Indeed, electrical stimulation could be envisaged on the treadmill but also in an overground situation and used to somehow couple movements evoked by intraspinal microstimulation (ISMS). ISMS in the hindlimbs with voluntary signals generated above the lesion. However, if electrical stimulation is to be used, which sites of the spinal cord should be stimulated and how? Electrical stimulation of dorsal roots was shown to be efficient to induce locomotor activity in decerebrate and spinal cats (Budakova 1971; Grillner and Zangger 1979) and in the in vitro neonatal rat (Marchetti et al. 2001). Epidural stimulation is also efficient in inducing locomotor rhythms in decerebrate and spinal cats (Gerasimenko et al. 2003, 2005; Iwahara et al. 1991b) as well as spinal rats (Ichiyama et al. 2005; Iwahara et al. 1991a). This approach is also promising in spinal cord injured patients where rhythmic activity was evoked by epidural stimulation (Carhart et al. 2004; Dimitrijevic et al. 1998; Herman et al. 2002; Minassian et al. 2004; Shapkova and Schomburg 2001). The optimal localization to induce locomotion with epidural stimulation differed between authors, however. In the spinal cat, Iwahara et al. (1991b) showed that segments L1 to L4 are as efficient as segments L5 to S2 to induce bilateral locomotion, whereas Gerasimenko et al. (2003), suggested that the border between L4 and L5 was the most efficient.

Intraspinal electrical microstimulation through microelectrodes was also used to generate movement of the hindlimbs. In the chronically spinalized frog and rat (Giszter et al. 1993; Tresch and Bizzi 1999), stimulation with a single electrode in the gray matter of the spinal cord seems to suggest that premotor circuits (at interneuronal levels) are organized into a limited set of modules, each of them inducing a specific force field. Similar topographic organizations were obtained in decerebrate cats (Lemay and Grill 2004; Mushahwar et al. 2004; Stein et al. 2002; Tai et al. 2003). However, to induce locomotion required stimulation of several sites of the spinal cord. For instance, to evoke locomotion-like movements in the hindlimbs of spinal cats, several electrodes were inserted near...
the motoneuronal pools of the hindlimb in the ventral horn at various lumbosacral segments (Saigal et al. 2004).

Bearing in mind that it would be advantageous to use a minimal number of implanted electrodes in the eventuality of chronic implants, we focused here on the use of a single electrode to evoke the full locomotor synergy instead of attempting to combine various sites of spinal stimulation. Further evidence suggesting this possibility was presented in a previous study where intraspinal injection of clonidine restricted to the L3 or L4 segment was sufficient to induce bilateral locomotion in spinal cats (Marcoux and Rossignol 2000). Thus if pharmacological stimulation of restricted regions of the cord can induce locomotion, it was reasoned that the same could also apply to microstimulation restricted to single spinal sites. We thus undertook to study systematically the various responses (locomotor and nonlocomotor) evoked through stimulation of a single electrode.

We aim at establishing the topographic organization of hindlimb responses and at determining the best sites to evoke locomotion. Through a single tungsten electrode, we applied trains of biphasic pulses at low current (<100 μA) from L3 to L7 at different depths and lateralities, thus stimulating both gray and white matter. Using kinematic and electromyographic analyses, we classified the responses obtained in three groups whether the responses involved one or both hindlimbs or had a rhythmic locomotor component. We report herein on the distribution of these responses before (pre) and after (post) an intravenous injection of clonidine, in subacute untrained spinal cats (1 wk after spinalization) and in chronic trained spinal cats that have recovered locomotion on the treadmill (3–5 wk). We found that locomotor responses as well as nonlocomotor responses could be evoked throughout the cord, but that each type of response was preferentially triggered from specific parts of the spinal cord. Also, for each type of response, the spinal distribution of the effective sites in the rostrocaudal, mediolateral, and dorsoventral axes did not vary greatly in the four conditions (subacute untrained or chronic trained, pre- or postclonidine), but the proportion of their occurrence varied postclonidine and with training. Part of those results have been published in abstract form (Barthélemey et al. 2002, 2005a).

METHODS

Eighteen cats of either sex (3.2–5.4 kg) were used for this study. Thirteen of these cats were spinalized and were confined to their cage in the following 5–7 days that preceded the acute experiment. Because no difference in the results was observed between the 5- and 7-day spinal cats, they will be referred to as subacute or untrained spinal cats. The remaining five cats were preselected for training, spinalized, and trained to walk on the treadmill (10 min, 3 times/day, 5 days/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. Results from those five cats were similar, and they will be referred to as chronic or trained spinal cats. 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.

Spinalization

All surgeries were performed in aseptic conditions and under general anesthesia. The cats received buprenorphine 0.01 mg/kg sc, 1 h before the surgery. After a preoperative medication (0.1 mg/kg im atropine maleate (Atravet), 0.01 mg/kg im glycopyrrolate, and 10 mg/kg im ketamine), anesthesia was induced with isoflurane 2% in 95% O2 by inhalation first through a mask, and then maintained through an endotracheal tube. A laminectomy was performed at the T13 vertebral. The dura was carefully opened and a few drops of xylocaine (2%) were applied on the surface of the spinal cord and then directly into the spinal cord with a 24-gauge needle at the T13 level on both sides. The spinal cord was severed completely with surgical scissors so that the ventral surface of the vertebral canal could be clearly visualized. Absorbable hemostat (Surgicel) was then used to completely fill up the space between the rostral and caudal ends of the spinal cord, thus providing hemostasis and insuring completeness of the section. Muscles, fascia and skin were then sutured in layers. For prolonged postsurgical anesthesia, a patch of transdermal fentanyl (Duragesic 25; 2.5 mg, 25 μg/h for 72 h) was sutured to the skin at the level of the sacrum. In some cases, a bladder catheter was inserted through the urethra and sutured to the perineum. At the end of the surgery, an antibiotic, Ayercilline, 40,000 IU/kg im, and an analgesic, Anafen, 2 mg/kg sc, were administered.

Postoperative care

After the surgery, the animal was placed in a heated incubator until it regained consciousness and expelled its endotracheal tube. It was then returned to its individual cage (104 × 76 × 94 cm) lined with a foam mattress in addition to absorbent tissues and with ample food and water supplies. The animal was attended to at least twice daily for manual bladder expression (if no catheter was in place), for general inspection, and for cleaning of the hindquarters. Although analgesia for the first three postoperative days was provided by the fentanyl patch, buprenorphine was administered (0.01 mg/kg sc every 6 h) when needed. An antibiotic, Apo Cephalex, 100 mg/d was given p.o. for 10 days or until the day of the acute experiment.

Acute experiments

Under general anesthesia (as detailed in the preceding text), 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 blood pressure. A laminctomy of L3–L6 vertebrae exposed the spinal cord segments from L3 to S1, and the cat was then mounted on a spinal fixation unit over a motor-driven treadmill. To ensure stability for subsequent intraspinal electrical 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 bones just below the iliac crest. A precocillar, postmamillary decerebration was performed with a spatula and the rostral nervous tissue was aspirated. Anesthesia was then discontinued. In some cases, assisted ventilation had to be used after decerebration. Dura was opened and the spinal cord covered with warm mineral oil. The spinal segments were identified by the most rostral and caudal roodlets of each dorsal root.

EXPERIMENTAL PROTOCOL. At least 1 h elapsed between the cessation of anesthesia and the start of stimulation. First, 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. In nine animals, motor responses evoked with ISMS were studied before injection of clonidine (500 μg/kg iv), and in all cats, ISMS was tested after an injection of clonidine intravenous. 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. 1998; 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). There was no difference between the results of cats that received clonidine only and those that received clonidine with naloxone. The experiment was terminated when either an important change of state occurred in the preparation, such as blood pressure drop, important decrease in excitability of the spinal cord or after locomotion was abolished by spinal lesions that were made at different spinal segments (discussed in a forthcoming paper).

**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 (around 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 (20–90 μA, 250–300 μs) were delivered as trains of 100- to 200-ms duration with an intra-train frequency of 300 Hz and a rate of 0.8 trains/s. Other parameters such as tonic stimulation were also explored but will be reported in a forthcoming paper.

The stimulation was applied one site at a time in different parts of the dorsal, lateral, and ventral areas of the L₃–L₇ segments and from the midline to 2.5 or 3 mm on either side. The electrode was held in a microdrive and was mounted on a stereotaxic manipulandum with x and y coordinates. Dorsoventral (where 0 is on the dorsal surface) and mediolateral displacements (where 0 is on the midline) were determined and noted. Based on the distribution of the dorsal roots, we divided each segment in four parts (e.g., rostral L₃, mid L₃, caudal L₃, and junction L₂–L₃). At each stimulating entry point, we descended the electrode while the trains of stimulation were continuously delivered, but stopped every 0.5 mm, as well as when the response changed, to assess the effects at that point. Therefore the responses illustrated for each point represent an average of several responses (around 8–10 stimulation trains). Stimulation tracks went from the dorsal surface to ~4–5 mm deep.

