Contribution of Cutaneous Inputs From the Hindpaw to the Control of Locomotion. II. Spinal Cats

L.J.G. Bouyer and S. Rossignol
Centre de Recherche en Sciences Neurologiques, Faculté de Médecine, Université de Montréal, Montréal H3C 3J7, Canada

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INTRODUCTION

After spinalization at the last thoracic segment, adult cats can regain a well-organized bilateral locomotor pattern of the hindlimbs after a 2- to 3-wk period of sustained training on a treadmill (Barbeau and Rossignol 1987a; Giuliani and Smith 1987; Lovely et al. 1990; Rossignol et al. 2000, 2002). The kinematics and electromyographic (EMG) pattern of such spinal locomotion on the treadmill. However, sensory inputs also appear necessary for the correct kinematic expression and adaptation to external conditions of this spinal locomotor pattern. Giuliani and Smith (1987) showed that if one hindlimb is deafferented by removing the dorsal root ganglia from L3 to S1–S2, normal spinal walking cannot be expressed by adult cats, even 4 mo after deafferentation. Instead, the deafferented limb steps erratically and makes contact with the dorsum of the paw. This is contrary to mesencephalic cats, which still display organized rhythmic activity after dorsal rhizotomy (Grillner and Zangger 1984). Therefore sensory inputs seem to be important for the functional expression of locomotion after spinalization but such global deafferentation does not give an insight on the respective contribution of the various sensory modalities to locomotion (reviewed in Rossignol 1996; Rossignol et al. 1988).

Proprioceptive inputs appear to play an important role in speed adaptation (reviewed in Grillner 1981; Rossignol 1996; Rossignol et al. 2000). With increasing belt speed, the cadence of locomotion increases and the cycle duration shortens. If the speed is increased sufficiently, interlimb coordination can even switch from alternating (walk) to in-phase coupling (gallop) (Forssberg et al. 1980b). Spinal cats can even adapt to differential speeds of separate treadmill belts (Forssberg et al. 1980b). It was also shown that manipulating the hip angle could exert potent effect on spinal walking such that hip flexion stopped locomotion whereas hip extension induced locomotion (Grillner and Rossignol 1978).

Studies involving perturbations of locomotion have also shown that cutaneous inputs play a role in adapting locomotion to perturbations. For example, if an object is placed in the path of the swing limb during treadmill walking, after the paw hits this object, a well-coordinated reaction will bring the foot around and above the object, in a stereotyped manner (stum-
bbling reaction; Forssberg 1979; Forssberg et al. 1975). Very little is known on how spinal locomotion is affected after cutaneous inputs have been removed. A few studies using neuromectomies or partial anesthesia of the hindlimb paw have suggested that the gross alternating rhythmic pattern persists after cutaneous feedback has been reduced or removed. Sherrington axotomized skin nerves in spinal cats and still observed some rhythmic activity of the hindlimbs (Sherrington 1910).

Our companion study on the other hand has shown that in intact cats, removal of all cutaneous inputs from the hindpaws on both sides results in well-identified but minor changes in the locomotor pattern. Is this the same for spinal cats? The present experiments were carried out to address 3 general objectives: 1) to characterize the specific role of distal cutaneous afferents in the expression and control of locomotion in cats spinalized at T13; 2) to define the adaptive capacity of the spinal locomotor system after a reduction in cutaneous feedback; 3) to determine whether compensatory mechanisms observed in intact cats remain after spinalization of cats denervated before spinalization [the ones reported in the companion study (Bouyer and Rossignol 2003a)]. Skin inputs from the hindlimb paw were removed by cutting several or all cutaneous nerves at ankle level, before or after spinalization, and the chronically implanted cats were recorded for several months. It will be shown that cutaneous afferents from the paws are essential for proper foot placement during spinal locomotion and also that the spinal cord shows adaptive capacities after partial cutaneous denervation.


M E T H O D S

Animals and general protocol

Four cats of either sex (3.7–4.5 kg) were used in this study for a total of 78 recording sessions. The experimental protocols used in this study were in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. For all surgical procedures performed in aseptic conditions, cats were premedicated [atratov 0.1 mg/kg subcutaneously (sc), glycopyrrolate 0.01 mg/kg sc, and ketamine 5–10 mg/kg, sc] and then intubated. General anesthesia was induced and maintained using isoflurane (1–2.5%).

Figure 1 summarizes the sequence of interventions for each cat. Two main protocols were used in this study. In the first protocol, 3 cats (“Denervated-Spinalized” DS1, DS2, and DS3) were denervated bilaterally in the intact state and allowed to recover for several weeks until they could walk on a horizontal ladder before they were spinalized. The prespinalization data of these 3 cats are reported in the companion study (Bouyer and Rossignol 2003a). They allowed a pre- and postspinalization comparison of the kinematics and EMGs in the same animals, with the same EMG recording electrodes. In the 2nd protocol, one cat was first spinalized, trained to walk on the treadmill until it reached a steady locomotor pattern, and then gradually denervated on the right side only (Spinalized-Denervated SD1). The gradual denervation consisted of cutting the 5 hindpaw cutaneous nerves on separate occasions during brief anesthesia (see following text). Neuromectomies were separated by several days to several weeks and treadmill training was provided daily to maximize recovery each time. Once a steady locomotor performance was reached after a neuromectomy, we proceeded to the next neuromectomy. Two or more recording sessions were made between neuromectomies.

