Changes in Corticospinal Efficacy Contribute to the Locomotor Plasticity Observed After Unilateral Cutaneous Denervation of the Hindpaw in the Cat

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INTRODUCTION

A recent study by Bouyer and Rossignol (2003a) showed that cutaneous denervation of the cat hindpaw leads to a transient deficit in treadmill locomotion that is characterized by a dragging of the hindpaw during the swing phase of locomotion. Cats rapidly compensate for this deficit, however, by modifying the level and the pattern of activity in hindlimb flexor muscles, especially those acting around the knee and ankle.

Several lines of evidence suggest that this functional recovery of locomotion might be mediated by a combination of spinal and supraspinal mechanisms. For example, in contrast to the situation in nondenervated animals (Barbeau and Rossignol 1987; Belanger et al. 1996; de Leon et al. 1998, 1999; Rossignol 2000; Rossignol et al. 1999), cats with a cutaneous denervation that are subsequently spinalized display marked hindpaw drag (Bouyer and Rossignol 2001, 2003b). This finding, which is also observed after lesion of motor nerves (Bouyer et al. 2001; Carrier et al. 1997) and dorsal rhizotomy (Goldberger 1977, 1988) suggests that denervation produces permanent changes in the spinal circuitry. At the same time, the deficits emphasize that the spinal plasticity is insufficient to completely compensate for the effects of the denervation, underlining the necessity of supraspinal descending influences for adequate recovery of function.

It is probable that at least a part of the supraspinal descending signal originates from the motor cortex. There is abundant evidence from lesion (Chambers and Liu 1957; Eidelberg and Yu 1981; Jiang and Drew 1996; Laursen and Wiesendanger 1966), single-unit recording (Drew et al. 2002; Kably and Drew 1998; Widajewicz et al. 1994), and intracortical microstimulation (ICMS) (Bretzner and Drew 2005a) studies, that the motor cortex plays an important role in the control of the hindlimb during locomotion in cats, particularly in situations that require a fine control over paw placement or limb trajectory. In addition, electrolytic lesion of the hindlimb representation of the cat’s motor cortex diminishes and delays the functional recovery after chronic cutaneous denervation of the hindpaw (Bouyer et al. 2000). Moreover, conditioning experiments have demonstrated that information from corticospinal and cutaneous afferent pathways is integrated on spinal interneuronal pathways that influence the level of motoneuron activity both in the anesthetized preparation (Flesman et al. 1988; Lundberg and Voorhoeve 1962; Lundberg et al. 1962; Pinter et al. 1982) and in the intact cat, during locomotion (Bretzner and Drew 2005b).

A contribution from the motor cortex to the functional recovery of locomotion after denervation is also supported by several lines of evidence in other species and in other tasks. For example, expansion of the motor cortical representation of different muscles has been reported after facial nerve section (Donoghue et al. 1990; Sanes et al. 1988, 1990) or forelimb amputation in adult (Sanes et al. 1990) and developing (Donoghue and Sanes 1987, 1988) rats. A similar expansion has been demonstrated in the cortical representation of the amputated arm compared with the unaffected side in humans (Cohen et al. 1991; Ridding and Rothwell 1995) and nonhuman primates (Qi et al. 2000; Schieber and Deuel 1997).

There is also evidence that changes in cortical organization may be complemented by changes in corticospinal efficacy. Enhanced responses to cortical stimulation, with respect to the unaffected side, have been reported in muscles proximal to an unusual side, have been reported in muscles proximal to an
implantation. Most of the surgical procedures used in these experiments were carried out on five male cats (weights 4.2–5.5 kg) trained to walk at a comfortable and constant speed (between 0.35–0.45 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods of 120 min. These same five animals were used in our previous experiments investigating the contribution of the motor cortex to the structure and the timing of hindlimb locomotion (Bretzner and Drew 2005a and b) during locomotion in the free walking cat.

Methods

Care and training

Experiments were carried out on five male cats (weights 4.2–5.5 kg) trained to walk at a comfortable and constant speed (between 0.35–0.45 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods of 120 min. These same five animals were used in our previous experiments investigating the contribution of the motor cortex to the structure and the timing of hindlimb locomotion (Bretzner and Drew 2005a and b) during locomotion in the free walking cat.

