Study of Cutaneous Reflex Compensation During Locomotion After Nerve Section in the Cat

Geneviève Bernard, Laurent Bouyer, Janyne Provencher, and Serge Rossignol

Groupe de Recherche sur le Système Nerveux Central, Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Université de Montréal, Montreal, Quebec, Canada

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Bernard G, Bouyer L, Provencher J, Rossignol S. Study of cutaneous reflex compensation during locomotion after nerve section in the cat. J Neurophysiol 97: 4173–4185, 2007. First published March 28, 2007; doi:10.1152/jn.00797.2006. In the cat, section of all cutaneous nerves of the hindfeet except the tibial (Tib) nerve supplying the plantar surface results in a long-lasting decrease in the intensity of Tib stimulation needed for a threshold response in flexor muscles and an increase in the amplitude of the phase-dependent responses recorded in various muscles during locomotion. Stimulating through chronically implanted nerve cuffs ensured a stable stimulation over time. The increase in reflex amplitude was well above the small increase in the amplitude of the locomotor bursts themselves that results from the denervation. Short latency responses (P1) were seen in flexor muscles, especially at the knee (semimembranosus) and ankle (tibialis anterior and extensor digitorum longus), with stimuli applied in the swing phase and also to a lesser degree in the later part of the cycle. Longer latency responses (P2) were increased in hip, knee, and ankle flexors, as well as in a contralateral extensor (vastus lateralis) when applied in late stance. Responses evoked from stimulating the proximal end of sectioned nerves were not larger than before neuroectomy. This suggests that the increased responsiveness to Tib stimulation is not simply caused by an increase in motoneuron excitability, because this would have resulted in a nonspecific increase of responses to stimulation of any nerve. It is concluded that the adult locomotor system is capable of central reorganization to enhance specific remaining cutaneous reflex pathways after a partial cutaneous denervation of the paw.

INTRODUCTION

Cutaneous reflexes originating from the foot are important for the control of foot placement during locomotion (as reviewed in Rossignol et al. 2006). Within the context of injury to cutaneous nerves of the paw, it is therefore particularly important to understand the potential for compensation by remaining cutaneous nerves through plastic changes in remaining reflex pathways. This study aims specifically at studying short- and long-term changes of responses evoked by stimulation, during locomotion, of the tibial (Tib) nerve supplying the plantar surface of the foot after other cutaneous nerves of adjoining cutaneous territories of the foot had been severed. Cutaneous reflexes show a functional modulation of amplitude during locomotion. Stimulation of the dorsum of the foot can generate well-organized phase-modulated patterns of corrective responses, as reviewed previously (Rossignol et al. 2006). For instance, a tap on the dorsum of the foot during swing in chronic spinal cats (Forssberg et al. 1975, 1976, 1977) or in intact cats (Forssberg 1979) evokes a robust response of the limb that rapidly withdraws the foot, which is followed by a flexion of the ankle and the hip to step over the obstacle and place the foot in front of it. However, when the foot is hit during stance, in the chronic spinal cat (Forssberg et al. 1975), there is a short latency response in the already active extensor muscles at the ankle but no response in the flexor muscles (Bélanger et al. 1988; Prochazka et al. 1978). Similarly, electrical stimulation of the dorsum of the foot (nerve or skin) during the swing phase evokes short-latency responses (~10 ms) in several flexor muscles (P1 responses of Duyens and Loeb 1980). In addition, a second response, P2, at ~25 ms, is often seen (Duyens and Loeb 1980; Forssberg 1979). These two reflex responses can be phasically modulated differentially within the step cycle (Abraham et al. 1985; Duyens and Loeb 1980; Forssberg 1979; LaBella et al. 1992).

Another aspect of functional phase-dependent and context-dependent modulation is seen when the direction of walking is reversed but the same skin area is stimulated in the intact cat (Buford and Smith 1993). A mechanical stimulus applied to the dorsum of the foot during swing of backward walking evokes a simultaneous co-activation of the knee and ankle flexors, leading to an increase of the backward swing. When, on the contrary, a perturbation is applied to the ventral surface of the paw during swing of backward walking, there are excitatory responses in the ankle flexor and knee extensor that withdraw the foot forward. Therefore even though the same skin area is stimulated, the pattern of muscle activation is quite different in forward and backward swing, but both responses achieve the same goal to remove the foot from the stimulus.

The above two examples suffice to suggest that the cutaneous reflex pathways must be organized such as to show the greatest potential for physiological adaptability. Indeed, there are several lines of evidence showing the differential control of cutaneous pathways to different synergistic muscles (Degtyarenko et al. 1996; Moschovakis et al. 1991), as well as the differential control of cutaneous nerves to ankle extensor synergists (LaBella et al. 1989). This suggests that, through di- and trisynaptic pathways, cutaneous reflexes may show a variety of responses depending on the source of stimulation and the time of stimulation within the cycle of a given rhythmical behavior. The complexity of the pathways involved is also seen in other work in the rat and the cat that shows a modular organization (Schouenborg 2003). This or-

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organization seems to be the result of a learning process starting right after birth and where withdrawal responses evoked by random stimulation of the foot during spontaneous movements become organized to remove the foot appropriately from the stimulus (Petersson et al. 2004). Such an organization can also be postulated in humans because the stimulation of cutaneous nerves innervating various parts of the foot gives rise to phase-dependent withdrawal reflexes that are adequate to remove the foot from the perturbation (Zehr and Stein 1999).

Given the importance of the cutaneous reflex responses to perturbation, it is thus surprising that the contribution of cutaneous inputs to normal locomotion has been underestimated. In fact, it has even been suggested that cutaneous inputs do not play a significant role in the step by step adjustment of foot placement in intact cats. This view probably resulted from previous experiments showing that the removal of cutaneous inputs from the hindlimbs did not prevent rhythmic stepping (Sherrington 1910). Sectioning cutaneous nerves in otherwise intact cats (Duyssens and Stein 1978) or infiltrating the central foot pad (Engberg 1964) or the dorsum of the foot (Forssberg et al. 1977; Prochazka et al. 1978) with a local anesthetic had similarly little effect on locomotion. However, sparing cutaneous innervation of the forelimb in deafened monkeys led to a recovery of limb use during ambulation (Mott and Sherrington 1895), indicating a particularly important role for this sensory modality during locomotion.