Figure 1 illustrates all sites (for all cats) that were stimulated in each of the four conditions tested by plotting the stimulation coordinates noted during the experiment. We plotted only the sites where a change in the kinematic or in the EMG occurred. All stimulation sites for one segment are merged on one representative section of that segment (taken from the middle of each segment). The most ventral and lateral sites were less investigated due to experimental time constraints (see also methodological considerations in the DISCUSSION).

Overall, subacute or untrained spinal cats were studied the most. As described in Table 1, the responses described in this paper were evoked in 61 stimulation tracks made in 6 untrained spinal cats preclonidine and in 210 tracks made in 12 trained spinal cats postclonidine. In each track, four to five different types of responses were observed successively. To assess stability of the responses, an average of 8–10 stimulation trains were tested for each response type. Results obtained in the untrained spinal cats will be compared with those obtained in trained spinal cats, from 36 stimulation tracks made in three cats preclonidine and 64 stimulation tracks made in five cats postclonidine.

**Recordings and analyses**

To record electromyographic (EMG) activity, muscles were implanted percutaneously using a 21-gauge needle with pairs of enamelled copper wires (32AWG), in the following muscles: semiten-dinosus (St), knee flexor and hip extensor; sartorius anterior (Srt), hip flexor and knee extensor; tibialis anterior (TA), ankle flexor; vastus lateralis (VL), knee extensor; and gastrocnemius lateralis (GL), ankle extensor.

The EMG signals were differentially amplified with AC-coupled amplifiers (bandwidth of 300 Hz to 10 kHz), and digitized at 1 kHz on a PC with a data-acquisition board. The EMG recording was synchronized to the recorded video images by means of a digital Society for Motion Picture and Television Engineers time code (SMPTE). This time code was recorded simultaneously on one digital channel as well as on the videotape. During locomotion, the onset and offset of the bursts of activity in muscles were detected automatically with a custom-designed software then verified and corrected manually when necessary.

For analysis of limb kinematics, reflective markers were placed on the bony landmarks of the left hindlimb: the iliac crest (the marker was thus on the lateral pin on the iliac bone and fixed relative to the frame), the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal joint, and the tip of the fourth or fifth toe. Video images of the locomotor movements were captured by a NTSC camera (shutter speed 1/1,000 s) at 30 frames/s and recorded on a video recorder (Panasonic AG 7350). Then, selected video sequences were digitized with a video grabber and the frames were de-interlaced yielding 60 fields/s with, therefore a temporal resolution of 16.7 ms.
between fields. The x and y coordinates of the different joint markers were then obtained. Angular displacement data and joint angles were calculated based on the relative position of the iliac, hip and knee markers). From both x-y coordinates of the recorded markers, displacement data and the calculated joint angle data, displays of stick diagrams and trajectories were generated using custom-made software. Stick diagrams of responses consisted of reconstruction of the actual hindlimb movement during the stimulation.

To evaluate and classify the responses obtained, videos of all experiments were reviewed and the responses were classified based on the presence of motor response in one or both hindlimbs and on the type of movement induced (be it flexion, extension or rhythmic locomotor movements). Although videos of experiments were only made of the left hindlimb, kinematics responses in the right hindlimb could still be seen. In the results, the ipsilateral hindlimb is located on the same side as the stimulation and the contralateral hindlimb is on the opposite side of the stimulation. The EMG data and the kinematics were used to determine the type of response induced, although sometimes stimulation induced a burst in all EMG channels recorded and kinematics became crucial. The use of both sets of data (kinematics and EMG) was also particularly important when the responses could be misinterpreted as being locomotor, such as ipsilateral flexion followed by a passive, treadmill-driven, extension of the limb. The presence of extensor muscle activity in the EMG could still be seen. In the results, the ipsilateral hindlimb is located on the same side as the stimulation and the contralateral hindlimb is on the opposite side of the stimulation. The EMG data and the kinematics responses consisted of reconstruction of the actual hindlimb movement during the stimulation.

### Statistics

Sites where different responses were evoked were compared with Student’s t-test and were considered to be significant if the probability of a type error was <0.05.

To determine if the trends observed in the localization of sites inducing specific responses were significantly different, as well as if the proportion of response types changed significantly between conditions, a χ² test with significance at the 5% level was used, with appropriate degrees of freedom depending on the populations of responses tested.

### Histology

At the end of the experiments, electrolytic lesions (1–5 mA, anodic DC current for 15 s followed by a cathodic DC current for 15 s) were performed. However, the location of stimulating sites on the figures is based on coordinates taken during the experiment from surface landmarks (midline, surface, dorsal root entry zone) and coordinates from the manipulandum. All cats were killed by an overdose of Sodium Pentobarbital intravenous and the spinal cord was then removed for histology.

The spinal cord was first placed in a fixative of 4% paraformaldehyde in phosphate-buffered saline (PBS) 0.2 M for ≥24 h. It was then transferred to a solution of 20% sucrose-10% paraformaldehyde in PBS, pH 7.3, at room temperature for cryoprotection. Segments of the spinal cord were then frozen individually in methylbutane cooled with liquid nitrogen. The spinal cord enlargements were cut into 20-μm transverse sections, put onto Superfrost Plus slides (Fisher) and Nissl-stained to facilitate histological identification. Drawings of spinal cord sections were based on the histology of two spinal cats and made with a camera lucida. Photomicrographs were taken with a Nikon Coolpix 995 digital camera custom-fitted on a Nikon Optiphot-2 microscope.

### RESULTS

#### General groups of responses in all conditions

Before drug injection, locomotor activity of the hindlimbs could not be elicited by the treadmill or perineal stimulation in any of the 18 cats tested, even a few hours after decerebration. The necessary extensive laminectomy as well as the sturdy fixation of the vertebral column by vertebral pins might be responsible for the absence of locomotion. Nonetheless, spinal reflexes were always present (e.g., withdrawal reflex in the ipsilateral hindlimb following a pinch of the paw), and most often perineal stimulation could elicit bilateral tonic flexion of the hindlimbs or tonic discharge in some muscles but no rhythmic activity. All such testing occurred while the treadmill was moving.

Typically, when stimulation was applied as the electrode was descended through the cord, different types of responses were evoked successively, and each one could be evoked over a 1- to 2-mm distance. The responses varied depending on the laterality and the segment stimulated. As an example, a complete sequence of responses induced along one stimulation track is shown in Fig. 2 for a 1-wk untrained spinal cat injected with clonidine intravenous. The stick figures were drawn to illustrate the movements recorded from video clips. ISMS at rostral L₃, 1 mm left of the midline evoked flexion of the ipsilateral hindlimb in the first mm below the surface (response A in Fig. 2). At 1 mm, ipsilateral flexion was accompanied by locomotion of the contralateral hindlimb (response B). The contralateral locomotion ceased at 2.7 mm below the dorsal surface and the amplitude of ipsilateral flexion diminished to the point of being barely noticeable at around 3 mm (response C). At 3.7 mm, ipsilateral flexion became vigorous again and was accompanied by a contralateral extension (response D). This response was observed until the ventral most area tested in this track. At each point assessed along the tracks, responses evoked were very stable and the same response could be evoked with each train of stimulation. However, the amplitude of the responses often decreased after several trains. When the electrode was raised back up toward the dorsal surface, the inverted sequence of responses was observed. This also confirmed the stability of the responses.

Because a variety of responses were evoked at distinct sites in the spinal cord, they were grouped based on the involvement of one or both hindlimbs, and the presence or absence of locomotor rhythms. These generic responses were similar enough in the different cats to be categorized in response groups. These groups were then mapped for L₃–L₇ segments at various lateralities and depths for all cats. The first group includes nonlocomotor responses that were evoked only in the hindlimb ipsilateral to the stimulation (flexion or extension responses). The second group includes locomotor flexion/extension responses, involving both hindlimbs simultaneously.
The third group consists of locomotor responses, whether uni- or bilateral. The kinematics and electromyographic description of those responses as well as their distribution in the spinal cord are presented in the following sections.

