Effect of stimulating the lumbar skin caudal to a complete spinal cord injury on hindlimb locomotion

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Effect of stimulating the lumbar skin caudal to a complete spinal cord injury on hindlimb locomotion. J Neurophysiol 113: 669–676, 2015. First published October 22, 2014; doi:10.1152/jn.00739.2014.—Sensory feedback from cutaneous afferents located in the hindpaws can also affect the hindlimb locomotor pattern in spinalized animals (recently reviewed in Panek et al. 2014). Mechanical or electrical stimulation of the skin of the foot dorsum during the swing phase produces a coordinated reflex response that serves to flex the stimulated limb while increasing extensor activity contralaterally (Forssberg et al. 1977). Moreover, following the complete loss of cutaneous inputs from the paw, the chronic spinalized adult cat can no longer properly place the paw at contact, and the weight support is severely reduced (Bouyer and Rossignol 2003). Cutaneous inputs from the hindpaw have also been shown to facilitate locomotor recovery following incomplete SCI in animal models (Muir and Steeves 1995; Smith et al. 2006).

Mechanoreceptors located in the hindlimbs are not the only source of sensory feedback that can influence the spinal-mediated locomotor pattern. Tonic stimulation of the skin of the perineal region is known to facilitate hindlimb locomotion in chronic spinalized animals (Barbeau and Rossignol 1987; Pearson and Rossignol 1991; Langlet et al. 2005; Hochman et al. 2012). In contrast, tonic stimulation of the skin of the back reduces or abolishes the capacity to generate locomotion. For instance, mechanical or electrical stimulation of the lumbar skin abolished fictive locomotor-like activity in curarized decerebrate rabbits with intact (Viala and Buser 1974, Viala et al. 1978) or transected (Viala and Buser 1974) spinal cords. This reduction in locomotor-like activity was attributed to the activation of Aδ fibers (Viala et al. 1978). More recently, it was shown that pinching the lumbar skin abolished locomotor-like activity in immobilized chronic spinalized decerebrate cats treated with clonidine (Frigon et al. 2012). This circuit from lumbar skin mechanoreceptors to spinal locomotor networks appears to have been conserved in humans, as pinching the lumbar skin in a motor complete SCI subject disrupted spontaneous involuntary rhythmic activity (Nadeau et al. 2010).

To date, the effect of stimulating the lumbar skin on real locomotion (as opposed to fictive locomotion) produced by the spinal pattern generator has not been reported. During fictive locomotion, phasic sensory feedback from the hindlimbs is absent. Sensory inputs from muscles of the hip and ankle, as well as cutaneous inputs from the hindpaw, can potently influence the output of spinal pattern generators (comprehensively reviewed in Rossignol et al. 2006). These sensory inputs could negate or influence the effect of cutaneous inputs from the lumbar area on the spinal locomotor network. Thus, the aim...
of the present study was to determine if mechanical stimulation of the lumbar skin disrupted a real spinal-generated locomotor pattern, where all sources of sensory inputs from the hindlimbs are present. We hypothesized that mechanical stimulation of the lumbar skin would disrupt hindlimb locomotion in chronic spinalized adult cats.

**MATERIALS AND METHODS**

**Ethical Information**

All procedures were approved by the Animal Care Committee of the Université de Sherbrooke and were in accordance with policies and directives of the Canadian Council on Animal Care. Six adult cats weighing between 3.5 and 4.7 kg were used in the present study. Three of these cats (BC, PB, and SA) were used in previous studies (Frigon et al. 2013; D’Angelo et al. 2014) to provide answers to other scientific questions. This is part of our ongoing effort to maximize the scientific output of each animal.

**Surgical Procedures**

Implantation and spinal transection surgeries were performed under aseptic conditions with sterilized instruments in an operating room. Butorphanol (0.4 mg/kg), acepromazine (0.1 mg/kg), and glycopyrrolate (0.01 mg/kg) were injected intramuscularly for sedation while ketamine and diazepam (0.11 ml/kg in a 1:1 ratio) were injected intramuscularly for induction. Cats were then intubated with a flexible endotracheal tube to deliver and maintain anesthesia with an appropriate isoflurane concentration (1.5–3%). The level of anesthesia was adjusted by monitoring cardiac and respiratory rate, by monitoring jaw tone and limb withdrawal when a pressure was applied to the paw. Body temperature was monitored using a rectal thermometer. During the surgeries, an antibiotic (0.1 ml/kg Convenia) was injected subcutaneously, and a transdermal fentanyl patch (25 μg/h) was taped to the back of the animal 2–3 cm from the base of the tail. Buprenorphine (0.01 mg/kg), a fast-acting analgesic, was also administered subcutaneously toward the end of the surgery and ~7 h later. After surgery, cats were placed in an incubator until they regained consciousness.