Surgical procedures

Implantation. Most of the surgical procedures used in these experiments, including details of general anesthesia, are reported in Bretzner and Drew (2005a,b). In brief, in three cats (MC23, 24, 27), microwire electrodes (Tri-ML insulated stainless steel: 25 µm diameter) attached to a miniature connector (Neuralynx: EIB27) were manually inserted into the posterior bank of the cruciate sulcus that contains the hindlimb representation of the motor cortex (Armstrong and Drew 1984; Bretzner and Drew 2005a; Nieoullon and Rispal-Padell 1976; Widajewicz et al. 1994). Microwires were always implanted into the right motor cortex. Appropriate positioning of the microwires was facilitated by recording neuronal activity and applying ICMS as the wires were inserted. The cortex was covered with a hemostatic material (Sterispon) and the microwire connector was attached to the cat’s cranium with dental acrylic. Microwires were also implanted in the right pyramidal tract at P7 (Drew 1993) in cats MC24–27 to allow comparison with the responses evoked by ICMS.

One to 2 wk after recovery from the initial surgery, multiple pairs of Teflon-insulated, braided stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record EMG activity during locomotion (see Bretzner and Drew 2005a for a list of these muscles and their major functions).

In addition, in cats MC24–27, cuff electrodes (Julien and Rossignol 1982) were also implanted around the saphenous, superficial peroneal, and tibial posterior cutaneous nerves of the right hindlimb, ipsilateral to the motor cortex and contralateral to the denervated hindlimb (Bretzner and Drew 2005b) (see following text).

Cutaneous denervation. After a period of 2–4 mo during which we obtained our control records, a unilateral cutaneous denervation of the hindpaw was performed according to the surgical procedures detailed by Bouyer and Rossignol (2003a). In brief, the five cutaneous nerves innervating the left hindpaw: the saphenous, sural, tibial, superficial peroneal nerves, and the cutaneous branch of the deep peroneal nerve were surgically transected. To prevent regeneration of the nerves, the proximal end was covered with a hemostatic material (gelfoam) and then a flexible vinyl polysiloxane cap (Reprosil, Dentsply International, Milford, DE). Receptive fields were tested daily with a sharp probe for the rest of the experiment to ensure the absence of regeneration. In cat MC25, the sural nerve was accidentally left partially intact, whereas all other nerves were cut. The sural nerve was completely transected 38 days later. Cat MC27 developed some allodynia and showed occasional episodes of dystonia that were treated with Baclofen, a γ-aminobutyric acid type B (GABA_B) receptor agonist acting mainly at the spinal level. Stimulation sessions in this cat were always performed 24 h after the application of Baclofen. Detailed data from this cat are not presented but the general trend of the results was the same in this cat as in the other two.

Pyramidotomy. To test the contribution of the corticospinal tract to the recovery of locomotion after the cutaneous denervation of the hindpaw, a pyramidotomy just rostral to the level of the decussation (Armstrong and Drew 1984) was performed in two cats, MC23 and 24. In brief, the pyramid was exposed at the level of the foramen magnum by a parapharyngeal approach and was divided with a pair of fine forceps. Recordings were pursued for about 20 days after this lesion.

Penicillin (Novopharm: 40,000 IU/kg, intravenous) and analgesics, buprenorphine hydrochloride (5 µg/kg), were provided at the beginning and at the end of each surgery, and for 48 h after each surgery. Antibiotics (cephadroxil: 100–200 mg/day) were administrated daily for the duration of the experiment. All surgical and experimental procedures followed the recommendations of the Canadian Council for the Protection of Animals and were approved by the local ethics committee.

Protocol

In initial experiments, we determined the threshold of all cortical and pyramidal microwires, as well as the cutaneous cuff electrodes, both with the cat at rest and at the onset of the swing phase during locomotion. Cortical and pyramidal microwires were tested by delivering trains of stimuli (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms), whereas cutaneous cuff electrodes were tested with a single pulse of the same duration. In addition, we also determined the strength at which each wire was stimulated for the duration of the experimental protocol. This intensity was set to ensure that the stimulation evoked robust responses during swing in most flexor muscles while ensuring that these responses were well below any saturation level. This guaranteed that both increases and decreases in the magnitude of the responses evoked after denervation would be detectable.