**Nonlocomotor ipsilateral responses**

**GENERAL DESCRIPTION OF THE UNILATERAL NONLOCOMOTOR RESPONSES.** At the start of the experiment, the hindlimbs resting on the moving treadmill were motionless. The stimulation was then applied along dorsoventral tracts, and the responses obtained were recorded and later grouped according to the kinematics from video clips and EMG recordings. Ipsilateral flexion represented ~38% of all responses obtained across conditions and constituted the most frequent response (cf. Table 2). It was characterized by a decrease in hip angle or, if no movement occurred at the hip, a decrease in the angle of the knee or ankle would be present. Flexion movements could occur at one or two joints only, but was mainly observed at all joints simultaneously. Different patterns of flexion were thus observed, notably one being composed of flexion at the hip and extension at the knee and/or ankle. Figure 3A illustrates such a response: the movement of the ipsilateral hindlimb was directed forward and upward and the joint angular excursion shows that flexion occurred at the hip and ankle joints (decrease in angle), with a slight extension (increase in angle) of the knee and metatarsophalangeal (MTP). The EMG averaging of eight consecutive responses shows bursts of activity in all ipsilateral muscles when the stimulation was first applied with a preponderant activation of flexor muscles LTA and LSrt. Another pattern of flexion is shown in Fig. 3B. The kinematics and joint angle pattern resembled the swing phase of the locomotor cycle: flexion occurred mainly at the hip, extension occurred at the knee and a small flexion followed by extension movement was observed at the ankle and MTP joints. However, no active extensor activity was observed in muscles following that overall flexion and the limb was passively extended by the moving belt of the treadmill after the flexion response.

ISM could also evoke extension of the ipsilateral hindlimb (a mean of 7% of all responses across conditions; cf. Table 2). Extension responses were determined by an increase in hip joint or, if no movement occurred at the hip, by an increase in knee and/or ankle joint angle. Two main profiles of extensions were observed. In the first one, the amplitude of extension at the joints was quite small and there was co-contraction of both flexor and extensor muscles. While the proximal part of the leg stiffened, the extension movement was observed at the ankle (not illustrated). The second type of extension was less frequent and consisted in a backward limb extension, where the limb was off the ground during the movement. In Fig. 3C, the stick figure shows an important extension at the hip, ankle and MTP and a smaller extension at the knee. The average angle excursion confirms that extension occurred at all joints and EMG averaging shows synchronous burst of activity in all ipsilateral muscles (including flexor muscles), with GL being more prominent.

The third ipsilateral response observed was a flexion followed by an extension (2% of all responses across conditions). In Fig. 3D, a clear flexion occurs at all joints during the stimulation followed by an active extension when the stimulation stops. This bout of alternating flexion/extension activity is reflected in the EMG averaging where LTA of the ipsilateral hindlimb is mainly active throughout the stimulation. When the stimulation stopped, a burst in LGL and LVL was triggered. Both flexion and extension movements were quite brisk and the hindlimb did not touch the treadmill at any point during those movements. Hence, we did not consider this response to be locomotor though it may be a prelude to a more sustained rhythmicity. Indeed, there was one more alternation between LS (knee flexor) and LVL (knee extensor) following that response. Note that small bursts of activity also followed the main response illustrated in Fig. 3, A–C. These after-bursts were observed in the untrained postclonidine condition and in both trained conditions, but they were absent from the untrained preclonidine condition.

Abduction and adduction could be observed alone or in combination with ipsilateral flexion and extension. In the latter cases, they were incorporated in the flexion or extension group. Pure abduction and adduction, other ipsilateral responses that were rarely evoked by ISMS (e.g., external rotation) as well as abdominal contractions were included in Table 2 under “other responses.” Furthermore, a zone of hypoexcitability was observed, where ipsilateral responses were barely noticeable or absent during stimulation. This zone occurred at depths varying from 2 to 4 mm below the dorsal surface, but only covered...
They corresponded to \( \sim 11\% \) of the responses across condition and were included in Table 2 under “faint/no response.” Finally, responses of extension followed by small flexion were rarely observed and were not classified in this study.

### TABLE 2. Distribution of responses per condition

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Untrained</th>
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<th>Post-clo (n)</th>
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<tr>
<td></td>
<td>Pre-clo (n)</td>
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<td>Post-clo (n)</td>
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<tr>
<td>A. Non-locomotor ipsilateral responses</td>
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<tr>
<td>Flexion</td>
<td>57 (6)</td>
<td>30 (12)</td>
<td>39 (3)</td>
<td>27 (5)</td>
<td>38</td>
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<tr>
<td>Extension</td>
<td>6 (4)</td>
<td>8 (8)</td>
<td>6 (3)</td>
<td>7 (4)</td>
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<tr>
<td>Flexion followed by extension</td>
<td>2 (1)</td>
<td>1 (4)</td>
<td>4 (2)</td>
<td>2 (3)</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>Other responses</td>
<td>2 (2)</td>
<td>2 (5)</td>
<td>0</td>
<td>1 (1)</td>
<td>1</td>
<td></td>
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<tr>
<td>Faint or no response</td>
<td>20 (5)</td>
<td>12 (11)</td>
<td>N/A</td>
<td>10 (4)</td>
<td>11</td>
<td></td>
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<tr>
<td>Total</td>
<td>87</td>
<td>53</td>
<td>49</td>
<td>47</td>
<td>59</td>
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<td>B. Non-locomotor bilateral responses</td>
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<tr>
<td>Ipsilateral flexion</td>
<td>6 (2)</td>
<td>9 (11)</td>
<td>15 (3)</td>
<td>10 (4)</td>
<td>10</td>
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<tr>
<td>Bilateral flexion</td>
<td>5 (4)</td>
<td>5 (8)</td>
<td>15 (3)</td>
<td>8 (3)</td>
<td>8</td>
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<tr>
<td>Bilateral extension</td>
<td>1 (1)</td>
<td>2 (4)</td>
<td>0</td>
<td>1 (1)</td>
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<tr>
<td>Other responses</td>
<td>12</td>
<td>18</td>
<td>34</td>
<td>19</td>
<td>21</td>
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<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>C. Locomotor responses</td>
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<tr>
<td>Ipsilateral locomotion</td>
<td>(&lt;1 (1))</td>
<td>1 (5)</td>
<td>1 (1)</td>
<td>(&lt;1 (1))</td>
<td>(&lt;1)</td>
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<td>Contralateral locomotion</td>
<td>0</td>
<td>11 (9)</td>
<td>15 (3)</td>
<td>17 (3)</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral locomotion</td>
<td>0</td>
<td>17 (8)</td>
<td>1 (1)</td>
<td>16 (4)</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>29</td>
<td>17</td>
<td>34</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Total responses</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table details the responses obtained for each condition as well as the number of cats tested in each condition in parentheses (\( n = \)). The first part details the non-locomotor ipsilateral responses (A), the second part, the non-locomotor bilateral responses (B), and the locomotor responses (C). For each type of responses, the percentage obtained in that condition is displayed as well as the number of cats in which that response was triggered in brackets. Those data are displayed for the four conditions tested. The last column corresponds to the mean percentage of that response across conditions. The last line corresponds to the sum of all responses (in %) in a given condition. For untrained preclonidine (pre-clo), \( n = 6 \); for untrained post clonidine (post-clo) \( n = 12 \); for trained pre-clo, \( n = 3 \); and for trained post-clo, \( n = 5 \). N/A: not assessed.

\( \sim 0.5–1 \) mm. They corresponded to \( \sim 11\% \) of the responses across condition and were included in Table 2 under “faint/no response.” Finally, responses of extension followed by small flexion were rarely observed and were not classified in this study.

DISTRIBUTION OF THE UNILATERAL NONLOCOMOTOR RESPONSES IN UNTRAINED SPINAL CATS. **Preclonidine.** Figure 4A shows the distribution of ipsilateral flexion (black), ipsilateral extension (gray) and ipsilateral flexion followed by ipsilateral extension (white) responses by segment (top), laterality (middle), and % Total responses.
depth (bottom). The value of each bar represents the percentage of each response out of all the other responses obtained by stimulating at that segment, laterality or depth respectively, in the four experimental conditions (untrained pre- and post-clonidine, trained pre- and post-clonidine). The total number of stimulation sites (N total) is displayed below each group of bars. In the segmental distribution histogram (top), responses obtained at various lateralities and depths were combined for each segment plotted on the x axis, thus only taking into account the criterion of segment. The same applies to the histogram of distribution per laterality (middle), where responses obtained at various depths and segments were combined for each of the laterality intervals plotted on the x axis, and for the depth distribution histogram (bottom) where responses obtained at various lateralities and segments are combined and plotted for each of the depth intervals tested. The y axis of all the graphics were optimized to clearly show the differences between depths, segments or lateralities. In front of a number indicates the inclusion of the first number in the interval, while the same symbol following a number indicates the exclusion of that number from the interval.

As was the case preclonidine, sites eliciting ipsilateral flexion were significantly different from the sites evoking ipsilateral extension. Indeed, on average, ipsilateral flexion was induced in all segments, at 1.1 ± 0.7 mm from the midline (laterality) and 1.4 ± 1.3 mm below the dorsal surface (depth). Sites evoking ipsilateral extension had a segmental distribution that was statistically different from those evoking flexion ($\chi^2; P < 0.01$) and were distributed in more caudal segments (see Figs. 4B, top, and 5, second column). They were also localized at more lateral sites (1.5 ± 0.9 mm; Student’s t-test $P < 0.05$) and more ventral sites (2.1 ± 1.8 mm; Student’s t-test $P < 0.05$).