**Spatin transection.** A small incision of the skin was made over the 12th and 13th thoracic (T12–T13) vertebrae. After carefully setting aside muscle and connective tissue, a small laminectomy of the dorsal bone was made. Lidocaine (xylocaine) was applied topically and aside muscle and connective tissue, a small laminectomy of the dorsal bone was made. Lidocaine (xylocaine) was applied topically and injected within the spinal cord in two to three different areas. The spinal cord was then transected with surgical scissors. A hemostatic agent (spongostan) was placed within the gap, and muscles and skin were sewn back to close the opening in anatomic layers. After spinalization, the bladder was manually expressed one to two times daily, and cats were monitored by experienced personnel. The bottom half of the animal was frequently cleaned in a warm soapy bath.

One week after spinalization, cats started treadmill training five times a week to recover hindlimb locomotion, with each session lasting 20–30 min. Initially, two experimenters moved the hindlimbs to reproduce a locomotor pattern with one of the experimenters holding the tail for support. The forelimbs were placed on a fixed platform ~1 cm above the treadmill belt. A Plexiglas separator was placed between the hindlimbs to prevent crossing. After a few days of training, stepping movements could be induced by stimulating the skin of the perineal region. Data collection started once the animals regained spontaneous stable hindlimb locomotion with full weight bearing and consistent plantar placement (>8 wk of training). During experiments, an experimenter held the tail to provide equilibrium.

**Electrode implantation.** Pairs of Teflon-insulated multistrain fine wires (AS633; Cooner Wire) were directed subcutaneously from a head-mounted 24-pin connector (Hirose Electric) in cats BC and SA or from a head-mounted 36-pin connector (Omnetics) in cats PZ, PB, BL, and FX and sewn into the belly of selected hindlimb muscles for bipolar electromyography (EMG). During experiments, the EMGs were bandpass filtered (30–1,000 Hz) and amplified (100–5,000 times) using a 16-channel amplifier (model 3500; AM Systems) and digitized at a sampling rate of 5,000 Hz using custom-made acquisition software.

**Experimental Protocol**

The effect of manually pinching the lumbar skin on hindlimb locomotor activity was evaluated. The lumbar skin over vertebrae L2–L7 was pinched with the thumb and index finger by the same experimenter (Frigon) in all sessions. In manual pinching trials, ~15 control steps were obtained, followed by 1 min of manual pinching and another ~15 control steps postpinch.

Mechanical stimulation of the lumbar skin was also made by a small bulldog clip weighing 2.75 g with a closing force of 520 g. The bulldog clip’s application surface was ~1 cm². Cutaneous stimulation was applied to seven different sites over the midline of vertebrae L2–L7. Trials were performed without (control) or with cutaneous stimulation at a treadmill speed of 0.4 m/s. In stimulated trials, the clip was applied by taking a small fold of skin while the animal was standing. Five seconds after applying the clip, the treadmill was started with an acceleration of 0.1 m/s². Data collection began once a speed of 0.4 m/s was attained, and the clip remained in place for the duration of the episode. The order of stimulation sites was randomly selected, and ~30 s of rest were given between episodes. Viala and Buser (1974) showed that the effectiveness of the pinch in disrupting fictive locomotor-like activity depended on the application surface. To minimize this effect, the clip was always applied to the back of the animal by the same experimenter (Hurteau). The first 15–20 locomotor cycles obtained after reaching 0.4 m/s were retained for analysis.

**Data Acquisition and Analysis**

**Kinematics.** Hindlimb locomotion was filmed from the left and right sides with two cameras (Basler AcA640-100 gm) at 60 frames/s with a spatial resolution of 640 by 480 pixels. A custom-made Labview program acquired the images and synchronized the cameras. Videos were analyzed off-line at 60 frames/s using custom-made software. Paw contact, defined as the first frame where the paw made visible contact with the treadmill surface, and liftoff, defined as the most caudal displacement of the limb, were determined for both hindlimbs. Cycle duration was measured from successive paw contacts while stance duration corresponded to the interval of time from paw contact to liftoff. Swing duration was measured as cycle duration minus stance duration.