Implantation of electromyographic electrodes

EMG electrodes were implanted chronically in selected muscles of the hindlimbs. Implantations were not identical for all cats. DS1 and DS2 were implanted with an emphasis on extensors, DS3 on ankle flexors, and SD1 was balanced (4 flexors and 3 extensors per leg). The muscles implanted and their main function were: sartorius anterior (Srt, hip flexor/knee extensor), semitendinosus (St, knee flexor/hip extensor), vastus lateralis (VL, knee extensor), tibialis anterior (TA, ankle flexor), extensor digitorum longus (EDL, ankle flexor), medial gastrocnemius (GM, ankle extensor), lateral gastrocnemius (GL, ankle extensor), soleus (ankle extensor), and extensor digitorum brevis (EDB, toe flexor). For each muscle, a pair of Teflon-insulated multi-strain fine wires (50 μm diameter, AS633; Cooner Wire, Chatsworth, CA) was led subcutaneously from head-mounted multipin connectors (CINCH Connectors, TTI, Pointe-Claire, Canada) and sown into the belly of the muscle for bipolar EMG recordings. All cats were implanted more than 6 wk before spinalization.

Spinalization

Cats DS1, DS2, and DS3 were spinalized 28, 76, and 55 days after the denervation, respectively, whereas cat SD1 was spinalized before being gradually denervated (Fig. 1). In all cats, the spinal cord was exposed at the level of the 13th thoracic vertebra by performing a
small laminectomy. The articular processes were spared to maintain the lateral rigidity of the spinal column. The dura was cut open and the spinal cord completely transected after local lidocaine application (Xylocaine, 2%). Hemostatic material (Surgicel) was then gently inserted inside the gap and the muscles and skin were sown back in successive layers to close the opening. Further details can be found elsewhere (Bélanger et al. 1996). Treadmill training started no earlier than 3 days after surgery.

**Analgesia**

During surgery, a fentanyl transdermal patch (Duragesic 25, Alza) was sutured to an area of shaved skin on the back of the cat. This patch slowly but continuously released fentanyl at a rate of 25 μg/h for a period of 72 h. When required, additional buprenorphine HCI (maximum of 0.01 mg/kg) was given intravenously (iv) every 6–8 h for 1–2 days.

**Cutaneous denervations**

The denervation protocol was explained in detail in the companion study (Bouyer and Rossignol 2003a). Briefly, the hindpaw was innervated by 5 cutaneous nerves. For cats DS1–DS3, these nerves were cut in both hindlimbs before spinalization, at the level of the ankle, and under general anesthesia. Their proximal end was embedded in a polymer cuff to prevent regrowth. For cat DS2, only partially denervated before spinalization (cf. companion study), the cutaneous branch of the deep peroneal nerve was cut 141 days after spinalization to complete the bilateral denervation. Daily treadmill training was continued for 20 days after this procedure. For cat SD1, cutaneous nerves were cut after spinalization. The denervation was performed in the right hindlimb only. In addition, the nerves were cut one at a time, and treadmill training was provided daily between successive neuroectomies. This protocol allowed for a progressive cutaneous denervation and provided time for compensation. The sequence of neuroectomies, the abbreviated name for each nerve, and the time allowed for compensation were as follows: 1) 40 days postspinalization: cutaneous branch of deep peroneal n. (DPc: compensation: 6 days); 2) 46 days postspinalization: saphenous n. (Sph: compensation: 15 days); 3) 61 days postspinalization: caudal cutaneous sural n. (CCS; compensation: 7 days); 4) 68 days postspinalization: superficial peroneal n. (SP; compensation: 42 days); 5) 110 days postspinalization: tibial n. (Tib; compensation: 71 days). Recordings were made no earlier than 6 h after a neuroectomy.

**Treadmill training after spinalization**

This method was presented in detail in previous publications (Barbeau and Rossignol 1987a; Bélanger et al. 1996). Briefly, the forelimbs of the cats were placed on a platform located over the front part of the treadmill, whereas hindquarters were placed over the moving belt. Training consisted of having the experimenter holding the hindquarters of the animal over a motorized treadmill belt and gently stimulating the perineum to evoke stepping movements. Cats DS1–DS3 were trained once or twice a day, 7 days a week, ≥15 min per session, for a minimum of 35 days. For cat SD1, control data collection began 26 days after spinalization, once the animal had reached a steady locomotor pattern with independent hindquarters weight support and plantar foot placement and no longer required perineal stimulation to express this complete walking pattern (Barbeau and Rossignol 1987a; Bélanger et al. 1996). After the onset of the progressive denervation, cat SD1 was trained according to the same schedule as cats DS1–DS3.

**EMG and movement recordings**

An experimental session consisted in recording the movements of the hindlimbs and associated EMG activity. Ankle flexors could not be recorded from cats DS1 or DS2 because signals from the chronically implanted electrodes in TA stopped providing reliable signals before spinalization. Reflective markers were placed over the iliac crest, greater trochanter, lateral epicondyle, lateral malleolus, metatarsophalangeal joint (MTP), and the tip of the 4th toe of the left hindlimb, for cats DS1–DS3, or right hindlimb for cat SD1. Video recordings of the walking cat were obtained at the same time as EMG activity and synchronization between the 2 was provided through an SMPTE time code generator (Skotell).

**Terminal experiment**

At the end of the chronic recording period, cats were prepared for a terminal experiment where their locomotor pattern, expressed in the absence of phasic sensory feedback (“fictive” locomotor pattern; Grillner and Zangger 1979), was recorded. The detailed methodology of this procedure was previously described elsewhere (Pearson and Rossignol 1991) and is only briefly summarized here. Under halothane gas anesthesia, the cat is installed into a stereotactic frame. The left and right popliteal fossae are opened, nerves from Sr, St, TA, and LGS are separated from surrounding tissue, cut, and placed into monopolar cuff electrodes. The opening is then closed with staples and the legs are left hanging. Decerebration is performed by transecting the brain stem just above the superior colliculus at a 50° angle. Gas anesthesia is then discontinued and the animal paralyzed (Flaxedil, initially 10 mg/kg and supplemented as required) and artificially ventilated for the rest of the experiment. Electroneurograms (ENG) were recorded (band-pass 1–10 kHz) using custom software.