Distribution of subtypes of ipsilateral flexion and extension was further assessed in a smaller number of animals postclonidine ($n = 7$). All patterns of flexion observed were distributed mainly dorsally and in all segments. However, forward flexion with dorsiflexion (Fig. 3B), which represented 11% of all flexion responses, was triggered from more lateral sites than the other types of flexion (1.7 ± 0.9 mm from the midline; Student’s t-test $P < 0.05$), 58% of the sequences being evoked at a laterality of ≥2 mm from the midline. Sites inducing flexion followed by extension were located in rostral and caudal segments, at all lateralties and in the dorsal area.

Postclonidine. After clonidine, ipsilateral flexion was still the most frequent response but formed a smaller fraction of all responses obtained (30%; Table 2). It was triggered in all segments, from all lateralities and dorsally (Fig. 4B), whereas ipsilateral extension was mainly evoked caudally, laterally and in the intermediate and ventral areas. Sites inducing flexion followed by extension were located in rostral and caudal segments, at all lateralties and in the dorsal area.
were evoked by a stimulation of the dorsal surface in the four cats in which it was observed, and in three of those cats, it was restrained to the L7 segment. Finally, flexion combined to abduction was mainly observed dorsally (0.75 ± 1.5 mm from the dorsal surface), while extension combined to either abduction or adduction were observed more ventrally (between 4 and 5 mm below the dorsal surface).

DISTRIBUTION OF THE UNILATERAL NONLOCOMOTOR RESPONSES IN TRAINED SPINAL CATS. Preclonidine. All responses described for untrained spinal cats were also observed in the trained condition. Before clonidine injection, ipsilateral flexion constituted 39% of all responses evoked (compared with 57% in the untrained spinal cat predrug) and was triggered in similar sites than in the untrained spinal cat (Fig. 4C). Indeed, it was evoked from all segments (\( \chi^2 \) between sites evoking flexion in the trained vs. untrained spinal cat was not significant), from all lateralities (\( \chi^2 \) not significant), and from all depths mainly dorsally (\( \chi^2 \) not significant). Sites evoking ipsilateral extension were mainly localized rostrally, medially, and at all depths. Thus they were localized differently than in the untrained spinal cat (\( \chi^2; P < 0.001 \)) with respect to segmental distribution, laterality, and depth. Ipsilateral flexion followed by extension occurred more frequently in the trained spinal cat and was evoked from rostral and caudal segments, in medial and dorsal root entry zones and in dorsal and intermediate areas. Although sites evoking ipsilateral extension were located rostrally and at all depth in the trained preclonidine cats, their distribution was statistically different from sites evoking ipsilateral flexion (\( \chi^2; P < 0.001 \)).

Postclonidine. After clonidine intravenous, ipsilateral flexion followed the same distribution as in the other conditions, but ipsilateral extension was evoked from rostral and caudal segments, mainly laterally and from dorsal and intermediate depths. Flexion followed by extension response was evoked from rostral segments and, as in the other conditions, was mainly elicited from medial and dorsal root entry zones and dorsal areas. The distribution of sites evoking ipsilateral flexion was statistically different from the distribution of sites evoking ipsilateral extension along the different segments (\( \chi^2; P < 0.01 \)), lateralities (\( \chi^2; P < 0.025 \)) and depths (\( \chi^2; P < 0.001 \); Fig. 4D).

Although the general distribution patterns are described by segments, laterality, and depth in Fig. 4, actual loci where these three ipsilateral responses were triggered are plotted by segments in the four conditions tested in Fig. 5. Each dot represents a type of response (ipsilateral flexion in blue, ipsilateral extension in red and ipsilateral flexion followed by extension in yellow), and the size of the dot reflects the number of times (n) a particular response type was triggered from a given locus. In the untrained spinal cat preclonidine, ipsilateral flexion was triggered in all segments but formed a higher proportion of the responses in the rostral segments, being the only ipsilateral response triggered in L4 and L5. Ipsilateral extension was triggered from those segments only after clonidine was injected, occurring more frequently in caudal segments and being triggered from more ventral areas than flexion. In the trained spinal cat, ipsilateral extension responses are less restricted to the ventral area and were triggered from rostral segments even prior to clonidine.

In summary, there was no statistical difference in the segmental (L3–L7) and mediolateral (from midline to 3 mm laterally) distribution of flexion responses in the four conditions tested. Although differences were observed in the pattern of distribution of flexion across depths (\( \chi^2; P < 0.001 \)), the responses were mainly triggered dorsally in all conditions. Extension was evoked caudally, laterally, and ventrally in the untrained spinal cat and the distribution was similar pre- and postclonidine. However, its distribution in the trained spinal cat (mainly preclonidine) was statistically different from the one.
observed in the untrained spinal cat ($\chi^2; P < 0.001$). Indeed, sites evoking extension were more spread out in the rostrocaudal and dorsoventral axis, and extension was mainly induced medially in the trained spinal cat preclonidine. Ipsilateral flexion followed by extension was mainly observed medially and dorsoventrally in all conditions. Furthermore, although the percentage of ipsilateral extension and ipsilateral flexion followed by extension did not vary greatly across conditions, the percentage of ipsilateral flexion decreased largely postclonidine and with training/chronicity.

Bilateral non locomotor responses

GENERAL DESCRIPTION OF THE BILATERAL NONLOCOMOTOR RESPONSES. This category includes responses evoked by ISMS in both legs simultaneously. Ipsilateral flexion accompanied by contralateral extension represented 10% of all responses across conditions (see Table 2). In Fig. 6A, the EMG averages show that muscles of both limbs are activated but mainly the VL and GL of the contralateral hindlimb (right) and the TA and Srt of the ipsilateral limb (left). The second response of this group (8% of all responses across conditions) is bilateral flexion and consisted of simultaneous flexion in both limbs In Fig. 6B, St and TA discharge simultaneously in both hindlimbs. Bilateral extension was the third response triggered in that group (2% of all responses) and translated either as the latter half of a gallop, a stiffening of both limbs or as a combination of stiffening in one leg and backward extension in the other leg. Figure 6C illustrates that VL and GL of both limbs are activated.

A few rare responses consisted in extension of the contralateral hindlimb only without ipsilateral flexion. Another response was ipsilateral extension with contralateral flexion which corresponded to 1% of all responses. Both those responses are included in Table 2 under Other Responses.

DISTRIBUTION OF THE BILATERAL NONLOCOMOTOR RESPONSES IN UNTRAINED SPINAL CATS. Preclonidine. Bilateral responses consisting in an ipsilateral flexion with contralateral extension was evoked in all segments, mainly rostrally (Fig. 7A), medially and in the intermediate and ventral areas. Bilateral flexion was triggered mainly rostrally, medially, and dorsally, being...
induced in significantly more dorsal sites than ipsilateral flexion with contralateral extension ($\chi^2; P < 0.001$). Bilateral extension was rarely observed in the preclonidine condition. It was triggered from the L6 segment, medially and at intermediate depths.

**Postclonidine.** After clonidine, ipsilateral flexion with contralateral extension, bilateral flexion and bilateral extension were evoked from all segments more or less equally and at all lateralities mostly medially (Fig. 7B). However, bilateral flexion was evoked from more dorsal locations ($1.6 \pm 1.4$ mm) than bilateral extension ($3.31 \pm 1.9$ mm; Student’s $t$-test $P < 0.05$) and than ipsilateral flexion with contralateral extension ($2.5 \pm 1.7$ mm; Student’s $t$-test $P < 0.05$). No statistical difference was observed between depths at which bilateral extension and ipsilateral flexion with contralateral extension were triggered.

**DISTRIBUTION OF THE BILATERAL NONLOCOMOTOR RESPONSES IN TRAINED SPINAL CATS.**

**Preclonidine.** Figure 7C shows that the distribution of sites inducing ipsilateral flexion with contralateral extension is similar to that of the untrained spinal cat, but that it is absent from L5 in the three cats tested in this condition (see Fig. 7C). Similarly, bilateral flexion was still triggered from all segments, mostly rostrally, from the medial and dorsal root entry zone and from dorsal areas. However, differences were observed in the distribution of sites evoking bilateral extension as it was triggered from rostral segments, dorsal root entry zone and from dorsal and ventral areas. This is contrary to the untrained spinal cat postclonidine where it was evoked from all segments, lateralities and mainly in intermediate and ventral areas.

**Postclonidine.** In the postclonidine state (Fig. 7D), distribution of ipsilateral flexion with contralateral extension, bilateral flexion, and bilateral extension were similar than in the untrained spinal cat postclonidine, and bilateral extension was triggered from all segments and all lateralities, which differed from the trained preclonidine distribution.