More recent work brings another perspective on the contribution of cutaneous afferent inputs to locomotion (Bouyer and Rossignol 1998, 2001, 2003a, b; Rossignol et al. 2002). After a complete bilateral cutaneous denervation of the hindfeet, otherwise intact cats could rapidly walk almost normally on a treadmill. The detailed EMG and kinematics analyses showed a long-term adaptation of swing and corresponding EMG. Cats could not, however, walk on the rungs of a horizontal ladder, although they did eventually develop a strategy to grasp the rungs while walking. After spinalization at T13, the same cats were incapable of placing the foot on the plantar surface even after several weeks of locomotor training, in marked contrast with nondenervated spinal cats that recover correct foot placement after spinalization (Barbeau and Rossignol 1987; Bélanger et al. 1996; Rossignol et al. 2000). The neuromodulated spinal animals could clearly generate alternate movements in proximal joints but dragged their feet on the surface of the belt while swinging and, during stance, walked with their toes curled under the foot. This shows the importance of cutaneous inputs in the expression of locomotion, at least in the spinal state. However, it was also observed that, after a partial cutaneous denervation that preserved only a minimal cutaneous input, spinal cats could walk again on the plantar surface within 8 days of the denervation. This suggested that after the neurectomy of certain nerves, the remaining pathways could be reorganized to provide an adequate signal to the cord, presumably through an increase in the excitability of central connections. Such plasticity in cutaneous pathways has also been shown by others. For instance, locomotor training on a treadmill modifies the excitability of transmission in cutaneous pathways in spinal cats (Côté and Gossard 2004). After spinalization, there was initially an increase in cutaneous reflex excitability, but locomotor training decreased this hyperexcitability of reflexes evoked mainly from the medial planter nerve (MPL) innervating the plantar surface of the foot, as if to normalize the gain of the reflex.

Because all the above physiological examples suggest that the cutaneous pathways are highly modifiable so that they are adapted to the phase and context of locomotion and that they show a potential for plasticity, it was decided to study specifically, in cats with an intact spinal cord, how cutaneous reflex responses evoked during locomotion from an intact cutaneous nerve could be modified on a long-term basis when other adjoining cutaneous nerves were sectioned. Part of this work has previously appeared in abstract form (Bernard et al. 2000).

**METHODS**

**General protocol**

Four adult cats were used in this study. In two of the cats (MP1 and MP2), the receptive field of the tibial nerve was mapped before and exactly 3 mo after the partial cutaneous denervation of the left hindlimb paw. In the other two cats (GB1 and GB4), chronic reflex recordings were made before and repeatedly after the same partial denervation. Each animal served as its own control; the short- and long-term effects of the partial denervation could be compared in the same animal, as well as the time-course of compensation, while keeping the number of animals very low. Cat GB1 participated in 34 recording sessions and GB4 in 48 sessions. Each session could last 60-90 min because hundreds of reflexes were evoked during locomotion. In cat GB1, it was possible to perform three complete control sessions before denervation and four complete sessions after denervation at 1.2 times motor threshold in the semitendinosus on days 1, 4, 9, and 51. The gap between days 9 and 51 was caused by two factors: nerves other than ipsilateral (i)Tib were tested or iTib was tested at other intensities. Only results at 1.2 times threshold are reported here for consistency, but similar results were obtained with slightly larger intensities. In cat GB4, three control sessions were also completed, as well as five postdenervation series at 1.5 times motor threshold in the tibialis anterior on days 1, 7, 14, 21, and 28.

Cats were initially trained every day for 2 wk to walk on a motorized treadmill continuously for at least 20 min at belt speeds of 0.35–0.5 m/s. After this training period, several hindlimb muscles were implanted chronically with EMG electrodes, and stimulation cuffs were placed around two cutaneous nerves.

Each recording session was divided into three parts: 1) NO-STIM: walking without nerve stimulation, to measure the short- and long-term effects of the denervation itself on the locomotor pattern; 2) STIM recruitment curves: walking with cutaneous nerve stimulation of varying intensities applied always at the same phase of the walking cycle, to measure objectively the motor threshold to nerve stimulation in several muscles; 3) STIM: walking with cutaneous nerve stimulation, to measure changes in reflex amplitude in different phases of the walking cycle as a function of time (short- and long-term compensation). Three control recording sessions were obtained over 2–3 wk to serve as a baseline before denervation. After this control period, the partial denervation was performed, and recordings were continued for 28 (GB4) and 51 (GB1) days. The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal.

**Chronic EMG electrodes**

After training, animals were prepared for implantation of EMG electrodes under aseptic conditions. Cats were pretreated with ketamine (10 mg/kg), acepromazine (Ayerst, Guelph, Ontario, Canada) (0.1 mg/kg), glycopyrrolate (0.01 mg/kg), and buprenorphine (0.01 mg/kg). They were anesthetized with isoflurane 1–2% through an endotracheal tube. Body temperature was monitored with a rectal...
thermometer and maintained within physiological range with a heating pad. Cardiac rhythm and respiration were also monitored during surgery.

Two 15-pin head connectors (CINCH) previously soldered to seven pairs of teflon-insulated stainless steel wires were secured to the cranium using acrylic cement (Bélanger et al. 1996). Each pair of teflon-insulated stainless steel wires were led subcutaneously and sown into the belly of hindlimb muscles for bipolar EMG recording. Before insertion, a small portion (~2 mm) of the teflon coating was removed from the wires to be inserted in the muscle. The remaining unpaired wire from each connector was placed under the skin of the neck to serve as a reference. Implanted muscles varied across cats but included semitendinosus (St; knee flexor and hip extensor); sartorius (Srt; hip flexor/knee extensor); vastus lateralis (VL; knee extensor); tibialis anterior (TA; ankle flexor); extensor digitorum longus (EDL; ankle extensor/toe extensor); and gastrocnemius medialis (GM; ankle extensor/knee flexor).