Once the stimulus intensities had been determined, one or more electrodes were stimulated on each day over a period of 2–4 mo. For each wire, we initially verified the threshold during the swing phase of locomotion and then applied stimulation, at the predetermined and fixed level, throughout the step cycle. Ten to 15 repetitions were made at each delay in the following order: 50, 150, 300, 500, 700, 900, 0, 1500, 3000, 5000, 7000, 9000, 15000, 30000, 50000, 70000, 90000, 150000, 300000, 500000, 700000, 900000.
100, 200, 400, 600, 800, and 1,000 ms after the onset of the activity in the anterior head of the left sartorius (lSrt). For cortical wires, the delay was then set to 50 ms and 15–20 stimuli were applied at intensities of 20, 25, 35, 50, 75, 100, and 150 μA. A similar procedure was also followed with stimulation of the pyramidal and cutaneous electrodes except that in these cases, the range of stimuli was determined separately for each electrode. During this control period, we tried to ensure that each electrode was tested a minimum of three times, evenly spaced over the control period. In addition, the impedance of the microwires was tested about once per week and we also tested each cortical wire for the presence of unit activity. When units were isolated, they were tested to determine whether they could be antidromically activated from one of the wires in the pyramidal tract. On selected days, we also recorded sessions of locomotion without stimulation to allow us to assess any changes in the background level of EMG activity either during the control period or after the denervation.

A similar protocol was used after the denervation with the exception that we placed more emphasis on stimulating each wire at different phases of the step cycle than on stimulating with different intensities at a single phase. This allowed us to check most wires at least once, and sometimes twice, in the week after the denervation.

In all experiments, evoked EMG responses were digitized on-line at a frequency of 5 kHz for ≥25 ms before and ≥150 ms after the onset of the stimulus train. EMGs were band-pass filtered between 100 Hz and 3 kHz. In addition, a continuous record of the EMG activity during locomotion was also digitized at 1 kHz. Most of the unstimulated sessions of locomotion were videotaped and a digital time code allowed these videos to be synchronized to the EMG recordings.

Data analysis

Data were analyzed as previously described (Bretzner and Drew 2005a,b; Rho et al. 1999). The responses evoked by all of the stimuli were computed rectified and averaged and plotted on a display monitor. The average activity from a similar time period taken from unstimulated cycles was superimposed on this display (see Fig. 2A and Bretzner and Drew 2005a; Drew and Rossignol 1984). The onset and offset of the response were determined manually using the interval of confidence (P < 0.01) of the SE of the mean of the control activity as a guideline. Evoked responses were included in the analysis if their latency was ≥50 ms and their duration exceeded 5 ms.

For the statistical analysis of phase-dependent responses, the net amplitude of the evoked EMG responses from each individual trial was computed by subtracting the mean area of the control response relative to the phase of the step cycle from that of the evoked response within a given latency range determined on the basis of the averages. Each individual value was then allocated to one of the 10 groups and means and SE of the responses were calculated (see Fig. 2).

To compare background EMG patterns between sessions before and after the denervation, data segments consisting of about 40 consecutive steps at a constant speed were chosen using the video-tapes of the experiments. The onset and offset of each burst of activity in a given muscle were selected by using an interactive custom software. These measures were used to calculate the burst duration and the integrated amplitude of the burst, as well as the phase of activity of the muscle relative to the onset of activity in the lSrt. For display purposes, the activity was computer rectified and averaged with respect to the onset of the period of activity in the lSrt.

To obtain a baseline value for our statistical analysis, we made a weighted average of all of the series of data obtained before the denervation and calculated the interval of confidence (P < 0.01) of the SE of the mean (see Fig. 2B). Averaged evoked responses measured after denervation were considered to be significantly increased or decreased if they fell outside of this confidence level.