In both pre- and postconditions, the dorsoventral difference in the distribution of sites evoking the three responses described above was present ($\chi^2; P < 0.001$). Indeed, ipsilateral flexion and contralateral extension as well as bilateral extension were mainly located in the intermediate and ventral zone, whereas bilateral flexion was mainly triggered dorsally.

Figure 8 shows the distribution of the loci where bilateral responses were evoked persegment in the four conditions tested. The main feature of these spinal cord drawings is that bilateral responses (mainly flexion and bilateral extension) were principally evoked medially. This was in contrast with the distribution of ipsilateral responses which were mainly triggered laterally. Indeed, in the untrained spinal cat postclonidine, bilateral responses were induced at a mean laterality of $0.7 \pm 0.7$ mm, which is significantly more medial than ipsilateral responses ($1.2 \pm 0.7$ mm; Student’s $t$-test $P < 0.05$). This differential distribution between ipsi- and bilateral responses was observed in all conditions ($\chi^2; P < 0.01$), except in the trained spinal cat preclonidine, where it was not significant. Another feature was that bilateral flexion was triggered in relatively more dorsal sites than bilateral extension, mimicking the distribution of ipsilateral flexion and ipsilateral extension respectively. For example, in the untrained spinal cat postclonidine, bilateral flexion was induced at $1.6 \pm 1.4$ mm below the dorsal surface, whereas bilateral extension was induced at $3.3 \pm 1.9$ mm (Student’s $t$-test $P < 0.05$; see especially Fig. 8, untrained post, segments L4–L7). Sites evoking ipsilateral flexion with contralateral extension were also located significantly more ventrally ($2.5 \pm 1.7$ mm below the dorsal surface) than sites evoking bilateral flexion ($1.6 \pm 1.4$ mm; Student’s $t$-test $P < 0.05$).

Overall, the spinal distribution of bilateral responses did not vary greatly in the different conditions, except for the trained preclonidine condition: Sites evoking ipsilateral flexion with contralateral extension were distributed more or less equally in all segments and lateralities and was mainly triggered in the intermediate and ventral areas in the four conditions tested (no statistical differences were observed).
Sites evoking bilateral flexion were distributed in all segments, mainly medially and dorsally in all conditions, whereas bilateral extension was either triggered in all segments or in rostral segments (trained pre), mainly medially and ventrally. Furthermore, although the percentage of bilateral extension remained relatively similar in all conditions, the proportion of ipsilateral flexion with contralateral extension and of bilateral flexion increased postclonidine and with training/chronicity.

Locomotor responses

GENERAL DESCRIPTION OF THE LOCOMOTOR RESPONSES. Three types of locomotor responses were induced. The first one was locomotion of the ipsilateral hindlimb only and was the less frequent form of locomotor response (~1% of all responses). It consisted of an upward and forward movement (flexion) of the ipsilateral leg during swing followed by an active extension on the treadmill during stance. Thus there was a clear alternation between flexor and extensor muscles in the ipsilateral hindlimb only and no locomotion in the contralateral hindlimb. The verification of the EMG activity was crucial for this response as certain patterns of ipsilateral flexion could resemble the swing phase of the locomotor cycle but were not considered to be locomotor responses because no active extension followed (see Fig. 3B). EMG activity during a bout of ipsilateral locomotion is depicted in Fig. 9A, where the stimulation applied on the right side of the cord elicits alternation between flexor (RS, RTA) and extensor (RVL, RGL) muscles of the ipsilateral hindlimb (right). EMG bursts are triggered in the contralateral St (left) evoking a flexion response at almost each stimulation, which alternates with the swing phase of the ipsilateral hindlimb (see RSt vs. LSt). No active extension is observed in the contralateral left hindlimb.

The second and most prevalent type of locomotor response was contralateral hindlimb locomotion (~11% of all responses in the trained and untrained postclonidine conditions). This response consisted of an alternation between flexor and extensor muscles and a forward and upward movement of the hindlimb contralateral to the stimulation during swing. Contralateral locomotion could be expressed without response in the ipsilateral hindlimb (less frequent) or could be accompanied by one of the three ipsilateral responses described earlier namely ipsilateral flexion, which was the most prominent, ipsilateral extension and ipsilateral flexion followed by extension. An example of contralateral locomotion with ipsilateral flexion is shown in Fig. 9B. The EMG traces show rhythmic and reciprocal activity between the flexor (LS, LS, RGL) and extensor (LVL) muscles of the contralateral hindlimb (left), while rhythmic synchronous EMG discharge are observed in the muscles of the ipsilateral limb (RS, RVL, RS).

The third type of locomotor response was bilateral hindlimb locomotion, which is defined as an alternation between flexor and extensor muscles in each hindlimb, a forward and upward movement of the hindlimbs during the swing phase of the cycle, and an out-of-phase alternation between both hindlimbs (represents 9% of all responses). As can be seen in Fig. 9C, locomotion was induced with trains of stimulation at L3 in a spinal cat postclonidine. The stick figures illustrate the amplitude of the movement during swing and EMG bursts are well defined and organized. Hence, the alternation between flexor and extensor muscles in each hindlimb is clear. There is also a well defined reciprocal activity between the left and right hindlimbs of the animal (see for example RSt vs. LSt and RGL vs. LGL). The electrolytic lesion in Fig. 9D displays an effective site (2 mm below the dorsal surface) evoking bilateral locomotion at the L4 segment.

DISTRIBUTION OF THE LOCOMOTOR RESPONSES IN UNTRAINED SPINAL CATS. Postclonidine. Because only a single locomotor response could be elicited in the six untrained spinal cats that were tested preclonidine, only the results obtained postclonidine will be presented. Figure 10A shows that the very few ipsilateral locomotion responses that were obtained (squared pattern) are scattered in all segments, lateralities and depths more or less equally.

Contralateral locomotion (solid fragmented bar) is evoked from all segments, from medial and dorsal root entry zones and from dorsal and ventral depths (Fig. 10A, bottom). To determine if the localization of effective sites for contralateral locomotion was correlated to the accompanying ipsilateral response, the distribution of sites was determined for each subtype. Each bar representing contralateral locomotion in the histogram is subdivided in three, whether contralateral locomotion is expressed with ipsilateral flexion (black), ipsilateral extension (gray), or ipsilateral flexion followed by extension.
Contralateral locomotion expressed with ipsilateral flexion is evoked in all segments and mainly in dorsal areas, similarly to the distribution of sites evoking nonlocomotor ipsilateral flexion. Contralateral locomotion with ipsilateral extension is mainly elicited in caudal segments and in ventral areas; this parallels the distribution of sites inducing ipsilateral extension. Contralateral locomotion expressed with ipsilateral flexion followed by extension is evoked in rostral and caudal segments, mainly dorsally. Dorsal localizations were also the most efficient to trigger ipsilateral flexion followed by extension alone. However, the mediolateral distribution of those three subtypes of contralateral locomotion is from more medial sites ($0.8 \pm 0.5$ mm) than the corresponding ipsilateral responses alone ($1.2 \pm 0.8$; Student’s $t$-test $P < 0.05$ mm). This mediolateral localization is similar to sites where bilateral nonlocomotor responses could be evoked ($0.7 \pm 0.7$ mm).

As shown in Fig. 10A (striped pattern), bilateral locomotion was triggered in all segments, was induced at all laterality, but mainly medially ($0.88 \pm 0.68$ mm) compared with ipsilateral responses ($1.2 \pm 0.8$ mm; Student’s $t$-test $P < 0.05$), and was almost exclusively evoked from dorsal localizations (mean depth: $0.5 \pm 0.8$ mm below the dorsal surface). The depth histogram illustrates that the majority of bilateral locomotion sequences were evoked between the dorsal surface and 3 mm deep, the efficiency decreasing quickly as the electrode progressed more ventrally. Both bilateral and contralateral locomotion were triggered from similar sites in the segmental and mediolateral axis, but bilateral locomotion was almost exclusively evoked from dorsal sites.

**DISTRIBUTION OF THE LOCOMOTOR RESPONSES IN TRAINED SPINAL CATS. Preclonidine.** Ipsilateral and bilateral locomotion were rarely triggered in this condition. However, contralateral locomotion with ipsilateral flexion was triggered in a proportion of 17%, and effective stimulation sites were mainly localized caudally in L6 and L7, laterally and in dorsal and intermediate areas (Fig. 10B). This distribution was different from that of the untrained spinal cat postclonidine ($\chi^2; P < 0.001$).