Stride length of the right hindlimb was measured as the horizontal distance travelled from liftoff to contact plus the distance travelled by the treadmill belt during the swing phase (swing duration multiplied by treadmill speed) (Goetz et al. 2012; Thibaudier and Frigon 2014). During experiments, a reflective marker was placed on the left and right greater trochanter. The relative position of the paw at contact and liftoff was measured as the horizontal distance between the hip marker and the front of the right toe at contact and liftoff, respectively. The percentage of steps with improper paw placement at contact and with paw drag was obtained by visual examination of the videos by the same experimenter (Hurteau). Improper placement of the paw at contact was defined as contact that was not plantigrade while paw drag was defined as a touching of the dorsum of the paw with the treadmill belt for more than three consecutive frames at the beginning of the swing phase.

**Electromyography.** The EMG of the anterior sartorius (Srt, hip flexor/knee extensor) and of either the lateral gastrocnemius (LG) (n = 2 cats) or vastus lateralis (VL) (n = 4 cats) was quantified. The EMG burst onsets and offsets were determined by visual inspection by the same experimenter (Hurteau) using custom-made software. Burst
duration was determined from onset to offset while mean EMG amplitude was measured by integrating the rectified waveform from onset to offset and dividing this value by burst duration.

**Statistical Analysis**

Statistical tests were performed with IBM SPSS Statistics 18.0. Wilcoxon signed-rank tests were performed for group data on kinematic durations (cycle, stance, swing), EMG burst durations, mean EMG amplitudes, stride lengths, relative paw positions, improper paw placements, and paw drags. For each parameter, the control value was compared with values obtained with cutaneous stimulation from L2 to L7. In each cat, 15–20 cycles were averaged. These individual means were then averaged for group data. Group data in Figs. 1–6 are means ± SD.

**RESULTS**

**Effect of Mechanical Stimulation of the Lumbar Skin on the Hindlimb Locomotor Pattern of Chronic Spinalized Cats**

Figure 1 shows an example of the EMG pattern of one cat before, during, and after manually pinching the lumbar skin over the L4 vertebra. Manual stimulation stopped hindlimb locomotion and abolished weight support. The limbs remained in flexion, as can be observed by tonic activity in Srt muscles. This effect persisted throughout the manual pinch even though we let the dorsum of the hindpaws make contact with the treadmill surface. Once the stimulation was removed, hindlimb locomotion recovered within a few seconds. A similar effect was observed in all six cats and at all sites (i.e., from L2 to L7) with manual pinching (data not shown).

To assess the effect of a more focalized mechanical stimulation on hindlimb locomotion, the lumbar skin was mechanically pinched with a calibrated bulldog clip. The effects were considerably less pronounced than with manual pinching and showed some variability between animals. Figure 2 shows examples of the hindlimb locomotor pattern of two spinalized cats without (control) and with mechanical stimulation of the skin at L4 during treadmill locomotion at 0.4 m/s. All panels are arranged in the same way with bilateral EMG activity of the Srt and an extensor muscle (LG or VL). Left and right hindlimb stance phases are shown below the EMGs. Mechanical stimulation of the skin at L4 reduced EMG activities of flexors and extensors in both cats, although the reduction was larger in cat BC. All six cats showed reductions in EMG activity, particularly at midlumbar levels. The duration of the stance phases was slightly reduced in cat BC and slightly prolonged in cat SA.

Figure 3 shows the effect of mechanical stimulation from L2 to L7 on cycle and phase durations across cats. Cycle and phase durations are expressed as a percentage of the control episode. Cutaneous stimulation had no significant effect on the duration of the cycle (Fig. 3A) and of the swing phase (Fig. 3C). There was a significant effect of cutaneous stimulation on stance duration at L4 only (Fig. 3B). Thus, small to no changes are observed on cycle and phase durations by localized mechanical stimulation of the lumbar skin.

To determine if cutaneous stimulation of the lumbar skin affected muscle activity, the burst durations and amplitudes of flexor and extensor EMGs of the right hindlimb were quantified. Figure 4 shows mean values ± SD of burst durations and amplitudes for the Srt and extensor muscles of the right hindlimb with cutaneous stimulation from L2 to L7 expressed as a percentage of the control episode across cats. Cutaneous stimulation significantly reduced Srt (Fig. 4A) and extensor (Fig. 4B) burst durations from L2 to L5. The burst amplitude of Srt was significantly reduced with cutaneous stimulation from L2 to L4 (Fig. 4C) while the burst amplitude of extensors was significantly reduced at all sites (Fig. 4D).