To compare changes in the threshold and the gain of the responses evoked by the motor cortex before and after denervation we determined the slope and the intercept of the relationship between response magnitude and stimulus intensity by fitting a nonlinear least-mean square curve according to the equation from Knash et al. (2003)

\[ y = \frac{m}{1 + e^{(h - x)/w}} \]

where \( y \) is the evoked EMG response, \( x \) is the current strength, \( m \) is the maximum evoked EMG response, \( h \) is the stimulus level producing a half-maximum evoked EMG response, and \( w \) is a measure of the width of the curve. This sigmoid curve is similar to the Boltzmann distribution of Devanne et al. (1997).

Histology

At the end of all experimental manipulations, small electrolytic lesions (20–50 μA DC cathodal current) were made through selected wires. The cat was deeply anesthetized and perfused per aortum with formaldehyde. The brain was removed, sectioned, and stained with cresyl violet. In cats with a pyramidal lesion, sections of the brain stem and the cervical and lumbar enlargements were stained using the Swank and Davenport method (Carleton 1967) to reveal degenerating myelin.

RESULTS

Behavior: functional recovery after a unilateral cutaneous denervation

The behavioral effects of a bilateral cutaneous denervation of the hindpaw have been described in detail by Bouyer and Rossignol (2003a). Because there were only minor differences in the effects observed after a unilateral denervation, the behavioral effects of our intervention will not be described in detail.

In brief, in all cats, the unilateral denervation induced a transient deficit that was characterized by the cat dragging the dorsum of the hindpaw along the treadmill during the swing phase of locomotion. This period generally lasted ≤2 wk. Inspection of the video recordings suggests that during this initial stage there was less excursion in all joints of the hindlimb as described by Bouyer and Rossignol (2003a). Later, all of the cats exhibited a prolonged stance phase whereby the limb was extended further behind the body than during the prelesion control. This was normally associated with an exaggerated knee flexion at swing onset. In addition, these cats with unilateral denervation normally exhibited a distinct limb, indicative of asymmetric locomotion.

Changes in EMG activity during the recovery period were, for the most part, compatible with these behavioral changes, and similar to those described by Bouyer and Rossignol (2003a), as illustrated for cat MC23 in Fig. 1. At 6 days after the denervation in the left hindlimb, the major change in activity was observed in the left tibialis anterior (lTA) with smaller changes in the lSrt, the extensor digitorum brevis (lEDB), and the vastus lateralis (lVL). At this stage the cat lightly dragged the dorsum along the treadmill belt. Subsequently, at day 10, there was an increase in the magnitude of the knee flexion and a major increase in the magnitude of the semitendinosus (lST) and the lEDB and a diminution in the activity of the lSrt and the lTA. The level of activity of the lVL was slightly increased. At this stage, the cat no longer dragged its hindpaw. Over the next 3 mo the lST remained elevated and there was a further progressive increase in the level of activity.
of the IVL: the level of activity of the Srt returned to slightly above control levels. There was also a significant increase of activity in the right VL, contralateral to the denervation. Similar compensatory changes in behavior and EMG activity were seen in the other four cats used in this study, albeit with some variations in the exact nature of the changes. In general, sustained increases in the level of activity of the left St and of the left and right VL were seen in all cats after the denervation, whereas changes in the TA and the Srt were more variable. Changes in the left lateral gastrocnemius, in all four
cats, were generally similar to, but smaller than, those observed in the VL. In one cat, MC24, the level of the EMG after the denervation was similar to that observed before the intervention. Nevertheless, the cat successfully compensated for the paw drag induced by the cutaneous denervation (see Discussion).

Stimulation of the motor cortex

Changes in corticospinal efficacy elicited after denervation were investigated in three cats (MC23, 24, and 27) by recording and comparing the EMG responses evoked by intracortical microstimulation during locomotion before and after denervation. Changes in corticospinal efficacy were essentially similar in all cats. However, because of the possible complications engendered by the occasional dystonia observed in cat MC27, emphasis will be placed on the results from cats MC23 and MC24.

During control locomotion (predenervation), stimulation of the motor cortex evoked phase-dependent responses in all recorded hindlimb muscles (see Bretzner and Drew 2005a; Fig. 2A) Both the absolute magnitude and the phase dependency of the responses were relatively constant over the period of the control recordings. As such, calculation of the weighted mean and the interval of confidence of the SE of the mean for these control recordings resulted in a narrow band mostly encompassing the individual responses (Fig. 2B). This interval of confidence was used as a basis for determining changes in response magnitude or phase dependency after the denervation.