Nerve stimulation cuffs

Chronic nerve stimulation was performed in cats GB1 and GB4 using implanted bipolar electrodes embedded in a cuff made of vinyl polysiloxane (Reprosyl from Dentsply International) (Julien and Rossignol 1982). Cuff electrodes were installed around the tibial nerve, just behind the Achilles’ tendon and around the superficial peroneal nerve at the lower third of the shank in both hindlimbs. The same cuffs remained around the nerves throughout the several months, thereby allowing for a stable stimulus delivery from session to session before and after denervation.

Receptive field mapping

Under general anesthesia, the peripheral receptive fields of the following nerves were measured in cats MP1 and MP2: tibial (Tib), superficial peroneal (SP), caudal cutaneous sural (CCS), saphenous (Saph), and the cutaneous branch of the deep peroneal (DPc). Each nerve was exposed after opening a small area of skin (2–4 cm), and a cuff electrode was temporarily placed around the selected nerve while taking great precaution not to overtly move it (see Fig. 1 for the location of electrodes). Electroneurographic signals from each cuff electrode was amplified (1,000–20,000 times) and band-pass filtered (3–10 kHz) and led to an audio-amplifier and an oscilloscope. The paw was placed over a millimetric grid, and its outlined was drawn. The receptive field of the selected nerve was mapped using von Frey hairs, a small brush, and small forceps. The entire surface of the paw, from the tip of the toes to the ankle joint, was systematically mapped three times onto four two-dimensional (2D) projection drawings representing the top, bottom, lateral, and medial view of the paw. An area of skin was considered responsive if action potentials could be detected either aurally or visually. All recorded nerves were cut except for the tibial nerve. The proximal end of the cut nerves was capped with Reprosyl to prevent or minimize regrowth. The receptive field of the tibial nerve was remapped using the same method 3 mo later.

Partial cutaneous denervation

Once the baseline recordings were completed in the intact state, a partial unilateral denervation of the left hindlimb paw was performed surgically in cats GB1 and GB4, removing tactile sensation from all but the plantar surface. Under general anesthesia and aseptic conditions, the following nerves were cut: SP, CCS, Saph, and DPc nerves in the left hindlimb (for more details, see Bouyer and Rossignol 2003a and see Fig. 1). SP was transected ≥1 cm distal to the stimulation cuff to avoid interfering with nerve stimulation. As was the case for mapping studies in other cats, the proximal end of the cut nerves was capped Reprosyl to prevent regrowth (Bouyer et al. 2001). This neurectomy protocol spared the left Tib nerve, thus maintaining cutaneous inputs from the plantar surface of the left hindpaw.

The adequacy of the neurectomy was checked periodically by a light pinch of the skin of the foot with small forceps to insure that such stimuli did not evoke withdrawal responses when applied to the denervated territories. It must be noted that all nerves sectioned conveyed purely cutaneous sensory afferent fibers at the level of the neurectomy, and therefore that the results presented here could not be caused by surgically induced motor impairment. The right hindlimb paw remained intact.

Postoperative care

Buprenorphine (0.01 mg/kg, sc, every 8 h) was given in the first 3 postoperative days to reduce discomfort. Animals also received antibiotics (cephatab or apo-cephalex, 1 cap of 100 mg/day, po) at the

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**FIG. 1.** Cutaneous nerves of the left hindlimb and their receptive fields: superficial peroneal (SP, foot dorsum), tibial (Tib, foot planta, central and digital pads), caudal cutaneous sural (CCS, lateral aspect of the paw and calcaneus), saphenous (Saph, medial aspect of the paw), and deep peroneal (DPc, cutaneous branch innervating digits 2 and 3). Maps obtained from cat MP1. Partial cutaneous neurectomy consisted of cutting 4 nerves (all but tibial) in the left hindlimb at levels shown by circles. The proximal stumps of the cut nerves were sutured to the inside of a polymer cuff to prevent regrowth. The right hindlimb was not denervated. L, lateral; V, ventral; M, medial; D, dorsal.
time of the surgery and for at least 10 days after. All animals remained healthy over the entire recording period.

**EMG recordings**

**LOCOMOTOR EMG.** All cats walked at 0.35–0.5 m/s during the NO-STIM and STIM walking periods. The EMG signals from the implanted hindlimb muscles obtained during locomotion were amplified (gains of 500–20,000) and filtered (100 Hz to 3 kHz) before being digitized on-line using custom software at a rate of 500 samples/s/channel.

**RELEX EMG.** During the STIM walking periods, in addition to the continuous acquisition of locomotor EMG, higher temporal resolution was obtained for the reflex responses by our custom software. Individual reflex responses were digitized at 2,000 samples/s/channel for off-line analysis.

**Nerve stimulation**

Threshold (T) of the motor responses was first established during the swing phase by using stimuli at a fixed latency relative to the onset of either the St (GB1) or TA (GB4) muscle burst (typically 0 ms). Threshold of stimulation was set as the minimal current value needed to evoke a short latency response (10 ms) in a flexor muscle (e.g., left St or left TA, depending of the cat) for 50% of the stimuli. Nerve stimuli were delivered as a multiple of this threshold. Cutaneous nerves were stimulated throughout the step cycle. At least 110–120 stimulations were given in each recording session for a given nerve. Both the Tib and the SP nerves were stimulated on both sides (only threshold data are presented here for the intact hindlimb), and more than one nerve could be tested in the same experimental session. Using custom software, rectified EMG activity from St or Srt was used to count steps and to measure step duration on-line. Step duration was divided into 10 bins of equal duration, and the computer triggered measurement software, onsets and offsets of EMG bursts were initially detected automatically using threshold values and were indicated by a major increase in reflex responses associated with more minor changes in baseline EMG activity.

**Data analysis**

**LOCOMOTION WITHOUT STIMULATION (NO-STIM).** Using custom analysis software, onsets and offsets of EMG bursts were initially detected automatically using threshold values and were indicated by cursors on the display. Obvious inaccurate detections (e.g., caused by spurious spikes) were corrected manually by placing the cursor at the appropriate place. EMG signals from the sciatic nerve were rectified, time-normalized to a cycle duration of 100%, and averaged over a number of cycles. The duration, phases of burst onset and offset, and amplitude of the EMG bursts were calculated for several muscles. Mean amplitude was calculated as the integral of the rectified EMG burst divided by its duration. Results from pre- and postneurectomy were compared.