In chronic spinal cats, spontaneous fictive locomotion is more frequent than in decerebrate cats (Pearson and Rossignol 1991); when spontaneous activity was not present, however, locomotion was triggered by injecting nialamide (50 mg/kg) and L-dihydroxyphenylalanine (L-DOPA; 80 mg/kg). In addition, clonidine was sometimes given [100 to 500 μg/kg (maximum), iv] to obtain good rhythmic activity. At the end of the experiment, cats were killed by a barbiturate overdose (Somnotol, 50–65 mg/kg). Cat DS1 unfortunately died soon after decerebration.

**Analyses**

**KINEMATIC ANALYSIS.** Joint angles and foot lifts/contacts were reconstructed off-line frame-by-frame from the video images using a Peak Performance motion analysis system (Englewood, CA) with a resolution of 60 fields/s. The knee angle was mathematically corrected for skin slippage by triangulation using postmortem leg segment lengths (femur andibia). The step cycle was divided into 4 phases (Philippson 1905): the swing phase was subdivided into flexion (F) and the first extension phase (E1), whereas the stance phase was divided into 2 extension phases, weight acceptance (E2) and push off (E3).

To quantify the modifications that occurred in the trajectory of the hindpaw during locomotion, horizontal paw position relative to the hip was measured from the video images at toe off (Fig. 2: l1) and at foot contact (l2) for each step in a walking session. The distance between l1 and l2 represents stride length. In addition, paw clearance was evaluated by measuring the peak vertical position of the MTP joint during the swing phase (Fig. 2E).

For cat SD1, the efficiency of the swing movement at lifting the paw off the treadmill belt was measured using a frame-by-frame video analysis of the spinal walking sessions. For each swing phase in a given session, the percentage of time the paw was in the air was measured and divided by total swing duration. This measure was called %lift (cf. Fig. 9A), which is 100% for intact cats and 0% for completely denervated spinal cats when the paw does not leave the surface of the treadmill.

EMG ANALYSIS. Electromyographic data recorded on tape were played back off-line and printed out using an electrostatic plotter.
(Gould ES-2000), along with their SMPTE time stamp. Data segments consisting of ≥10 consecutive steps at constant speed were chosen using the EMG printouts and videotapes of the experiments. EMG data of these chosen segments were then digitized using custom software at 1,000 samples/s per channel on a 200-MHz Pentium computer. Burst duration, amplitude, and relative timing of chosen data segments were measured by manually placing cursors using custom analysis software. To compare EMG patterns between sessions (e.g., Fig 4), EMGs were rectified, normalized to 256 points per step, and averaged over ≥10 consecutive steps. To quantify changes in EMG activity in each muscle, the area under each rectified EMG burst was divided by its duration, a measure called mean amplitude.

Electroneurogram (ENG) Analysis. Electroneurographic data obtained during the terminal experiments, were played back off-line and printed out using an electrostatic plotter (Gould ES-2000), along with their SMPTE time stamp. Data segments where several nerves were regularly active over a number of consecutive steps were selected and digitized at 2,000 samples/s per channel on a 200-MHz Pentium computer using custom software. A snapshot of one of these data segments is presented in Fig. 6. Because fictive locomotion ENGs represent the output of the locomotor system in the absence of phasic sensory feedback, comparing the fictive ENG patterns to EMG patterns obtained late after the denervation (e.g., Fig. 4) allowed us to better identify the central and peripheral contributions to the locomotor pattern after denervation.

Statistics

Differences between pre- and postdenervation data were evaluated using a one-way ANOVA (Glantz 1992) using SigmaStat statistics software (Jandel Scientific). Data from ≥3 preneurectomy sessions were pooled and compared with each postdenervation session. For normal and equivariant data, parametric ANOVA was used, followed by Dunnett’s post hoc comparison against a single control (the pooled preneurectomy data). If data failed the normality or equivariance tests, the nonparametric Kruskal–Wallis one-way ANOVA on ranks was used instead, followed by multiple comparisons versus a control group using Dunn’s method (Glantz 1992).

RESULTS

This section is divided into 3 parts so as to present the results according to the order of denervation/spinalization and the extent of the cutaneous denervation. For comparison with other studies, it should be recalled that cats with normal sensory feedback recover locomotion with plantar foot placement and hindquarters weight support in 14–21 days after spinalization when daily treadmill training is provided (Barbeau and Rossignol 1987a; Bélanger et al. 1996).

Spinalization after a complete cutaneous denervation

Figure 2 compares the kinematics of locomotion in a completely denervated cat before and after spinalization. Although only modest changes in kinematics were observed after the neurectomy in the intact state (Fig. 2A and companion study, Bouyer and Rossignol 2003a), the same cat presented an important and permanent locomotor deficit after being spinalized. This deficit was mainly characterized by the inability to lift the foot during the swing phase, to place the foot on the plantar surface during stance, and to fully support the weight of the hindquarters. Instead, the cat dragged the dorsum of the foot across the treadmill belt during the whole swing phase (Fig. 2B) and the paw remained plantarflexed throughout stance. Partial weight support was possible after spinalization, but even with vigorous perineal stimulation, complete hindquarters weight bearing was never achieved even after 35 days (cat DS1) or 56 days (cat DS3) of treadmill training. Figure 2C–E compare the kinematics of locomotion of denervated cat DS1 before and after spinalization. Figure 2C shows that the step cycle duration at 0.2 m/s was reduced by 55% of its prespinalization value (1,241 ± 100 ms control; 679 ± 162 ms, 10 days postspinalization). Stance duration was proportionally more affected than swing. Figure 2D shows that stride length was...
substantially reduced (distance between $l_1$ and $l_2$; cf. METHODS), the paw remaining behind the hip joint even at foot contact ($l_1$). Finally, Fig. 2E shows that the paw did not clear the treadmill belt during swing, but was rather dragged across on every step, whereas before spinalization the MTP joint reached about 2 cm above the belt.