After the denervation, there were frequently large, significant increases in the magnitude of the evoked responses. Such changes were seen from all sites in all three cats. Figure 3 illustrates a typical example of the responses evoked by cortical stimulation before and after cutaneous denervation during locomotion in cat MC24. Before the denervation, stimulation during swing evoked a weak, but significant, short-latency decrease in activity in the hip flexor Srt, together with transient increased responses in the knee flexor St and the ankle flexor TA (Fig. 3A, left). Two days after the denervation, the decrease in Srt activity produced by the cortical stimulation was lost and there was a small increase in the amplitude of the St and the TA. In addition, the increase in activity in the TA was substantially prolonged, leading to a major increase in the overall magnitude of the response. At 40 days, the short-latency responses in both the St and the TA were clearly more pronounced, as was the longer-latency response in the TA. In addition, the stimulus now also evoked a substantial longer-latency response in the St. Short-latency–increased responses were now also evoked in the Srt.

Pronounced responses after denervation were also observed during stance (Fig. 3B). Before the denervation, stimulation evoked very small responses in TA and EDB, and a small transient decrease in the level of activity of the VL. Two days after the denervation, the responses in TA and the EDB were larger and there was also a clear evoked response in the St. The transient decrease in activity of the VL was also much more pronounced. At 40 days, responses in St, TA, EDB, and in the extensor VL were still more pronounced and there was also a clear response in the Srt. Note that all of these responses in the flexor muscles occurred at a time in the step cycle when these muscles were inactive during locomotion.

FIG. 2. Phase dependency of the responses evoked by stimulation of one wire (D4) in the motor cortex of cat MC23. A: computer-rectified and averaged responses evoked in St by stimulation of the motor cortex at 7 different phases of the step cycle (there were no evoked responses in phases 0.5–0.7). Filled regions, in this and all other figures, indicate net responses for which we measured the integrated area under the curve (see METHODS). Thin solid line indicates the average EMG activity in unstimulated cycles. B: net integrated responses are plotted as a function of the step cycle for 3 different days before denervation. Shaded area illustrates the 99% CI of the SE of the control responses as calculated from the weighted average of all 3 days. Units on the ordinate are arbitrary. N, number of stimulated cycles.

Inspection of the traces in Fig. 4A shows that the phase-dependent responses in both the Srt and the St were increased 2 days (dotted line) and 40 days (solid line) after the denervation as compared with the control responses (shaded area). Similar increases in the magnitude of the phase-dependent responses were also observed in other flexor muscles, such as the TA, the ankle flexor, extensor digitorum longus (EDL), and
the EDB. Note that in the St and the TA (not illustrated), there were increases in the magnitude of both the short-latency responses and the overall responses (inset box). In the VL, the depression of activity evoked by the stimulation during stance before denervation became more pronounced after the denervation. Similar responses were observed in the other recorded extensors, including the lateral and medial heads of the gastrocnemius, the flexor digitorum longus, and the soleus. Increased responses were also observed in the hip extensor, the biceps femoris.

Similar changes in the phase-dependent responses were evoked by stimulation from most other cortical sites in cat MC24 as well as in the two other cats, as illustrated in Fig. 4B for one example taken from cat MC23. The only major difference in the responses evoked in this animal was the lack of any long-latency responses in the St and the TA.