**REFLEX RESPONSES (STIM).** Figure 5 summarizes how cutaneous reflex responses were quantified. Average responses from each of the 10 phases of the step cycle (Fig. 5A) were used to construct phase-response curves for each muscle. Cutaneous reflexes usually consists of two reflex responses: one at short (P1, ~10 ms) and the other at longer (P2, ~25 ms) latencies (Fig. 5, A–C). Latencies varied slightly across muscles, but care was taken to always use the same time window to analyze responses from a given muscle before and after denervation. For P1 and P2, reflex response curves obtained on different postneurectomy days were plotted and compared with the level of reflex amplitude preneurectomy (as in Figs. 7 and 8). Reflex amplitude was measured by subtracting the nonstimulated locomotor EMG underlying the response window obtained from the nonstimulated cycles from each stimulated cycle (Fig. 5, A–C) (Rossignol et al. 2006). It should be emphasized that, with this measurement method, the amplitude of the reflexes is represented by the integrated EMG activity above the baseline locomotor EMG activity. Such a method allows us to better isolate the changes in baseline locomotor EMG caused by the denervation, from the changes in reflex response amplitude. For example, in Fig. 9, comparing the percentage change in baseline locomotor activity and percentage change in reflex responses, one can appreciate major increase in reflex responses associated with more minor changes in baseline EMG activity.

**RECRUITMENT CURVES.** To have an objective measure of threshold at different times before and after denervation, specific walking sessions were dedicated to threshold measurements, where stimulation current was systematically varied while the phase of stimulation within the step cycle was kept constant. Recruitment curves were then reconstructed off-line, plotting reflex amplitude for a given muscle as a function of stimulus intensity. Figure 4 shows one example of this analysis. After all stimulations for one session were plotted, a sigmoid curve was fitted to the data using commercial plotting software (Sigmaplot). A logistic three-parameters equation of the following form was chosen:

\[
y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}
\]

This fit described the data very well for many muscles, with \(R^2 > 0.9\). Parameter \(a\) represented asymptotic reflex amplitude. An amplitude of 10% of the asymptotic value was considered to be an objective representation of the reflex threshold. The fitted equation was solved for 10% of max

\[
threshold = x_0 \left(1 - \frac{a}{x_0}\right)
\]

**Mean reflex/EMG ratio**

The modifications of reflex amplitude associated with the neurectomy were sometimes accompanied by small modifications in background EMG activity. Because the amplitude of reflex responses can be influenced by the level of background EMG (automatic gain control; Matthews 1986), it was necessary to also consider this factor in our analysis. To do so, a mean reflex/EMG ratio was calculated as done by others (Duyens et al. 1993). Briefly, average reflex responses from each of the 10 phases of the step cycle were summed and divided by the sum of the corresponding background EMG recorded in the same time bin as the reflexes. To conform with the method and for this analysis only, the nonsubtracted reflex responses were used. A mean reflex/EMG ratio value above 1 represents an overall facilitation, whereas a value below 1 would represent an overall inhibition. In Fig. 10, the mean ratios were also expressed as percentages of the ratios obtained before the neurectomy in the control period. It must be noted that this method is influenced by the phase modulation that cutaneous reflexes undergo during the step cycle. However, comparing the mean reflex/EMG ratios obtained in several conditions from the same cat, after stimulation of the same nerve at the same intensity, allows to evaluate if the modification in the reflex responses are only proportional to change in background EMG or represent a premotoneuronal modulation.
Statistics

For comparing locomotor EMG before and after neurectomy, data from three control sessions were first compared and, if compatible, pooled together to serve as a control to which postneurectomy data were compared with using ANOVA. When significant changes were detected by the ANOVA, multiple comparisons against the control group (proneurectomy) were performed using Dunnett’s method. Level of significance was set at 0.05.

To compare reflex responses, because reflex amplitude is modulated according to its time of arrival within the step cycle (phase modulation), we considered each of the 10 phases (values in each bin) as being independent of each other. For each phase, comparison was made before and after denervation using ANOVAs, similarly to what was done for locomotor EMG.

Results

Peripheral receptive field of the tibial nerve

Two cats (MP1 and MP2) were operated on for the purpose of determining the peripheral receptive fields of the five nerves innervating the hindlimb paw, namely the Tib, SP, CCS, Saph, and DPc. Touching the paw surface using von Frey hairs, a small brush, and small forceps, while recording directly from each of the nerve mounted on hook electrodes, the receptive fields were carefully mapped on four 2D projections of the paw. A representative summary is shown in Fig. 1 for cat MP1. Qualitatively, little difference was seen between cats with this method. The receptive field of the Tib nerve was remapped 3 mo after the partial denervation, using the same method to assess whether the damage to other nerve induced any significant changes in the receptive field of the remaining intact Tib nerve. It was found that the extent of the receptive field measured 3 mo after partial denervation was very similar indeed to that established before the denervation. It must be noted, however, that various factors (exact hair length, skin movement, and projection errors on 2D surfaces) limit the extent of our conclusion because this method gave only a gross evaluation of the borders of each receptive field. Even if some changes in receptive field territory did occur, such changes would not affect the reflex results obtained with chronic cuff electrodes, because the whole nerve was stimulated before and after denervation.

Locomotion without stimulation (No-STIM)

To measure changes in reflex responses during locomotion after the partial denervation, it was important to first determine if the locomotor pattern itself was affected by the neurectomy. Indeed, a significant shift in burst onsets/offsets or burst amplitude may falsely be interpreted as a change in the gain of reflexes when comparing reflexes before and after denervation. For instance, a doubling of locomotor EMG amplitude in a given muscle and a doubling of a reflex response when comparing before and after might be falsely interpreted as a change in the gain of the reflexes, whereas it merely represents an automatic gain control (see Methods). Although our reflex measurements are taken above the baseline locomotor activity, it was still important to determine if the denervation changed the timing and the amplitude of the EMG bursts.