Figure 3A is a stick figure reconstruction of a complete step 30 days after spinalization. Although the paw was dragged on the surface and never placed rostral to the hip joint, the leg was nevertheless actively moved forward during the swing phase, and back during stance. Average rectified EMG activity from several hindlimb muscles obtained from the same walking session (Fig. 3B) shows that a well-defined phasic bursting was present. The knee flexor semitendinosus (St), knee extensor vastus lateralis (VL), and ankle extensor soleus (Sol) expressed the timing of activation typical of spinal cats with normal sensory feedback (Barbeau and Rossignol 1987a; Bélanger et al. 1996).

Recordings from several distal muscles in cat DS3 (Fig. 4) show that the paw drag deficit was accompanied by synchronous muscle activation across the ankle joint (iEDL, iGM, and iGL), not typically observed 51 days postsurgical in cats with normal sensory feedback (Barbeau and Rossignol 1987a; Bélanger et al. 1996). Figure 4A shows the locomotor pattern of cat DS3 before spinalization, 41 days after the complete cutaneous denervation. One can see the orderly activation sequence during the swing phase of the knee flexor St, followed by ankle flexor EDL, and toe flexor EDB. These muscles then pause during extension, where knee (VL) and ankle (GL, GM) extensors are active. Such a locomotor synergy is similar to predenervation; only amplitude changes are seen in knee and ankle flexors after denervation (cf. companion study). After spinalization (Fig. 4B), IS becomes activated later in the step cycle, as previously reported in cats with normal sensory feedback (Bélanger et al. 1996). This shift in knee flexor activity probably contributes to toe drag at the beginning of swing (Bélanger et al. 1996), but does not prevent spinal cats with normal sensory feedback from lifting the paw during swing or from properly placing the plantar surface of the paw in contact with the treadmill during stance. However, additional changes are present in the EMG pattern of the denervated spinal cat. A synchronous activation of ankle flexor EDL with ankle extensors GL and GM is observed during stance (Fig. 4B). This coactivation is not present in spinal cats with normal sensory feedback (Bélanger et al. 1996). Such a phase shift in ankle flexor activity influences the control of locomotion during both swing and stance. Coactivation remained even after more than 50 days of treadmill training in the spinal state.

Results from cat SD1 after the complete unilateral denervation (see Spinalization followed by a gradual cutaneous denervation of one hindpaw below) present similar changes in ankle flexor activity as cat DS3, suggesting that this abnormal muscle pattern is a general phenomenon after a complete cutaneous denervation and spinalization.

To see whether a more normal activation could be reinstated using pharmacological stimulation, clonidine, an $\alpha_2$-noradrenergic agonist, that normally improves the locomotor pattern in spinal cats (Barbeau et al. 1987b) was administered to cat DS3, 8 days (not shown) and 48 days after spinalization (Fig. 5). The stick figure reconstruction in Fig. 5E shows that clonidine allowed the foot to clear the ground at the end of swing. Furthermore, the swing movement amplitude was greatly increased as compared with Fig. 5B. However, the foot landed on the tip of the toes and stance was performed in this posture (Fig. 5F), which was different from predrug (Fig. 5C), yet not as good as in cats with no denervation (e.g., Fig. 9A, last drawing on the right). The raw EMG traces in Fig. 5D show that ankle antagonists coactivation remained.

To look at this pattern in more detail, foot lift and contact times were extracted from the video recordings and combined with the EMG data concurrently obtained (cf. DUTY; Fig. 5G, bottom). Small-amplitude bursts were sometimes seen in TA, EDL, or EDB during the swing phase with close examination of individual cycles and these are reflected as small deflections in the relevant EMG traces.

To see whether the long-term expression of a locomotor pattern showing a cocontraction of ankle flexors and extensors would be reflected in the fictive locomotor pattern obtained in

![Figure 3](http://jn.physiology.org/DownloadedFromHttp://Jn.Phpysiology.org/By10.22032247/onNovember3,2016)
the absence of phasic sensory feedback (CPG), a terminal “fictive locomotion” experiment was performed (see METHODS). The fictive pattern of cat DS3 is presented in Fig. 6. Note that activity in the LGS (ankle extensor) nerve is now out of phase with the one from the TA (ankle flexor) nerve. Furthermore, hip (Srt), knee (St), and ankle (TA) flexors discharged synchronously, which is typical of DOPA-induced fictive locomotion in normal cats. Therefore the fictive locomotor pattern of this denervated spinal cat shows temporal characteristics similar to nondenervated spinal cats (Pearson and Rossignol 1991), including an alternated activation between ankle flexors and extensors. This is in contrast to the spinal EMG locomotor pattern observed during actual treadmill walking in the same cat (Figs. 4 and 5) where a clear cocontraction of ankle flexors and extensors was consistently present for the several weeks preceding this terminal experiment.

**Spinalization after a partial cutaneous denervation**

In cat DS2, the somesthesic denervation performed before spinalization was incomplete. Figure 7A shows the outline of the cutaneous receptive field preserved by sparing the DP nerve bilaterally, as evaluated by a meticulous pinching of the skin with small forceps. Although mild responses were elicited by some small areas on the side of the paw, by far the areas of greater sensitivity corresponded to toes II and III.