As shown in Fig. 3, cutaneous stimulation had little to no effect on kinematic cycle and phase durations. To determine if
cutaneous stimulation of the lumbar skin influenced other kinematic parameters, stride length and the position of the paw relative to hip position at contact and liftoff were measured. Figure 5 shows the stride length and the horizontal distance of the front of the toe relative to the hip marker at contact and liftoff with cutaneous stimulation from L2 to L7 expressed as a percentage of the control episode across cats. Although cutaneous stimulation did not significantly affect stride length at any stimulation site (Fig. 5A), there was a potent effect on the positioning of the paw at contact and liftoff. Cutaneous stimulation significantly decreased the forward placement of the paw at contact (Fig. 5B) while increasing the backward placement of the paw at liftoff (Fig. 5C).

Cutaneous stimulation also affected the way the hindpaw was placed on and lifted off the treadmill surface. The hindpaw could make contact with the treadmill surface that was not plantigrade and the dorsum could drag at liftoff. The percentage of steps with improper nonplantigrade paw placement at contact and paw drag at liftoff was calculated in control and stimulated trials. Cutaneous stimulation significantly increased...
the percentage of steps with improper paw placement at contact with stimulation from L2 to L5 (Fig. 6A) and the percentage of steps with paw drag at liftoff with stimulation from L2 to L7 (Fig. 6B).

DISCUSSION

This is the first study to show the effects of stimulating the lumbar skin during real hindlimb locomotion (as opposed to fictive locomotion) in a chronically spinalized animal. Consistent with our hypothesis, the results show that stimulating the lumbar skin disrupted hindlimb locomotion. While manually pinching the skin completely stopped hindlimb locomotion and abolished weight support, more focalized cutaneous stimulation with a calibrated clip produced less dramatic effects. Specifically, cutaneous stimulation with the clip reduced the EMG activity of hindlimb flexors and extensors and altered paw position at contact and liftoff. Cutaneous stimulation also led to a greater number of steps with improper nonplantigrade placement at contact and paw drag at liftoff. Therefore, cutaneous stimulation of the lumbar skin alters the excitability of spinal circuits involved in generating locomotion and weight support.

Comparison with Other Studies

In chronic (1 mo) spinalized decerebrated cats (Frigon et al. 2012) that were immobilized but not curarized and in decerebrate curarized rabbits (Viala and Buser 1974), manually pinching the lumbar skin completely abolished locomotor-like activity. In the present study, pinching the lumbar skin from L2 to L7 with the index finger and thumb also completely stopped hindlimb locomotion for as long as the stimulation was applied (see Fig. 1). It also abolished weight support, inferred by a reduction in extensor activity (Fig. 4). Thus, manually pinching the lumbar skin is as effective in stopping actual hindlimb
locomotion as it is in immobilized and/or curarized preparations. On the other hand, although mechanical stimulation of the lumbar skin with the bulldog clip, particularly at midlumbar levels, disrupted the hindlimb locomotor pattern, it did not abolish it. The difference between manual pinching and the clip is most likely due to application surface. While the bulldog clip applied a pressure over \( \sim 1 \text{ cm}^2 \), manual pinching performed in this study (around 4 cm\(^2\)), by Frigon and colleagues (2012), and by Viala and Buser (1974) stimulated a much larger cutaneous area. Viala and Buser (1974) showed that reducing stimulation area increased the threshold to abolish locomotor-like activity. Therefore, we propose that the differences in effect between manual pinching of the skin and the use of the clip were primarily due to the larger area of stimulation and the greater number of receptors recruited by the manual stimulation.

**Distributed and Localized Effects**

In the present study, the most consistent effects of cutaneous stimulation were observed on EMG burst amplitudes and durations (Fig. 4), on the relative position of the paw at contact and liftoff (Fig. 5), and on the number of steps showing improper paw placement at contact and paw drag at liftoff (Fig. 6). The most consistent significant effects on these parameters were found with cutaneous stimulation at midlumbar levels, around L4. These results are in agreement with those observed on curarized decerebrate rabbits with or without spinal transection where the most effective site was around L4 (Viala and Buser 1974). In cats, spinal segments L3–L4 are critical rhythmogenic elements of the spinal locomotor network (Marcoux and Rossignol 2000) and are important relay sites for group I and group II inputs from hindlimb muscle afferents (Cavallari et al. 1987; Edgley and Jankowska 1987). In cats, spinal segment L3 is located between the rostral articular processes of L3 and L4 vertebrae while L4 and L5 spinal segments are primarily found between L4 and L5 vertebrae (Elrdige 1984). In the cat, inputs from the skin overlying the L3–L7 vertebrae enter the spinal cord through the T12–L4 dorsal roots (Hekmatpanah 1961). Although there is a rostrocaudal organization, there is substantial overlap between dermatomes. For example, cutaneous inputs from the skin overlying vertebrae L2–L5 can enter the spinal cord via three to four different dorsal roots (Hekmatpanah 1961). Cutaneous receptive fields can also expand following SCI (Andersen et al. 2004; Schouenborg et al. 1992), resulting in greater overlap. As such, it is difficult to ascribe the effects of stimulating a specific cutaneous site on a particular spinal segment.