Figure 5 shows that the denervation resulted in a rapid increase in the magnitude of the evoked responses in all muscles, and from most cortical sites in cats MC23 and MC24; similar results were seen in MC27. In 13/18 (72%) wires recorded from cats MC23 and MC24, the magnitude of the response in the St increased twofold with respect to the control responses within 10 days after the denervation. Moreover, in 9/18 (50%) wires, this increase in magnitude was observed within the first 2 days after the denervation. With respect to the maximum values attained, at all except two sites, the increase in the magnitude of the responses was ≥50% of the largest values observed within 3 wk of the denervation. In the St, final increases of ≥250% of control magnitude were observed in 14/18 (78%) electrodes and in 8/18 (44%) cases, changes of >500% were seen. In the VL, responses were also pronounced, in this case representing a more pronounced decrease in activity (plotted as a negative value in Fig. 5). Overall, the increase in the magnitude of the responses late in the recovery period in the two cats averaged 1,042% (median 658%) in the Srt, 1,235% (median 391%) in the St, and -788% (median -497%) in the VL.
Stimulation through most cortical electrodes evoked increased responses in most of the muscles that we recorded. However, inspection of Fig. 5 illustrates that the relative change in activity evoked in the different muscles varied from one electrode to another. For example, the wire D4 (thick black line in Fig. 5A) was one of the most effective electrodes in producing increased activity in the St but evoked relatively weak changes in Srt, when compared with those evoked from other electrodes. In contrast wire D6 in the same cat (dotted blue line in Fig. 5A) produced relatively large increases in the level of activity in the Srt and relatively weak responses in the St. Similar differential effects were also observed for many of the electrodes in cats MC23 and MC27. These differential effects are quantified in Fig. 6 for wires D4 and D6 from cat MC24 as well as for two other wires A2 (orange line in Fig. 5A) and A6 (brown line in Fig. 5A) in the same cat. Inspection of this figure clearly shows the differential nature of the responses evoked in Srt and EDB by wires D4 and D6 and shows a similar property for wire A2, which also produced the largest percentage increases in the Srt. In contrast, wire A6 produced similar increases in the Srt and EDB but only small changes in the activity of the St and TA.

In addition to increasing the relative magnitude of responses evoked by the motor cortex at any one stimulus intensity, the denervation also increased the gain of the response. Figure 7, A and B illustrates the relationship between the magnitude of the response evoked in the St at the onset of swing and the intensity of the stimulation for two electrodes in cat MC23. Before the denervation (dashed lines), increasing stimulus intensity produced a relatively linear increase in the response magnitude up to a value of 75 μA. Intensities greater than this value produced little further increase in magnitude, leading to the plateau observed in the graphs. After the denervation, the gain of the relationship was modified. For example, comparison of the graphs obtained at days 32 and 41 in Fig. 7A with the control graphs clearly shows that the magnitude of the response for any one intensity of stimulation was greater after the denervation. Moreover the slope of the relationship at days 32 and 41 was greater than that during the control measures. In addition, in most wires, it was also clear that the amplitudes of responses evoked at the lowest intensities used were also increased (Fig. 7B). This suggests that there was also a decrease in the threshold of the responses, although because we used a fixed series of intensities for these experiments, this was not tested directly.

Application of the Boltzmann equation to this data (see METHODS) showed that the slope of the relationship between response magnitude and stimulus intensity was clearly increased after the denervation, and this increase in gain was progressive. For example, the data illustrated in Fig. 7C show a significant relationship between the slope of the relationship and the time after denervation for wire B1. In the case of wire
B6 (Fig. 7D), there was also a positive relationship between slope and the length of time after denervation but, in this case, it was not significant. Altogether there was a positive relationship between slope and time postdenervation for 9/11 wires, indicating an increase in the gain of the relationship over time; in only wire B1, however, was the relationship significant at the $P < 0.05$ level.

To determine whether these increased relationships were simply a result of an increased background of EMG activation resulting from the denervation (see Fig. 1), we plotted the values for the slope of this relationship as a function of the integrated value for the EMG calculated from the unstimulated cycles at the same phase as the stimulation was applied. The results of this analysis are shown in Fig. 7, E and F for wires B1 and B6. Both of these wires showed a negative, nonsignificant relationship between slope and background EMG activity. Overall, all 11 electrodes showed a negative relationship between these two values and this was significant in three out of 11, which suggests that the changes in the magnitude of the responses evoked by the motor cortex are largely independent of any changes in the background EMG activity.

To determine whether there was any relationship between the magnitude of the effects evoked in different muscles before the denervation and the extent of the increase in magnitude observed after denervation we rank-ordered the wires in cats.
MC23 and MC24 according to the magnitude of the response evoked in the St before the denervation (Fig. 8; see also Fig. 7 in Bretzner and Drew 2005a). The results shown in Fig. 8 for MC24 were typical of those also obtained in MC23. In general, the electrodes that evoked the largest responses in the St before denervation (at the bottom of each display), also evoked the largest responses after the denervation (Fig. 8A, left). However, the wires evoking the smallest responses before denervation (at the top) also evoked large responses after denervation. As such, the largest percentage increases in cat MC24, with respect to the values before denervation, were obtained from the wires that were the least effective before the denervation (Fig. 8B).