Contrary to completely denervated cats, DS2 eventually recovered plantigrade locomotion and weight support after spinalization. DS2 expressed plantigrade locomotion and weight support present 98 days after spinalization. It must be noted that perineal stimulation was necessary to evoke stepping even this late postspinalization. This is different from non-denervated spinal cats, which would sustain their own locomotion even this late postspinalization. Figure 7E shows the plantar paw placement and weight support present 98 days after spinalization. It must be noted that perineal stimulation was necessary to evoke stepping even this late postspinalization. This is different from non-denervated spinal cats, which would sustain their own locomotion even this late postspinalization.

Local anesthetic (Xylocaine 2%) was then injected under the skin between toes 2 and 3 to temporarily inactivate the remaining cutaneous inputs on both sides. Effectiveness of the anesthesia was verified by pinching the skin with a pair of small forceps. Response to pinching in flexor EMG was completely blocked for more than 20 min. When the cat was put on the treadmill after such restricted anesthesia, plantar foot placement and weight bearing were immediately lost. The paws were dragged across the treadmill during swing and stance (Fig. 8B). The kinematics of the locomotor pattern observed after local anesthesia was very similar to that present in completely denervated animals (cf. Fig. 3A). Figure 8D shows average angular displacements pre- and postlocal anesthesia. Cycle duration was not normalized so as to emphasize the differences between the 2 situations. Although flexion at the hip, knee, and ankle were triggered synchronously, the switch to extension at the knee and ankle was normally associated with the second half of hip flexion (E1 phase; Philippson 1905) was missing after anesthesia. Plantar foot placement and weight bearing returned as soon as the effects of the local anesthetic wore off (not shown). Unilateral anesthesia was also tested and resulted in a unilateral deficit that was very similar (not shown).

The cutaneous branch of the DP nerve was then cut bilaterally 141 days postspinalization in this cat. This neurlectomy resulted in a loss of plantar foot placement and weight bearing. No recovery occurred over the following 20 days, despite daily treadmill training (Fig. 8C). Overall, local anesthesia and completion of the denervation produced the same kinematic deficit (Fig. 8E). The cat’s posture was not identical postanesthesia and postdenervation (Fig. 8, B and C) simply because the experimenter could not maintain the animal at exactly the same height over the treadmill belt during these 2 recording sessions. This small disparity explains the differences in angular position for the hip, knee, and ankle (Fig. 8E), but is of no functional consequence because similar deficits were present in both situations.

Figure 8, F–H summarize quantitatively some of the kinematic changes that occurred after anesthesia/complete denervation. In addition to causing foot drag, dorsal foot placement, and reduced weight bearing capacity, it can be seen that removing skin inputs (temporarily or permanently) substantially reduced step cycle duration.

**Spinalization followed by a gradual cutaneous denervation of one hindpaw**

Seven days after spinalization, cat SD1 (no cutaneous denervation at this time) started to express plantar foot placement during locomotion. Three weeks after spinalization, it walked
Cat DS3, Pre-drug

40 min post-clonidine 100 μg/kg i.p.
steady at a treadmill speed of 0.4 m/s with full hindquarters weight support without the need of perineal stimulation.

The cutaneous nerves of the right hindpaw were then sequentially cut (cf. Fig. 1). As an index of recovery of locomotion, the percentage of lift (the inverse of drag) during the swing phase was measured. The %lift (cf. Methods) was measured in several walking sessions before and after each denervation and is plotted in Fig. 9B. A 100% lift during swing means that the foot is lifted throughout the whole swing phase, whereas a 40% lift means that the foot drags on the surface of the treadmill for 60% of the swing phase.

Before denervation, cat SD1 had on average a 71 ± 19% lift. This value is similar to that of other nondenervated spinal cats (Bélanger et al. 1996). The gradual denervation began by cutting the cutaneous branch of the DP nerve, to serve as a control for cat DS2 that could walk in the spinal state with only this source of cutaneous input. As can be seen in Fig. 9B, this had little consequence on the degree of foot drag. Saphenous and caudal cutaneous sural, the nerves that have receptive fields on the medial and lateral aspects of the paw, respectively, were then sectioned. No significant decrease in %lift was observed after cutting these 3 cutaneous nerves.

Two cutaneous nerves were left at this time: SP and Tib. Temporarily blocking either of these nerves using local anesthetics produced the same deficit; paw drag during swing and stance, and no hindquarters weight bearing. This finding suggests that both of these nerves were important for locomotion at this stage of the denervation. The SP nerve was cut next. As suggested by the local anesthesia, cutting this nerve decreased the %lift (i.e., increased paw drag). Notwithstanding the fact that the paw dorsum was now insensitive, compensation nevertheless occurred rapidly, reaching a steady state after 8 days of locomotor training. Despite a return of planter paw placement during stance, the %lift after compensation remained statistically lower than pre-SP neurectomy. The remaining cutaneous nerve, Tib, was then cut, 42 days after SP. Planter paw placement was lost and never recovered despite another 71 days of treadmill training and having intact sensory feedback from the contralateral leg.

EMG patterns before and after SP neurectomy are presented in Fig. 10. To allow for direct comparison, the vertical scale for any given muscle is constant across conditions. Before SP denervation (Fig. 10A), right VL burst duration spans about 50% of the step cycle, as would be expected during symmetric alternating gate. St, St, and EDL have a single burst of activity, during the period where VL is silent.