**Postclonidine.** Few bouts of ipsilateral locomotion were evoked in the dorsal part of L3 segment. Contralateral rhythms were still triggered with a higher prevalence in caudal segments (Fig. 10C), medially and dorsally, with a peak in the ventral area ($[4-5]$). Bilateral locomotion was mainly evoked from segments L3-L6 and L7, from medial and dorsal root entry zones and from dorsal areas. Thus there was a significant difference in the segmental distribution between bilateral and contralateral locomotion ($\chi^2; P < 0.001$).

Figure 11 shows the distribution of effective sites for unilateral locomotion responses (ipsi- and contralateral) per segment. In the postclonidine conditions, unilateral locomotion was mainly induced medially and dorsally but was not triggered laterally. However, ventromedial areas were also efficient in L6 and L7 segments of the untrained-post condition. In the trained preclonidine condition, contralateral locomotion

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**FIG. 10.** Distribution of locomotor responses in the 4 conditions tested. Proportion of ipsilateral locomotion (squared pattern), contralateral locomotion (solid bar) and bilateral locomotion (striped pattern) evoked by segment (top), laterality (middle), and depth (bottom) in untrained spinal cats postclonidine (A) and in trained spinal cats pre- (B) and postclonidine (C). Graphic reads as Fig. 4. Each bar of contralateral locomotion on the histograms is divided to represent the percentage of contralateral locomotion with ipsilateral flexion (black), with ipsilateral extension (gray), and with ipsilateral flexion followed by extension (white).
was mainly evoked in L₆–L₇ and was triggered from more lateral sites (1.2/0.5 mm; Student’s t-test \(P < 0.05\)). Indeed, after injection of clonidine the proportion of ipsilateral responses decreased and more bilateral and locomotor responses were triggered, mainly in rostral segments L₃–L₅. A similar observation was done in the trained spinal cat: while bilateral responses diminished with clonidine, the locomotor responses increased, mainly in rostral segments. Ipsilateral responses remained relatively similar before and after clonidine.

Differences in the proportion of responses were also observed between 1 wk-untrained and 3–5 wk-trained spinal cats before clonidine was injected (\(\chi^2; P < 0.001\)). More specifically, ipsilateral responses were less numerous in the trained

![Unilateral locomotor responses](image1)

![Bilateral locomotor responses](image2)

**Effect of clonidine and training/chronicity on proportion of responses evoked**

The proportion of ipsilateral, bilateral, and locomotor response was statistically different in the untrained spinal cat before and after clonidine (\(\chi^2, P < 0.001\)). Indeed, after injection of clonidine the proportion of ipsilateral responses decreased and more bilateral and locomotor responses were triggered, mainly in rostral segments L₃–L₅. A similar observation was done in the trained spinal cat: while bilateral responses diminished with clonidine, the locomotor responses increased, mainly in rostral segments. Ipsilateral responses remained relatively similar before and after clonidine.

Differences in the proportion of responses were also observed between 1 wk-untrained and 3–5 wk-trained spinal cats before clonidine was injected (\(\chi^2; P < 0.001\)). More specifically, ipsilateral responses were less numerous in the trained
spinal cat, whereas bilateral and locomotor responses were more frequently triggered. The effect of training/chronicity was, however, not observed in the postclonidine condition. No statistical difference in the proportion of ipsilateral, bilateral, and locomotor responses between the untrained and trained spinal cats was found.

Changes in proportion applied to specific responses. Electrical stimulation evoked predominantly ipsilateral flexion in all conditions tested, but its occurrence diminished in postclonidine and in trained condition due to the emergence of more complex responses. Table 2 shows that in the untrained spinal cat preclonidine, 57% of all responses evoked were ipsilateral flexion compared with 30% postclonidine, 39% in trained spinal cats preclonidine, and 27% in trained spinal cats postclonidine. Alternatively, apart for one bout of ipsilateral locomotion induced, no locomotor responses could be evoked in untrained spinal cat pre clonidine compared with 29% after clonidine, 17% in the trained spinal cat preclonidine, and 34% in the trained spinal cat post clonidine. It thus seemed that some sites that elicited ipsilateral flexion could induce locomotor responses after clonidine or in trained chronic spinal cats.

To confirm this correlation between decrease of ipsilateral flexion and increase of more complex responses, the same stimulating tracks were compared before and after clonidine in an untrained and in a trained spinal cat. Figure 13 illustrates responses obtained along a stimulation track made before and after an injection of clonidine intravenous (left side of the figure). In the preclonidine condition, ISMS first elicited an ipsilateral flexion when the electrode tip touched the dorsal surface at L6. The electrode was descended ventrally, and at 3 mm below the dorsal surface, a decrease in the amplitude of flexion was observed to the point of being abolished. At 4 mm, ipsilateral extension with contralateral flexion was triggered, and at 5 mm, ipsilateral flexion was evoked again. Postclonidine, ipsilateral flexion was elicited in the first 2 mm. From 2 to 4 mm below the dorsal surface, a zone of hypoxicitability was encountered where ipsilateral flexion became very faint. At 4 mm, ipsilateral extension with contralateral flexion was triggered followed at 5 mm deep by bilateral extension interrupted by sequences of ipsilateral extension with contralateral locomotion. Therefore ipsilateral flexion induced ventrally preclonidine was no longer evoked postclonidine and was replaced by a bilateral and a locomotor response. In the trained spinal cat, ISMS was applied at L4. Prior to clonidine injection, ipsilateral flexion was induced in the first 2 mm. As the electrode was descended, bilateral flexion was induced from 2 to 3 mm below the dorsal surface. At 3 mm, ipsilateral flexion was triggered again, and at 4.5 mm, ipsilateral flexion was accompanied by contralateral extension. After clonidine, ipsilateral flexion with contralateral locomotion was induced from the dorsal surface to 1.8 mm. Bilateral locomotion was then observed until 2.8 mm, where the response in the contralateral hindlimb disappeared and very faint ipsilateral flexion occurred, but was quickly no longer noticeable as the electrode is descended. At 4.4 mm, ipsilateral flexion with
Discussion

ISMS applied with one electrode at different lateralities and depths in segments L₁–L₇ in chronic spinal cats, pre- and postclonidine, triggered three main types of hindlimb responses: nonlocomotor ipsilateral, nonlocomotor bilateral, and locomotor. The distribution of sites where each response was preferentially evoked generally followed preferential gradients in the rostrocaudal, mediolateral, and dorsoventral axis. These gradients remained stable in the pre- and postclonidine conditions in untrained spinal cats spinalized 5–7 days earlier but were more varied in the chronic trained spinal cats (3–5 wk), mainly before clonidine was injected. Notably, extension responses (ipsi- and bilateral) were more frequently triggered in rostral segments and in dorsal areas in the trained spinal cats. However, some of the differences observed might be due to the smaller sampling done in these cats. Although the distribution was similar, the prevalence of certain responses varied greatly across the four conditions (trained, untrained, pre- or postclonidine). Indeed with clonidine and/or training, the percentage of simple ipsilateral flexion decreased and was paralleled by a higher occurrence of more complex locomotor responses.

Methodological considerations

In these experiments, it was very difficult to trigger locomotion with the usual stimuli (treadmill movement, perineal stimulation) without adding a pharmacological stimulation, even in trained spinal cats which had recovered locomotion on the treadmill. Since the cats were spinalized about 1 wk prior to the acute experiment, a spinal shock cannot explain this difficulty in triggering locomotion. Rather it might be explained by the spinal fixation used to immobilize the cat. Such sturdy fixation was required to rigidly suspend the animal and diminish movements of the spinal cord secondary to respiration or limb movements, an essential condition to insure repeated and accurate stimulation of the same spinal loci with a rigid electrode. The fixation undoubtedly provided noxious stimulation that might have contributed to a change in the excitability in the spinal cord, making the expression of locomotion more difficult or often incomplete.

In these experiments, ventral- and lateral-most regions were somewhat less studied than dorsal and medial regions (see Fig. 1). The risk of damaging the electrode and the cord was high in ventral-most regions, and they were not assessed as frequently as other regions. Furthermore, each track could take 10–15 min and, to ensure stability of the preparation when comparing sites of stimulation, we had to limit the number of tracks in any given experiments.

Gradient of distribution were consistent in every cat, and differences between localization of different types of responses were found to be significant. However, in each condition, the variability of responses observed between cats was important. Hence, the mapping of localizations is not identical for all cats, but the distribution gradients are the same for all cats. Furthermore, each response was determined by observation of videos and corresponding EMG. With this method of analysis, we might have missed some responses because the EMG recordings were limited to a few muscles and the movements analyzed in details only in the sagittal plane. The responses were also grouped in large categories (flexion, extension, locomotion) involving the ipsilateral and/or the contralateral sides. This might have ignored more subtle patterns but surely has captured the main categories of responses. Furthermore, the aim of the present research was to find sites of predilection to induce locomotion, an emphasis was put on these sites whose location is described here. A more detailed account of the various characteristics of locomotion evoked from different sites is the subject of a forthcoming paper.