Figure 2A shows the averaged rectified EMG activity during treadmill walking at 0.4 m/s before (control trace in black) and 1 day after (blue traces) the partial neurectomy in cat GB1. No stimulation was applied during these runs. It can be seen that only a small change in muscle activation occurred specifically in the EDL muscle on the side of the neurectomy. No unloading of the denervated leg or change in left/right stance duration that could have been associated with limping occurred, because VL activity remained similar to control on both sides. The EMG data could also be nicely superposed even after 51 days (Fig. 2B).
Overall, the effects of the partial denervation on the locomotor pattern were small and specific. There were no major changes in step cycle duration associated with the partial denervation (Fig. 2C). For cat GB1, the mean control step cycle duration at 0.4 m/s was 994 ± 50 (SD) ms (n = 183; average of the 3 control sessions before day 0). One day after the neurectomy, step cycle duration remained the same (mean = 1,008 ± 62 ms, n = 33, P > 0.16). Fifty-one days after the neurectomy, the mean step cycle duration decreased to 949 ± 72 ms (n = 36; P < 0.001), which, although statistically significant relative to the pooled control data, remained within the normal restricted range seen between individual control days for this cat (949–1,017 ms). For cat GB4, no statistical difference was found in the step cycle duration after neurectomy (P > 0.13).

Figure 3 details the mean burst amplitude of different muscles and their duration in three control sessions before the denervation (day 0) and early (days 1, 2, and 7), as well as 51 days after denervation. Although EDL activity was somewhat reduced for the first 7 days or so, it was increased at day 51 (Figs. 2B and 3E; P < 0.01). Cat GB4 also showed an increase in EDL activity (data not shown). No changes were observed in the discharge pattern (phase and amplitude) of other limb flexors such as Srt, St (Figs. 2B and Fig. 3, A and B) or even EDL’s agonist TA (data not shown), contrary to what has been shown for complete cutaneous denervation of the hindpaw either unilaterally (Bretzner and Drew 2005) or bilaterally (Bouyer and Rossignol 2003a).

The fact that EDL activity was changed as early as 1 day after the partial cutaneous denervation with no concomitant change in other muscle activity suggests that, overall, the cats were walking qualitatively the same way, but that removing SP, CCS, Sural, and DPs caused a specific deficit in the ability to activate EDL during the swing phase of walking. Furthermore, the subsequent gradual increase in EDL activity over time, associated with the absence of reinnervation (as tested by pinching the paw with small forceps), suggests that the animals gradually compensated for this initial deficit.

**Locomotion with stimulation (STIM)**

Threshold of Motor Response. Figure 4 shows the relative current strength necessary to reach threshold in iSt (GB1) or iTA (GB4). Threshold was calculated from recruitment curves obtained in specific experiments where stimulus intensity was systematically varied.

Recruitment curves obtained before and after denervation (Fig. 4A) showed that threshold could change significantly.
after denervation. Resulting thresholds in iSt or iTA as a function of time after the neurectomy in cats GB1 and GB4 are plotted in Fig. 4, B–E.

For responses evoked by the intact left Tib nerve, the threshold gradually decreased after denervation to ~70% of preneurectomy values (Fig. 4, B and D). Note that the changes with stimulation of the right Tib are minimal for GB1 (Fig. 4B) and somewhat more pronounced in GB4, at least initially (Fig. 4D). In Fig. 4C, the threshold to evoke responses with stimuli to the cut left SP nerve proximally to its section is transiently decreased and returns to normal at day 51. The same applies to GB4 (Fig. 4E), but in this case, the threshold continues to rise for at least a month after the neurectomy. In both GB1 and GB4, there was no change in amplitude of the responses evoked by stimulation of the intact right SP, indicating that the changes seen in reflex responses were specific to the denervated side. Comparing the motor threshold in different muscles as a function of time after denervation in cat GB4, in the control state, iSrt and iTA have a lower threshold than iSt and iEDL. By 28 days postneurectomy, all muscles had a lower threshold, iSrt and iTA having the lowest. The decrease in threshold in percent of control value was in the same range for all muscles. The most stable thresholds across time after denervation were obtained in TA and EDL (data not shown).

Differential modulation of short- and long-latency responses

Figure 5 shows a control reflex testing session in cat GB1, 12 days before denervation. The step cycle was divided into 10 phases (y-axis) and synchronized to the onset of Srt activity (time 0). In Fig. 5A, two reflex responses can be observed: an early response (P1) at ~8 ms and a late response (P2) at ~25 ms. The two dotted lines define windows of 8–20 and 26–46 ms that include the onset and offset of these two responses. These two responses are of different amplitude and duration, and their maximum is reached at different points in the cycle. It is important to note here that different amplitude scales (indicated on the right side) were used to optimize the display for the response in each phase. This is particularly important when looking at the P2 responses, which are much larger in phases 0.8–1.0 than in phases 0.1–0.3, even though they might appear roughly the same on the display. This phase modulation of response amplitude is better seen in Fig. 5, B and C, where the P1 and P2 responses are plotted on a uniform ordinate scale. In Fig. 5C, the P2 response is largest between phases 0.7–1.0, at a time when the P1 response (Fig. 5B) reaches its minimum.

The amplitude of these responses is not simply a function of the size of the underlying locomotor EMG. Figure 5B shows the total St EMG (gray squares) integrated over the preset time window (8–20 ms). For each phase, this total EMG includes both the underlying baseline locomotor EMG (white squares) and the reflex responses (black squares), which are also plotted individually to clearly extract the reflex response above the baseline EMG. Note that the locomotor EMG is increased in phases 0.8–1.0, whereas the P1 responses are increased starting in phase 0.9 and remain increased until the next phase 0.1. Between 0.8 and 0.9, although the baseline EMG is high, the reflex response reaches its minimum, below the locomotor EMG, suggesting a potential inhibitory response. Also, for the P2 response (Fig. 5C), the large total EMG in phases 0.8–1.0 is caused principally by the large reflex response, because the increase in the underlying locomotor EMG is rather modest.

Modulation of reflex responses after denervation

In Fig. 6, two reflex testing sessions from the same cat are superimposed much in the same format as Fig. 5A, except that the baseline EMG was removed for the sake of clarity. The thin line represents responses in a control session, whereas the thick lines are the responses obtained 4 days after the partial denervation. The responses are obtained at the same relative threshold established on each day, although the current needed to reach such a threshold was lower after denervation as detailed in Fig. 4. In Fig. 6, it can be seen that both reflex responses (P1 and P2) were modified after the denervation, and according to a different phase-specific pattern. These changes are detailed in the subsequent figures.