By 24 h after cutting SP, the paw was dragged during swing and plantarflexed during stance. Frame-by-frame video analysis reveals that the swing phase of the denervated leg was composed of 2 rapid forward limb movements for each stance of the intact leg. This 2:1 movement was reflected in the EMG pattern (Fig. 10B). For every burst of VL in the intact left leg (coVL), there were 2 bursts in St, St, and EDL. In addition, the timing between flexor bursts was modified, with St, EDL, and EDB nearly in phase and >20% lagging St. Finally, left VL amplitude was higher, indicative of increased weight bearing by the intact leg.

With training, compensation occurred, paw placement and weight bearing returned, and the 2:1 movement disappeared. Thirty-four days post SP neurectomy, burst duration and timing returned toward preneurectomy values, for most of the recorded muscles. St amplitude was significantly diminished, however (cf. Fig. 10E, arrow).

One day after cutting Tib, the last ipsilateral cutaneous nerve (Fig. 10D), paw placement was permanently lost, but the 2:1 swing movement was not observed. The EMG pattern was now similar to that of cats completely denervated before spinalization (cf. Fig. 4), with distal flexors and extensors being coactivated during stance.

Considering all 4 locomotor EMG patterns, it is interesting to note that the 2:1 swing pattern present after cutting SP (Fig. 10B) seems to be an intermediate between the predenervation (Fig. 10A) and postcomplete denervation (Fig. 10D) EMG patterns.

In addition, this figure suggests that contrary to other muscles, St activity was reduced after SP cut and did not recover.

To look into more details at the effects of the progressive denervation on St activity, Fig. 10E presents the time course of changes in St burst amplitude, duration, and timing as a function of time after spinalization. The timing is expressed relative to the end of the intact leg VL burst. For the 2:1 swing pattern, only values for the first burst of activity are shown. In general, it can be seen that, as for paw drag, no major changes were observed until SP was cut (cf. arrow). After SP neurectomy, however, St amplitude was significantly reduced and did not recover. St burst duration was increased once the cutaneous denervation was complete. The timing of the St burst was not changed, except for the 1st session post SP neurectomy (cf. Fig. 10B). The reduction in burst amplitude accompanied by an increase in burst duration shown in Fig. 10D was not attributed to the averaging on VL, given that the burst by burst amplitude measurement presented in Fig. 10E showed a similar result.
DISCUSSION

The first main original finding of this study is that in the complete absence of cutaneous inputs from the hindlimb paw, spinal cats cannot place the foot on the plantar surface or bear weight during spinal locomotion, even after months of treadmill training. Therefore cutaneous inputs from the hindlimb paws seem to be crucially important for the proper expression of locomotion after spinal transection. The second main finding is that in the spinal state, cats can adapt their hindlimb locomotion to progressive lesions of cutaneous afferents, thus suggesting the presence of some intrinsic mechanisms of spinal plasticity independent of supraspinal structures. These 2 interrelated findings will be discussed.

The importance of cutaneous inputs for spinal locomotion

The observation that spinal cats cannot place the foot properly on the plantar surface and adequately support the weight of the hindquarters raises a number of issues, some of which are methodological. For instance, the complete denervation protocol required the cutting of the tibial nerve at ankle level, the only mixed nerve that was axotomized as part of our protocol, carrying axons to several intrinsic foot muscles (Engberg 1964). However, the deficit in spinal walking was not the result of a motor paralysis caused by this denervation because the same cats could properly place the foot before they were spinalized (cf. companion study Bouyer and Rossignol 2003a). Obviously, if important muscles had been denervated by the neurectomy, one could have expected a permanent deficit before and after spinalization. Furthermore, cat DS2, which only had DP intact and not Tib, could nevertheless express plantar foot placement even after spinalization. Therefore intrinsic foot muscles innervated by the tibial nerve (or their afferent feedback) are not essential for plantar foot placement during level walking on a treadmill, in the normal or in the spinal cat. Our results suggest that the walking deficit seen after the denervation was genuinely related to the missing cutaneous inputs.

In cat DS2, a small fraction of the normal cutaneous input was left by sparing the DP nerve (Fig. 7). After spinalization, DS2 gradually recovered plantar foot placement and weight bearing, contrary to the completely denervated cats. The fact that DS2 could express locomotion with drastically reduced hindpaw cutaneous inputs shows that only a small amount of cutaneous input is required for cats to express a functionally useful locomotor movement after spinalization. However, cat DS2 presented some locomotor deficits compared with spinal cats with intact sensory feedback, such as a premature onset of swing (E3; Philippson 1905). However, these deficits were minor compared with what was seen in completely denervated cats.

The immediate and reversible loss of paw placement on injection of local anesthetics into the remaining cutaneous receptive fields of DS2 further shows that cutaneous feedback is necessary not only to initially express, but also to later

FIG. 7. A: receptive field of remaining cutaneous sensitivity in cat DS2, 98 days postspinalization, as evaluated using systematic pinching of skin with small forceps. B and D: raw EMG activity of several hindlimb muscles during treadmill walking at 0.2 m/s, early (3 days) and late (98 days) after spinalization, respectively. C and E: corresponding stick figure reconstructions of one step cycle.
maintain the locomotor pattern. Results from SD1 confirmed this finding because this cat lost plantigrade locomotion even if the unilateral denervation was progressive and performed after spinal locomotion had first been expressed with normal sensory feedback.

Cat SD1 also showed that the cutaneous branch of DP was not essential as such for paw placement during spinal locomotion, given that cutting this nerve before the other nerves did not statistically increase paw drag (Fig. 9) or change the spinal EMG locomotor pattern (Fig. 10). Thus some cutaneous information is necessary for paw placement, but it can also originate from the tibial nerve, provided the cat is trained on the treadmill for a few days. Further experiments will be required to know whether the other 3 cutaneous nerves, with receptive fields that do not contact the ground, could also provide adequate feedback for walking.