In the present study, trains of stimulation were used to induce the responses and allow the hindlimb to return to its initial position. When locomotion was induced, trains were used with the aim of controlling the locomotor frequency. However, we have also used tonic stimulation to induce locomotion and preliminary data were presented previously (Barthélémy et al. 2002).

Elements activated by the stimulation

Using a single electrode and relatively low intensities of electrical stimulation (20–90 μA), it is estimated that the stimuli used (<100 μA) would not spread further than a diameter of 1 mm (Bagshaw and Evans 1976; Gustafsson and Jankowska 1976; Jankowska and Roberts 1972; Lemay and Grill 2004; Porter 1963; Tai et al. 2003; Yeomans et al. 1986). Thus most responses might be explained by elements that are in the vicinity of the electrode tip, such as dendrites, cellular bodies, and fibers (ascending and descending). Another evidence of the short range of the direct stimulation is the occurrence of sites where the response was faint or absent. In those loci, mainly prominent at intermediate depths, the amplitude of the response diminished significantly and was often completely abolished at the same intensity of stimulation that induced frank responses at sites located just above or below.

ISMS is a nonphysiological means of activating groups of neurons, and it is difficult to determine with certainty which elements the stimulation is activating. It will activate neurons directly or synaptically, through afferents from the periphery, axons from other interneurons, or descending pathways. Nonetheless, the reproducibility and characteristics of the major responses described is consistent with the localization of interneurons and motoneurons involved in certain reflex pathways.

Nonlocomotor ipsilateral responses

Ipsilateral flexion and ipsilateral extension. Some of the responses described in this paper correspond to classic reflexes first described by Sherrington, such as the classic flexion reflex. Different flexion patterns, as mentioned in this study, were also observed by Sherrington and dependent on which nerve was stimulated. He referred to them as “local sign” (Sherrington 1910). This is undoubtedly the basis for the numerous functional withdrawal responses also described in the rat (Schouenborg 2002). Other reflexes included the “direct extension” and
the “extensor thrust,” which are likely to correspond to the two patterns of extension discussed here. These responses might correspond to direct activation of extensor motoneurons or to the activation of various groups of afferents or propriospinal fibers impinging on extensor motoneurons. For the backward extension, responses were mainly observed in L7, corresponding to the localization of hip extensor motoneurons. Also, the higher occurrence of flexion and extension responses laterally might be explained by the localization of motor nuclei innervating limb muscles that are localized laterally in the ventral horn compared with those innervating axial muscles (McHanwell and Biscoe 1981; Nicolopoulos-Stournaras and Iles 1983; Vanderhorst and Holstege 1997).

The dorsoventral distribution of ipsilateral flexion and extension responses may reflect a topographical organization of descending pathways and the interneurons that mediate their action. Indeed, vestibulo- and reticulospinal pathways that descend in ventral and ventrolateral quadrants mainly excite extensor muscles via interneurons located ventrally in the gray matter. (Armstrong and Edgley 1984; Drew and Rossignol 1984; Orlovsky 1972a; Orlovsky and Pavlova 1972). Cortico- and rubrospinal pathways, as well as some reticulospinal pathways, descend in the dorsolateral funiculi and activate mainly flexor muscles as well as distal muscles via interneurons located more dorsally in the spinal gray (Armstrong and Drew 1984a,b; Kuypers 1982; Orlovsky 1972b). More specifically, the dorsolateral localization of forward flexion with dorsiflexion might be explained in the untrained spinal cat by stimulation of remaining cortico- and rubrospinal fibers (Jiang and Drew 1996) or, in trained chronic cats, by stimulation of propriospinal fibers activating similar targets as the corticospinal tract.

We cannot disregard the possibility that descending axons from supralumbar levels, such as long propriospinal pathways from the brachial enlargement (English and Lennard 1982; Jankowska et al. 1974), and supraspinal levels may be activated by ISMS, especially in animals lesioned only 1 wk before. However, it is unlikely that neurotransmitters are still present in these axons at longer periods based on work on noradrenergic pathways done in 9- to 15-day chronic spinal cats (Anden et al. 1972). Moreover, the distribution gradients were similar in untrained spinal cats spinalized 5–7 days prior to the experiments and in trained spinal cats, spinalized 21–35 days previously and in which descending axons have most likely degenerated. This suggests that descending pathways do not constitute in a preponderant manner in the response obtained here and implies that stimulation preferentially activates postsynaptic elements as suggested by Tresch and Bizzi (1999) and Lemay and Grill (2004), either directly or through intraspinal axons.

This flexor/extensor polarity was also evidenced in the decerebrate cat (Lemay and Grill 2004; Mushahwar et al. 2002; Tai et al. 2003). Localization of flexor and extensor motoneuronal pools, suggesting that flexor motoneurons are located more dorsally in the ventral horn might also contribute to the dorsoventral gradient of responses described here. However, in the cat, such distribution has been evidenced in the brachial enlargement but does not seem to be present in the lumbosacral enlargement (Sterling and Kuypers 1967; Vanderhorst and Holstege 1997).

**Ipsilateral flexion followed by extension**

Although a clear alternation between flexor and extensor muscles was observed, ipsilateral flexion followed by extension was not considered to be a locomotor response because the hindlimb did not touch the treadmill during extension and the movements were very abrupt. Because there was no real swing or stance phase in these motions, it could not be interpreted as a step. This type of response was also described in the decerebrated and spinal animal (Sherrington 1910) and was called “rebound extension following on reflex flexion.” It was suggested that there were mutual inhibitory connections between interneurons transmitting late excitation to flexor and extensor muscles in acute spinal cats injected with DOPA (Jankowska et al. 1967). Stimulating high-threshold afferents (named flexor reflex afferents-FRA) produced a flexor discharge as well as inhibited interneurons to the extensor muscles ipsilaterally. At the end of the stimulation, a rebound effect might induce a discharge in the extensor of the ipsilateral hindlimb. The authors suggested that those alternating discharge may constitute evidence for a half-center organization of the network released after DOPA as a basis for locomotion. Interestingly in our experiments, ipsilateral flexion followed by extension was only elicited from dorsal sites as was bilateral locomotion.

Such pathways of interneurons might also account for the small bursts of flexor and extensor activity alternating after stimulation has stopped and which were more evident post-clonidine and with training/chronicity (see Fig. 3, A and B). Thus, those responses might reflect an excitability level, concordant with a locomotor state of the spinal cord and represent the basis of a step.

**Nonlocomotor bilateral responses**

Sites where bilateral responses were triggered were mainly observed medially. Although we cannot rule out a spread of the current across the midline, stimulation of dorsal and ventral commissural pathways may explain that medial localization. Indeed, axons of neurons in the lateral aspect of the dorsal horn cross the midline within the posterior commissure and terminate in corresponding areas of the contralateral dorsal horn (Petko and Antal 2000; Petko et al. 2004). Those neurons are integrated in spinal sensory circuits and may be activated by our stimulation to produce bilateral responses such as bilateral flexion. Ventrally, commissural interneurons located in lamina VIII and neighboring lamina VII (Harrison et al. 1986) project contralaterally to other interneurons or to contralateral motor nuclei directly (Birinyi et al. 2003; Edgley et al. 2003; Harrison et al. 1986), and receive inputs from the group I afferents. Because of the input they receive and their projections, they are thought to be interposed in crossed reflex pathways (see Jankowska 1992) and could mediate crossed reflex actions accompanying the flexion reflex from proprioreceptors (Matsuyama et al. 2004). Stimulation might have activated these interneurons directly or indirectly, through other interneurons that make synapses on these commissural interneurons. One candidate would be dorsal horn interneurons that are thought to contribute to crossed reflexes from group II muscle afferents (Edgley et al. 2003). This ventromedial localization of elements that would induce contralateral responses was also observed in the spinal cord of other species, notably in the...
Interneurons subserving flexion-extension reflexes, and activated by reflex afferent inputs, might also be involved in generation of the bilateral responses because they are thought to activate contralateral extensor muscles as well as flexor muscles ipsilaterally (Lundberg 1979). However, there are numerous crossed pathways that could give rise to a variety of crossed responses (Holmqvist 1961). They could be involved in the generation of ipsilateral flexion with contralateral extension or the opposite response, ipsilateral extension with contralateral flexion. The latter response, rarely triggered in the present study, was only briefly mentioned by others (Lemay and Grill 1998; Kjaerulff and Kiehn 1996).

### Locomotor responses

**UNILATERAL LOCOMOTION.** Ipsilateral locomotion was the least frequent of the three locomotor responses evoked in this study and a clear gradient for it is difficult to establish. As mentioned before, a pattern of ipsilateral flexion followed by passive extension by the treadmill could resemble ipsilateral locomotion, but there was no activity in extensor muscles and was thus not considered as locomotor.