Figures 7 and 8 show the effects of the denervation on reflexes evoked by stimulating the Tib nerve in cats GB1 and

![Figure 5](http://jn.physiology.org/)

**Figure 5.** Reflex responses in St to tibial nerve stimulation at 1.0 T, 12 days before denervation. Treadmill speed of 0.5 m/s. A: step cycle was divided into 10 phases, and responses were grouped according to phases of stimulation within step cycle triggered on the knee flexor iSt, which presents a sharp burst of activity at the end of stance-beginning of swing. For each phase, reflex responses consist of averaged rectified EMG activity above baseline locomotor activity indicated by dotted line. Time windows defined by vertical dotted lines define areas of integration of P1 and P2 responses applied to all phases. In all cases, locomotor EMG was subtracted from total response. Note that vertical scale (amplitude) is different for each phase to optimize display and emphasize differences in phase modulation of P1 and P2 responses. For instance, P1 responses are seen mainly in early phases, whereas P2 responses in later phases are much greater than in early phases. B: phase plots showing total EMG (gray squares) integrated in time window 8–20 ms (P1 responses) and locomotor EMG (white square), as well as the reflex itself above baseline (black squares). C: same for P2 responses with integration between 26 and 46 ms.
GB4, respectively. For cat GB1, the left column of Fig. 7 shows the effects of the denervation on P1 reflex amplitude. Horizontal rectangles represent the phases during which the muscle is active during walking. The denervation had little effects on muscle timing (data not shown). The amplitude of the early reflex response was increased as soon as 1 day after the denervation in EDL (Fig. 7E) in phase 0.3 (end of swing phase), with a reflex amplitude now reaching 298% of peak control amplitude ($P < 0.05$). In addition, reflex activity in phase 0.7 (mid-stance during which there is normally no locomotor EMG) was also augmented to 129% of control maximum ($P < 0.05$). This early after the denervation, reflexes showed no change before day 9 (highest response), followed by normalization at 159% of control maximum in bin 0.9. For St, there was no change until day 9, with days 9 and 51 being very similar. EDL day 9 shows a transient large increase in response in bins 0.8–1.0. Finally, coVL only showed significant changes on day 51.

FIG. 7. Phase plots showing a summary of reflex amplitude modulation to tibial nerve stimulation at 1.0 $\times$ T in cat GB1. A, C, and E: P1 responses measured in 3 muscles on the ipsilateral side of denervation (iSrt, iSt, iEDL). Average of 3 control sessions (black), day 1 (blue), day 4 (brown), day 9 (green), and day 51 (red) after partial denervation. All data points are expressed as a percentage of peak control reflex amplitude recorded in each muscle. Horizontal rectangles represent phase of activity of each muscle activity during locomotion in the control period. B, D, and F: P2 responses in the same muscles, whereas G shows responses in coVL together with phases of locomotor discharges of coVL. ($*P < 0.05$; ANOVA).
Figure 8 shows that largely similar changes in reflex amplitude were observed in cat GB4. In this cat, St EMG was lost postdenervation and could not therefore be analyzed. However, TA was implanted together with EDL in this cat to look at reflex changes in synergistic muscles.

Reflex activity from TA was also measured, and followed a similar time-course of changes as EDL, even though baseline EMG activity did not change. There is a clear increase in P1 responses in TA and EDL that is not so evident in iSrt. The inhibitory P2 responses seen in Fig. 8D was caused by the silencing of a prolonged low level activity detected in the muscle during stance.

The overall pattern of reflex responses could be interpreted in the following manner. Stimuli applied in the early swing generate P1 responses in flexors of the knee and ankle to rapidly avoid an impeding obstacle, whereas the responses in hip flexor iSrt are not increased much. Stimuli applied in the late stance would tend toflex the hip while flexing the knee to remove the foot. Increase in contralateral weight support through contralateral P2 responses occurs only with stimuli arriving toward the later half of the contralateral extensor burst.

**Time-course of changes of the reflex responses**

Figure 9A shows the time-course of the gradual increase in reflex responses in St to Tib nerve stimulation, starting 4 days after denervation in cat GB1. For St, significant reflex increases occurred first in phases 0.1–0.3, which represent the swing phase. By day 9 after denervation, phase 1.0, representing the stance to swing transition, also became significantly augmented.

As shown in Fig. 9B, these increases in reflex response did not result from correlated increases in baseline EMG activity. The largest increases in St reflexes amplitude occurred actually in a phase where the baseline locomotor EMG activity was not changed (phases 0.1–0.3). When such changes did occur in baseline EMG activity, the changes in reflex amplitude were much larger.

**Responses to the stimulation of other nerves**

Other nerves were at times stimulated, such as SP, which was cut as part of the partial denervation. However, stimulation of this nerve could still generate reflex responses. Figure 9C shows that reflex changes were essentially absent in left St when left SP was stimulated at days 1, 9, and 51 postdenervation. Only two points in bin 1 (days 1 and 51) reached statistical significance, and they did so because they were lower than control. No EMG changes were seen either (Fig. 9D). Two testing sessions are presented for day 9. Their superimposibility confirms the stability of responses on a given day. Therefore the reflex changes observed with stimulation of iTib were specific to pathways activated by this nerve and not the result of an unspecific increase in motoneuronal activity that would have led to an increased response of reflexes regardless of the nerve stimulated.

**Comparison of reflex modifications with the level of background EMG**

In this study, some discrete changes in locomotor EMG activity sometimes occurred after denervation (e.g., Figs. 2, 3,
and 9). To account for this, mean reflex/EMG ratios were calculated for St, Srt, and EDL and are plotted in Fig. 10 as a function of time after the neurectomy. For St and EDL, it can clearly be seen that the reported reflex changes to Tib stimulation cannot simply be explained by an augmentation in the locomotor EMG, because the reflex ratio became greatly augmented after denervation (>250% of control), whereas it was very stable preneurectomy. Furthermore, the increase was gradual over the first 9 days and remained substantially elevated even at 51 days. For Srt, the stimulation of the same nerve had no visible effect on the reflex/EMG ratio. Stimulation of the cut SP produced no change in reflex ratios calculated for this nerve. This result is compatible with the conclusion from Fig. 9, i.e., that the reflex modifications are nerve-specific.