Comparison with other denervation models

The cutaneous denervation model is rather unique because it removes specifically a sensory modality from the foot. It is of interest, however, to compare these findings with those of other models of neurectomies involving muscle nerves that not only remove some specific proprioceptive inputs from these muscles but also permanently remove the muscles from the locomotor pattern.

Carrier et al. (1997) studied the effects of cutting the TA and EDL muscle nerves in one leg on the control of locomotion. These investigators showed that the order of denervation and spinalization could produce very different modifications in the chronic spinal locomotor pattern after such neurectomy. When the neurectomy was performed in the otherwise intact cat, there were few changes in the locomotor pattern except a consistent small increase in knee and hip flexors. However, the locomotor pattern exhibited after spinalization was very asymmetrical and made of abnormally large knee flexions and repetitive banging of the foot on the treadmill. Yet, when the same neurectomy was performed in chronic spinal cats, no erratic movements were observed. Instead, the cat walked with a reduced flexion at the ankle. Carrier et al. (1997) interpreted these results as an indication that when the CNS is intact, compensatory changes after a muscle nerve axotomy are distributed at the level of the lumbar spinal cord as well as at other, more rostral levels of the CNS. By performing the spinalization after compensation had occurred, only spinal components of this distributed adaptive plasticity were expressed, giving rise to an erratic walking pattern presumably because the dynamic interaction between spinal and supraspinal mechanisms normally needed for functional compensation was now missing.

The first 3 cats used in the present study were also denervated before spinalization. Is it possible that the lack of paw placement and weight support as well as the abnormal cocontraction of ankle muscles observed constituted the equivalent of the erratic pattern found after a muscle nerve axotomy? To
answer this question, a 4th cat (SD1) was first spinalized and then gradually denervated. Contrary to the results obtained after ankle flexors neurectomies (Carrier et al. 1997), cat SD1 exhibited the same locomotor deficit once completely denervated as the cats that were completely denervated before spinalization. Therefore the deficit in weight bearing and paw placement appears mainly related to the removal of cutaneous inputs and not to the expression of spinal compensatory changes that occurred before spinalization. After complete denervation, other compensatory changes not detected in the present experiments (i.e., change in the gain of proprioceptive reflex pathways) may have occurred and will be studied in other experiments. This is very likely, given that the present experiments have also shown some evidence of spinal plasticity.

The adaptive capacity in the spinal cat

Over the last decade, there has been a growing body of evidence in the literature suggesting that the adult spinal cord is capable of adaptive plasticity (cf. Patterson and Grau 2001). This includes the locomotor system, which appears less hard-wired than previously thought based on muscle tendon and nerve transposition studies (reviewed in Bouyer and Rossignol 2001).

In some models of spinal plasticity, such as operant conditioning of the H-reflex, the modification of spinal synapses requires the integrity of certain descending pathways, such as the corticospinal tract (Chen and Wolpaw 1997). For locomotion, however, supraspinal pathways may not always be required. In a recent study, we showed that chronic spinal cats can rapidly compensate their locomotion after the nerve to the lateral gastrocnemius and soleus muscles has been cut (Bouyer et al. 2001). These results suggest that, at least for proprioceptive inputs, spinal locomotor networks can implement adaptive changes in the absence of supraspinal inputs.

Results from cat SD1 suggest that locomotor plasticity after spinalization also extends to the cutaneous system. The complex protocol used to study this cat allows us to discuss several aspects of the adaptive capacity to a reduction in cutaneous feedback. First, the gradual unilateral denervation in this chronic spinal cat suggests that a substantial reduction in skin feedback is necessary before deficits in paw placement become visible. Cutting DPc, Saph, and CCS nerves removed feedback from the medial 2 digits, and the medial and lateral surfaces of the hindpaw from the ankle to the MTP joint. Nevertheless, this large reduction in feedback did not produce statistical changes in paw drag, as meticulously measured using frame-by-frame video analysis (Fig. 9B). Only after SP was also removed, with a receptive field covering the dorsum of the paw, did we see paw drag appear. Second, spinal adaptive mechanisms can solve complex situations. After SP was cut, the paw was dragged on its dorsal side, which had just been denervated by this last neurectomy. During walking with the paw drag, the spinal locomotor system received only indirect cutaneous feedback from the tibial nerve. Intrinsic mechanisms could nevertheless use this faint and not necessarily
appropriately phased information to produce compensation within 8 days, with only 15 min of locomotor training per day. Third, although effective, the adaptive capacity of the spinal locomotor network to a reduction in cutaneous feedback is limited. Once the last cutaneous nerve was cut, plantigrade walking and weight support in that limb were lost for as long as we kept the animal, despite daily treadmill training. Even if the denervation was gradual, spread out over 70 days, and interleaved with regular training, the chronic spinal cat did not "learn" to use proprioceptive inputs from the denervated leg, or any inputs from the contralateral leg to compensate the paw drag. However, the remarkable finding is that even after spinalization, the cat was capable of adapting its locomotor output to a sensory damage up to that ultimate stage.

Mechanisms for compensation

CLONIDINE. One potential mechanism that could have accounted for the deficit in spinal walking of the completely denervated cats is a nonspecific reduction in spinal cord excitability caused simply by removing a very large amount of feedback, leading to a lack of recruitment of distal muscles. This possibility was ruled out by administering clonidine to the spinal cats. Clonidine is an adrenergic alpha-2 agonist known to improve spinal locomotion and to have a preferential activation effect on flexor muscles (Barbeau et al. 1987b). Although this drug increased hip and knee flexion, thereby allowing the foot to clear the ground during swing, it did not lead to plantar paw placement during stance (Fig. 5, E and F) or larger ankle flexor activation during swing (Fig. 5G). Therefore when present, cutaneous inputs from the paw do more than simply provide a nonspecific increase in spinal cord excitability. They are likely involved in properly timing the activation of ankle flexors.