A previous study proposed that the cessation of fictive locomotor-like activity in decerebrate curarized rabbits was mediated by slow adaptive fibers, since the effect could last for several minutes (Viala and Buser 1974). Viala et al. (1978) attributed this effect specifically to A6 fibers from the dorsal lumbar skin. However, we found that a slight pressure to the lumbar back could reduce weight support in chronic spinalized adult cats (data not shown), indicating that activation of non-nociceptive afferents could also be involved. Another study also showed that gentle pressure to the dorsolumbar skin could inhibit locomotor-like movement in curarized decerebrate rabbits (Viala and Buser 1974). Physical immobility and a lack of responsiveness to external stimulation can be induced by different forms of manual restraint in numerous species, including insects, crustaceans, fish, amphibians, reptiles, birds, rats, rabbits, and primates (Gallup, Jr. 1974). In rabbits, positioning the animal on its back can produce immobilization for several minutes (Viala et al. 1978). In cats, it has been documented that immobilization can be induced by placing clips along the dorsal midline, principally at the cervical level (Pozza et al. 2008). This technique is frequently used in veterinary practice to gently restrain cats, although its efficiency varies across cats (Pozza et al. 2008).

**Functional and Clinical Implications**

What is the functional significance of the pathway from the lumbar skin to spinal networks that generate locomotion and/or weight support? In some mammals, including cats, mothers carry their newborn by gently biting the skin of the neck, and it has been hypothesized that the pathway from the lumbar skin could have a similar function (Pozza et al. 2008). In certain mammals, a pathway from the lumbar skin that depresses the excitability of the spinal locomotor network could be to facilitate mating behavior (Van der Horst and Holstege 1998). However, it could be that mechanical stimulation of the lumbar skin simply elicits a transition to a crouching locomotion, as would be observed when an object contacts the back of the animal. As such, the purpose of this pathway would be to move the body away from the stimulus, similar to the local sign withdrawal first described by Sherrington (1910). Indeed, crouching locomotion is characterized by a more caudal position of the paw at contact and liftoff (Trank et al. 1996), similar to what was found with cutaneous stimulation of the lumbar skin (Fig. 5).

The pathway from the lumbar skin to spinal networks appears to have been conserved in humans (Nadeau et al. 2010). In the clinic, cutaneous stimulation of the lumbar skin...
cooled could be used to reduce rhythmic involuntary muscle spasms in spinal cord-injured subjects, since it has been proposed that rhythmic involuntary muscle spasms, or myoclonus, are produced by spinal pattern generators (Beres-Jones et al. 2003; Brown et al. 1994; Bussel et al. 1988; Calancie 2006), similar to those that generate locomotion. Moreover, it was recently proposed that transcutaneous stimulation of the back could be an effective means of activating the spinal locomotor network (Gorodnichev et al. 2012). As the results of the present study indicate, tonic transcutaneous stimulation of the back in spinal cord-injured patients could activate circuits that depress weight support and locomotor activity, such as those from the lumbar skin. We recommend that studies investigating the effects of transcutaneous stimulation of the lumbar area or those that stimulate mechanoreceptors of the lumbar skin with a contact (e.g., harness or pad) should consider its potential inhibitory effect on locomotor activity and weight support.

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DISCLOSURES

No conflict of interest exists.

AUTHOR CONTRIBUTIONS

Author contributions: M.-F.H. and A.F. conception and design of research; M.-F.H., Y.T., C. Dambreville, C. Desaulniers, and A.F. performed experiments; M.-F.H. analyzed data; M.-F.H., Y.T., and A.F. interpreted results of experiments; M.-F.H. and A.F. prepared figures; M.-F.H. and A.F. drafted manuscript; M.-F.H., Y.T., C. Dambreville, C. Desaulniers, and A.F. edited and revised manuscript; M.-F.H., Y.T., C. Dambreville, C. Desaulniers, and A.F. approved final version of manuscript.

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