**Discussion**

**Summary of the results**

This study showed that, in the cat, section of all cutaneous nerves of the hindfeet except the Tib nerve, whose receptive field covers the plantar surface of the foot, results in a significant, gradual, and long-lasting increase in the amplitude of the reflex responses to Tib nerve stimulation and a decrease in the threshold to evoke responses from this nerve. This increase in responsiveness occurred during specific phases of locomotion and was above the small increases in the amplitude of the locomotor bursts of these same muscles that occurred after the denervation.

Furthermore, the changes in the early and late reflexes were found to be distributed specifically to certain muscles. The increase of the early P1 response evoked in the early part of the swing occurred in muscles more directly involved in removing the foot from ground such as the knee flexor St and ankle flexors such as TA and EDL, but to a lesser extent in the hip flexor/knee extensor Srt. The longer latency P2 responses were increased in hip, knee, and ankle flexor muscles, as well as in the crossed extensor muscle, especially with stimuli applied later in stance.

Reflex responses evoked by the stimulation of the cut end of sectioned nerves remained unchanged or decreased after the partial denervation, contrary to the intact Tib nerve. This suggests that the increase in the excitability of the flexor motoneuron pools. The short-latency P1 and longer-latency P2 responses were both affected by the denervation, but followed separate phase-specific changes. This again suggests that the increase responsiveness is not simply caused by an increase in motoneuron excitability, because this would result in an unspecific increase of both P1 and P2 responses.

The partial denervation used in this paper produced only small changes in the locomotor pattern itself. It is, however, of interest to indicate that the gradual increase in reflex responsiveness seen in this study occurred predominantly in muscles (St, EDL) that showed most changes after a complete cutaneous denervation (i.e., including the Tib nerve) in cats with an intact spinal cord (Bouyer and Rossignol 2003a).

**Measurement of chronic changes in cutaneous reflex responses**

Although the reflex modulation results presented here concern only two cats, it should be realized that this was achieved through 82 distinct recording sessions each lasting several hours.

A few points of methodology need to be discussed. First, the lowest possible strength of stimulation that could evoke the smallest responses in the knee flexor St or the ankle flexor TA was used. The reasons for this were that we wanted to minimize the discomfort of the cat, and indeed, cats could walk steadily for long periods of time without seemingly being aware of the stimulus. In such experiments, it is crucial to have runs of very stable walking because it is virtually impossible to study the phase dependency of reflexes in cats walking irregularly. The other advantage of using such a low current is that kinematic changes induced by the reflexes are minimal, and therefore there are little effects on the step cycle duration and in EMG bursts, which can easily disturb the automatic trigger system based on EMG detection.

Second, it was important to determine whether the changes in the amplitude of reflexes represent true changes in gain (i.e., a multiplicative factor in the transmission through the reflex pathway) or only a scaling of the reflex responses to the small changes in locomotor EMG (automatic gain control; Matthews 1986). Analyses have shown that the changes in the relevant flexor muscles were relatively small (maximum of 193% of control for St) compared with the changes in the amplitude of the reflexes, which could reach 931%. In one muscle (EDL), there was a slight modification in the temporal profile of its discharge, with a prolongation of the locomotor burst to phases 0.3–0.4. Although it could account for some shift in the phase-response curve for this muscle, this change could not explain the appearance of new responses in phases 0.6–0.9. In addition, Fig. 10 compares directly the size of the cutaneous reflexes with the size of the background EMG. The calculated mean reflex/EMG ratios (Duyssens et al. 1993) increased gradually and dramatically for St and EDL after the neurectomy.
Therefore the reported changes in reflex amplitude cannot be simply attributed to changes in background level of locomotor EMGs. Changes in reflex amplitude at comparable background EMG levels have also been reported in humans when the task executed by the subjects is changed (Baken et al. 2006). While task specificity and long-term plasticity are different neural mechanisms, they both point out the flexibility of cutaneous reflexes at the premotoneuronal level. Another example of this premotoneuronal modulation during walking in the cat is the fact that the highest reflex response often occurs outside of the locomotor EMG burst, i.e., when motoneurons are not actually discharging.

Third, it is well known that cutaneous reflexes can be quite labile and subjected to various tonic or dynamic influences (Rossignol et al. 2006). For instance, stimulating the same nerve at rest in two different positions of the cat (sitting, lying down on one side or the other) can lead to significant changes in responses, especially with low strength of stimulation. Therefore all the thresholds were defined during locomotion by triggering the stimulation at a fixed point in the step cycle (early swing). This is a much more stable situation that can be repeated day after day with minimal variation. The motor thresholds to nerve stimulation, established before and after neurectomy, thus seem to really indicate significant changes in excitability. The changes in threshold were almost the same for the two cats. We observed a reduction of \(\sim 40\%\) of the mean control value in the threshold of the left Tib nerve (intact nerve in the denervated limb) and no changes in the contralateral nerves thresholds, e.g., right Tib and right SP nerves (2 intact nerves). For the threshold of the left SP (cut nerve in the denervated hindlimb), we observed first a reduction (40–60\% of the mean predenervation value) rapidly followed by an increase in the threshold (110–120\% of the mean control value for GB1 cat and 300\% of the mean control value for GB4 cat).

These results suggest that there were some specific excitability changes occurring in the intact cutaneous nerve when the sensory fields of adjacent nerves innervating the foot are cut. This represents a true plastic change in the gain of cutaneous reflexes that could result from several mechanisms.

**Mechanisms of plasticity**

**SPROUTING.** On the basis of published reports (Bajrovic et al. 1999; Diamond et al. 1992; Dubovy and Aldskogius 1996; Jackson and Diamond 1983, 1984), one could have expected in our preparation a certain degree of sprouting of the Tib nerve, because the adjacent cutaneous territories were denervated. However, the receptive field of the Tib nerve remained essentially the same 3 mo after the denervation and therefore does not suggest that there was major peripheral sprouting. Furthermore, in our experimental situation, it is unlikely that collateral sprouting from the Tib nerve that was already inserted in a polymer cuff electrode arrangement could explain the increase in response. Indeed an increase peripheral sprouting distal to the stimulating electrode would not change the number of axons stimulated within the cuff.