CENTRAL PATTERN GENERATOR (CPG). Changes in the CPG pattern are at the moment very unlikely, given that the fictive locomotor pattern of denervated spinal cats (Fig. 6) was similar to that of nondenervated spinal cats, with ankle flexors and extensors reciprocally activated (Pearson and Rossignol 1991). Therefore the locomotor deficit present after the cutaneous denervation does not seem to result from a plastic modification of the centrally generated, spinal locomotor pattern. Central drive for ankle flexors was therefore available during the swing phase. By comparing the fictive and denervated locomotor patterns, our results suggest that during real locomotion, central drive was not adequately facilitated to bring the ankle flexor motoneuron pools above firing threshold during swing when cutaneous inputs were absent. As a result, stride length was reduced and paw drag increased. Increased paw drag in turn led to toe underturning, which stretched ankle flexors during stance, thereby activating stretch reflexes in these muscles that were synchronized with normal ankle extensor activity.

CHANGES IN CONTRIBUTING REFLEXES. Results from cat SD1 show that cutaneous inputs from the same limb were required for compensation of paw placement. This result is surprising considering that tri- and polysynaptic connections exist between skin primary afferents and contralateral flexor motoneurons (reviewed in Burke 1999) and are modulated during locomotion (reviewed in Rossignol et al. 1988). These contralateral inputs were apparently not adequate to correct for the ipsilateral deficit.

In this cat, no perineal stimulation was provided during testing, before or after denervation, to avoid nonspecific increases in cord excitability that would have confused data interpretation. Further studies are required to find out the specificity of the cutaneous input necessary for the control of paw placement. Patterned phasic electrical stimulation of the proximal end of the cut nerves might provide valuable information.

SUPRASPINAL MECHANISMS FOR COMPENSATION. In the companion study (Bouyer and Rossignol 2003a), we have shown that removing all cutaneous inputs from the hindlimb paw caused only small changes in level walking in intact cats. This could have been the result of either a rapid compensation from the CNS or to an unimportant role of cutaneous inputs in the
control of locomotion. The cats from the companion study were included as part of this study and followed after spinalization, where supraspinal structures are no longer available, to address a complementary question on the role of spinal mechanisms in adaptive capacity of the intact locomotor system.

After spinalization, an important paw drag was present in denervated cats. EMG recordings show that this deficit resulted from a great reduction or lack of activation of ankle and toe flexors during swing. These muscles became activated during stance, resulting in an abnormal maladaptive coactivation with extensors that reduced stride length and impeded plantar paw placement. Furthermore, in cat SD1 it was shown that St extensors that reduced stride length and impeded plantar paw stance, resulting in an abnormal maladaptive coactivation with fl from a great reduction or lack of activation of ankle and toe cit resulted fi

anisms in adaptive capacity of the intact locomotor system. Address a complementary question on the role of spinal mechanization, where supraspinal structures are no longer available, to were included as part of this study and followed after spinal-control of locomotion. The cats from the companion study, we saw that nonspinal cats do not present a paw drag deficit during locomotion after the same denervation. Their muscle activity in knee and ankle flexors is in the appropriate phase, and amplitude is augmented, not reduced.

By comparing the effects of the denervation before and after spinalization, we therefore suggest that the lack of major deficit after the cutaneous denervation in the nonspinal cats was the consequence of a supraspinal compensation. From the changes in EMG activity in St, we propose that this compensation must involve neural structures that can selectively increase the activity of knee (and ankle) flexor motoneurons. Recording and stimulation studies in awake walking cats have shown that the motor cortex can perform this function. We therefore suggest that this structure might be involved in the compensation after the cutaneous denervation in the intact cat. To test this hypothesis, experiments involving a cutaneous denervation in motor cortex lesioned cats are currently under way in the laboratory. Preliminary results indeed support the idea that the motor cortex contributes to compensation in the intact cat, given that larger walking deficits are observed after the same denervation in cortex-lesioned animals (Bouyer et al. 2000).

In conclusion, the results from this study and the companion study together show that cutaneous inputs do participate to the control of paw placement during locomotion. Their role can be most appreciated after spinalization, where compensation from supraspinal structures is absent. Previous work (Sherrington 1910) led us to believe that removal of cutaneous inputs had little effect on rhythm generation, which—although true—minimizes their importance for the proper kinematic expression of the locomotor pattern. Furthermore, our work shows quite clearly that, whereas the intact cat may use alternative sensory cues (e.g., proprioceptive), the spinal cat appears unable to do so. The fact that spinal cats cannot compensate for the paw placement deficit suggests that cutaneous inputs may represent the default sensory modality used for the correct placement of the foot during locomotion. This fits very well with the idea that a number of cutaneous reflexes participate in the fine control of foot positioning in animals (Schouenborg and Kalliomaki 1990; Schouenborg and Weng 1994; Schouenborg et al. 1992) and humans (Van Wezel et al. 1997; Zehr and Stein 1999).

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Present address of L. Bouyer: Department of Rehabilitation, Universite Laval, and Center for Interdisciplinary Research in Rehabilitation and Social Integration (CIRRIS), IRDPQ-Site Francois-Charon, 525 Blvd Wilfrid-Hamel, Room 1318, Quebec, G1M 258, Canada (E-mail: Laurent.Bouyer@rea. ulaval.ca).

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