Contralateral locomotion was the most prominent locomotor pattern evoked by ISMS. In the untrained spinal cat, ipsilateral flexion for each stimulation accompanied by a continuous contralateral locomotion is evoked from both dorsal and ventral areas but was preferentially evoked from the dorsomedial area. On the other hand, ipsilateral extension responses evoked by each train and contralateral locomotion was mainly evoked from the ventromedial area. This dorsoventral segregation is not as clear in the trained animals because contralateral locomotion with ipsilateral flexion or ipsilateral extension could similarly be evoked dorsally and ventrally. Matsuyama et al. (2004) showed that most lamina VIII commissural interneurons discharge rhythmically during fictive locomotion. Similarly, Kiehn and colleagues described a similar population of commissural interneurons that is involved in production of locomotion in the neonatal rat (Butt et al. 2002; Kiehn and Butt 2003). Those interneurons were probably activated by ISMS and may be central in the pathways producing contralateral locomotion.

Ipsilateral flexion with contralateral locomotion was also observed by Sherrington. A noxious stimulus to a limb could induce a flexion reflex in the ipsilateral hindlimb accompanied by stepping of the three other limbs or, for spinal cats, of the contralateral hindlimb (Sherrington 1910). This response seen in the cat, dog, and rabbit was interpreted to mean that the irritated foot was withdrawn from harm while the others ran away. Because there is a relative independence of central pattern generators for both limbs, it is not surprising that ISMS can evoke locomotion on one side. Work in the chronic spinal kitten has clearly established that one hindlimb can walk while the other is prevented manually from walking (Grillner and Rossignol 1978).

It is not surprising either that contralateral locomotion could be more easily evoked than ipsilateral locomotion. Often the stimulation applied ipsilaterally perturbed the ipsilateral locomotor cycle, whereas the contralateral cycle was only slightly perturbed. It is then possible that the ipsilateral stimuli imposed either a reset of the cycle or a sequence of excitation and inhibition that profoundly perturbed the ipsilateral cycle. The medial localization of sites inducing contralateral locomotion could suggest activation of various pathways through ipsilateral and commissural interneurons. It is of great interest to mention here work that showed that c-fos labeled interneurons after a prolonged bout of locomotion were located medially in the cord (Dai et al. 2005). It could be that our stimulation could, in these cases, activate mainly such population of interneurons.

**Bilateral locomotion**

Sites that would effectively induce a smooth and well-coordinated bilateral locomotion were almost exclusively located dorsally, in dorsal and dorsolateral funiculi, as well as dorsal laminae. Thus stimulation of afferent pathways or of interneurons receiving these afferent inputs seems to evoke bilateral locomotion, whereas stimulation deep in the intermediate gray did not evoke locomotion with our paradigm of electrical stimulation, using a single electrode. This dorsal localization is not surprising as numerous studies have demonstrated the efficiency of epidural and subdural stimulation in the decerebrate and spinal cat (Gerasimenko et al. 2003; Iwashara et al. 1991b) as well as dorsal funiculi (Grillner and Zangger 1979). Stimulation of ventral parts of the spinal cord with a single electrode did not induce bilateral locomotion contrary to other studies where stimulation through groups of electrodes localized in the motoneuronal pools of segments L₅ to S₁ induced locomotion-like patterns (Saigal et al. 2004) or locomotion (Guevremont et al. 2003) in chronic spinal cats. The different technique used in that study might account for the difference.

Both contralateral locomotion and bilateral locomotion were evoked from all segments tested but preferentially from caudal segments. This was especially clear for the contralateral locomotion in the trained spinal cat preclonidine. This finding relates to other studies showing that segments L₃–L₅ contain interneurons active during the production of locomotor pattern as revealed by localization of c-fos (Dai et al. 2005) or by evoked potential analysis (Noga et al. 1995).

Results concerning optimal rostrocaudal localization of electrodes to induce locomotion differ in the literature. Earlier reports by Iwashara et al. (1991b) showed that epic- and subdural stimulation of the preenlargement L₁–L₄ segments was as effective in inducing stepping as the L₅–S₁ segments in acute mid-thoracic spinal cats. Conversely, the border between L₄ and L₅ was found optimal to induce locomotion with epidural stimulation in chronic spinal cats (Gerasimenko et al. 2003). It was also shown that stimulation through groups of electrodes were more efficient to induce locomotion in chronic spinal cats when applied to more caudal segments (Guevremont et al. 2003).

The dorsal localization inducing locomotion suggests that the stimulation may activate afferences to the central pattern generator as first observed by Brown (1914). Indeed, locomotion requires activation of propriospinal pathways, such as the commissural interneurons described earlier for bilateral responses and that are located in the lamina VIII. Other propriospinal pathways include interneurons contacted by group II afferents (group II interneurons). They are located within...
L6–L7 segments, at the border of L4–L5 and of L5–L4 and some of them contact motoneurons in L4–S1 directly via the ventrolateral funiculus (Edgley and Jankowska 1987a,b; Lundberg et al. 1987a–c). Some of those interneurons in L4 were shown to be rhythmically active during locomotion (Shefchyk et al. 1990), and half of the interneurons located in L6–L7, project ipsilaterally within the lateral funiculus to the L4 segment (Riddell and Hadian 2000). Previous experiments have shown that the L4 segment was essential for the induction of locomotion (Langlet et al. 2005; Marcoux and Rossignol 2000) and preliminary evidence show that they would also be essential in the locomotion induced by ISMS applied in caudal segments (Barthélemy et al. 2002, 2005b). This will be discussed more fully in a forthcoming paper.

Effect of clonidine and training

ROSTRAL SEGMENTS. The more frequent involvement of the contralateral hindlimb and the more complex responses triggered after injection of clonidine intravenous was especially true at segments L2–L3. For instance in untrained spinal cats, preclonidine, ipsilateral extension, and bilateral extension were mainly induced ventrally in caudal segments. Postclonidine, those responses were evoked from rostral as well as caudal segments and at all depths of the spinal cord. In trained animals, contralateral locomotion was almost exclusively evoked from caudal segments, but postclonidine, rostral segments as well as caudal segments were efficient to induce it. The fact that clonidine increases the locomotor responses mainly in rostral segments L3–L4 and L5 may suggest the preponderant activation of the subpopulation of commissural interneurons receiving input from group II afferents that are mainly located in midlumbar segments and that were shown to be modulated by monoamines (Bras et al. 1989, 1990; Noga et al. 1992).

State of excitability of the spinal cord

ISMС was applied in intact cats that were then decerebrated and spinalized (Mushahwar et al. 2004). The authors noted a change in the excitability of neuronal elements that made the flexor muscles more dominantly activated in acute spinal cats. This corresponded to the “flexion release” described by Sherrington where the flexion reflexes were increasingly present following acute spinalization. In the present study, the untrained preclonidine condition exhibited an important proportion of flexion but in postclonidine or training condition the proportion of flexion responses elicited decreased and allowed for a greater variety of responses. In fact, the changes brought about by clonidine were similar to that obtained with locomotor training and chronicity. Thus clonidine may have activated pathways that become more excitable with chronicity and training.

Intraspinal electrical stimulation as a tool to restore locomotion

It is thus possible to induce locomotion with ISMS delivered through a single electrode in combination with clonidine. Locomotor responses could also be triggered in a spinal cat that recovered locomotor abilities. Compared with other intraspinal approaches, ISMS delivered through a single electrode has clear advantages. First, it means that stimulating several sites of the spinal cord to induce locomotion is not necessary because one electrode is enough to evoke the complete bilateral pattern. It thus diminishes the number of electrode necessary to evoke locomotion. In the eventuality of chronic implants, the size of the implant could be minimal, causing minimal damage of the spinal cord. Bilateral locomotion was induced at low intensity; this also suggests minimal negative effects. Furthermore, ISMS also has clear advantages over peripheral neuromuscular stimulation where multiple surface or indwelling electrodes have to be used to ensure proper activation of the different muscle groups in the proper sequence.

Locomotion could be induced in 20% out of all the sites tested in the spinal cord. However in the efficient area (dorsally) the % of success increased greatly, neighboring 50% efficiency (see Fig. 10, depth graphic, the first two bars). Thus if the electrodes were placed in the optimal area, locomotion could be evoked at a higher rate, suggesting that this technique could be suitable for rehabilitation purposes.

In conclusion, ISMS through a single electrode is thus efficient to evoke locomotion and constitutes a promising technique to restore locomotion after spinal cord injury. The spinal cord mapping of nonlocomotor and locomotor responses evoked by ISMS as shown here should help focusing on important spinal sites and guide further chronic implants. The mechanisms by which locomotion may occur with ISMS are still largely unknown, but preliminary results indicate that the efficacy of ISMS relies on the integrity of the midlumbar segments (Barthélemy et al. 2005b).

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