After section of various cutaneous nerves of the hindpaw, it is also possible that central axons of cut nerves and the intact Tib nerve have sprouted. Central sprouting after peripheral nerve injury has been suggested in mammals (Cameron et al. 1992; Koerber et al. 1994; Mannion et al. 1996; Shortland and Woolf 1993). However, a reassessment with new tracers of central sprouting by intact nerves (Wilson and Kitchener 1996) suggests that, at least in the adult, plastic changes in cutaneous pathway excitability depend more on the “increased efficacy of preexisting, but previously undetected somatotopically inappropriate primary afferent collaterals.” This conclusion agrees very well with our results, which suggest principally a change in the efficacy of specific groups of motoneurons to re-establish the adaptive function.

**Changes in the excitability of transmission in cutaneous pathways**

Some evidence was given above to suggest that the reported modifications in cutaneous reflexes cannot simply be attributable to a change in the excitability of motoneurons. First, the changes in muscle discharges during locomotion after neurectomy are much smaller than the changes of the reflex responses. It has been shown previously that cutaneous pathways may be differentially controlled from segmental and suprasegmental inputs (Burke et al. 1970). In this experimental situation, we cannot discard the possibility that the motoneurons responding to the stimulation are different (at least in part) from those recruited during locomotion. The increased in reflex would nevertheless represent a change in a specific subpopulation of motoneurons. Second, stimulation of nerves other than Tib does not yield increased responses (see Figs. 9 and 10), and responses evoked by Tib nerve stimulation are increased only in certain muscles. Third, there is the differential effect of the neurectomy on P1 and P2 responses. P1s were much more increased than P2s, while occurring under very similar motoneuronal excitability levels. One could also have envisaged the possibility that the differential modulation of the P1 and P2 responses results from the recruitment of different motoneurons. This is unlikely because it was shown previously with intracellular recordings that P1 and P2 responses are present in the same motoneuron (Andersson et al. 1978).

**Interneuronal and presynaptic mechanisms**

As mentioned in the Introduction, several lines of evidence suggest functional plasticity in cutaneous pathways, and these changes undoubtedly involve interneuronal and presynaptic mechanisms.

Evidence of plasticity in cutaneous pathways also comes from observations made in chronic spinal cats after cutaneous nerve section (Bouyer and Rossignol 2003b). In a progressive denervation paradigm in spinal cats, the locomotor pattern of the hindlimbs recovers after section of each cutaneous nerve until all nerves are cut. The recovery occurring during the period of partial denervation suggests that inputs from other cutaneous nerves are involved because the foot placement recovery disappears after the selective removal of all cutaneous inputs from the paw. When all cutaneous nerves are cut, the animal is still capable of performing rhythmic hindlimb locomotor movements but is incapable of correctly placing the foot on the plantar surface.

Another form of plasticity was shown in spinal cats trained to walk on a treadmill (Côté and Gossard 2004). Using intracellular techniques, it was shown that the amplitude of cutaneous responses recorded in motoneurons was in general re-
duced and, more specifically, in nerves of the plantar surface of the foot, as if the training had reduced the hyperexcitable cutaneous reflexes in the spinal cat to reach a more normal level. As suggested by these authors, the most likely interpretation of these changes lies in the excitability changes of the interposed interneurones. However, presynaptic mechanisms could contribute as primary cutaneous afferents are normally subjected to presynaptic control during locomotion (Gossard et al. 1989). Evidence of presynaptic changes after peripheral nerve injuries is, however, not convincing, as suggested by Rudomin and Schmidt (1999).

**Supraspinal controls**

Although several mechanisms may be involved in changing the excitability of cutaneous pathways after neurectomy, it seems, at least in intact cats, that the motor cortex may play an important role as shown by two experiments. First, it was reported that the recovery of foot placement during locomotion after cutaneous nerve section observed in intact cats was lost after spinalization, suggesting that the maintenance of the recovered pattern (Bouyer and Rossignol 2003a) depended in great part on supraspinal controls (Bouyer and Rossignol 2003b). Furthermore, lesions of the motor cortex largely abolished the foot placement recovery in cats with a complete unilateral cutaneous neurectomy (Bouyer et al. 2000). Based on these findings, experiments were designed to study changes in muscle responses evoked by cortical microstimulation with chronically implanted electrodes in the motor cortex on the side opposite to the denervated hindlimb or in the pyramids (Bretzner and Drew 2005). Evoked responses were markedly increased in flexor muscles with cortical microstimulation and to a lesser extent by stimulation of the pyramids. This suggests that there is probably a change in excitability of corticospinal pathways occurring both at the cortical and spinal levels after such neurectomies.

**Significance of the change in reflex responsiveness**

The significance of this work is that, after denervation of certain skin territories of the hindpaw, there are specific changes in the excitability of transmission through other cutaneous pathways. When the foot is denervated except for its plantar surface, there is a change in the gain of the reflex responses evoked by stimulation of the remaining Tib nerve. We have shown that this increase represents a real increase in gain of the reflex and not only an additive increase of the responses riding over an increase of baseline locomotor EMG resulting from the neurectomy itself. Because other inputs did not evoke an increase in responses of the same muscle and because some muscles not principally involved in removing the foot did not produce larger responses either, it is believed that the change in gain of these reflexes evoked by the Tib nerve in certain muscles represent a true specific plastic change. We interpret this to mean that perturbations applied to the foot could then be more easily detected by the remaining intact Tib nerve and yield corrective responses. One could for instance surmise that the increased gain in the reflexes evoked from the remaining intact nerve (here the Tib nerve) could serve to enhance the detection of mechanical inputs to other parts of the foot that could be transmitted to the receptive field of the nerve. It is known for instance that minute stretches of the skin between the toes during locomotion can evoke activity in cutaneous receptors (Loeb 1981). Such might be the case not only to provide important cutaneous signals during unimpeached locomotion but also when meeting obstacles. Because the dorsum of the foot is largely denervated, an increased sensitivity of the borders of the receptive field of the Tib nerve may lead to a more secure stumbling corrective reaction (Forssberg 1979).

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