Similar modifications were also seen in the other muscles. For example, the largest responses evoked in the Srt (Fig. 8A, middle) were from those wires that evoked the smallest responses in the St. Nonetheless, as a percentage of the predenervation control values, the largest increases in activity were observed from the wires causing the largest responses in the St. The changes in the responses evoked in the TA (Fig. 8, right) resembled those observed in the St, and this despite the fact that the wires producing the largest responses in the TA before denervation were clearly different from those producing the largest responses in the St (see Bretzner and Drew 2005a).

Cortical versus spinal plasticity

In an attempt to determine the extent to which the increased corticospinal efficacy might arise from changes in the efficacy of the motor cortex or spinal interneuronal networks, we quantified changes in the magnitude of the responses evoked by the pyramidal tract and crossed cutaneous reflexes after denervation in three cats (MC24–26). Because EMG responses evoked by stimulation of the pyramidal tract are likely to be independent of the level of cortical activity (see Discussion), they provide an indication of the effect of changes in excitability of spinal interneuronal networks on corticospinal efficacy. Similarly, stimulation of the cutaneous nerves also provides an indication of changes in spinal excitability that are independent of changes in cortical excitability.
Stimulation of the pyramidal tract

Figure 9A illustrates a typical example of the responses evoked during the swing phase of the step cycle by stimulation of the pyramidal tract before and after cutaneous denervation in cat MC26. Before denervation, stimulation during swing evoked an increase in activity in the hip flexor Srt and the knee flexor St (see also Fig. 9B, left). There were also increases in activity in the ankle flexor TA and the knee extensor VL (not illustrated). After the denervation, there were increases in the magnitude of the responses in most flexor muscles that, at plateau, attained 178% of the control in the Srt and 327% of control in the St (Fig. 9A, B, and C, left). There was no change in the depression of the activity observed in the extensor muscles (not illustrated). Similar changes in the magnitude of the Srt were observed in cat MC25, whereas the change in the magnitude of the St was slightly larger (593%) (Fig. 9B and C, middle). In cat MC24, the effects of the pyramidal stimulation were unchanged after the denervation (Fig. 9B and C, right).

Stimulation of cutaneous crossed reflexes

The magnitude of the cutaneous crossed reflexes produced by the three nerves tested—the superficial peroneal, saphenous, and tibial nerves—was unchanged by the denervation. This is clearly shown in Fig. 10, A and B (left) for recordings made from the left Srt and TA after stimulation of the right superficial peroneal nerve. Almost identical findings are illustrated in Fig. 10B (middle and right) for stimulation of the same nerve in two other cats.

Pyramidotomy

To determine whether the integrity of the corticospinal tract is necessary for the changes in the magnitude of the cortical-evoked responses after the denervation we attempted to lesion the pyramidal tract at the level of the medulla oblongata in two cats. In one of these cats (MC23) the lesion almost completely transected the pyramidal tract as illustrated in Fig. 11A. Inspection of this figure shows the physical extent of the damage in the right pyramid (top) and the course of the degenerating fibers at the level of, from top to bottom, the pyramidal decussation, the cervical, and the lumbar spinal cord. Degenerating myelin is clear in both the left dorsolateral and right ventromedial funiculus at the cervical level of the spinal cord and is also visible in a similar location in the lumbar spinal cord, demonstrating that corticospinal fibers innervating lumbar regions were transected by the lesion. After this lesion, the cat dragged the dorsum of both the forepaw and the hindpaw along the treadmill during the swing phase of locomotion (Fig. 11B). In the forelimb, this deficit recovered over a period of a few days, whereas the hindlimb continued to drag lightly for the remainder of the experiment (20 days). Microstimulation through all of the cortical wires at the same intensities used before the lesion was ineffective in producing muscles responses after the pyramidotomy, either at rest or during loco-
motion. Figure 11C shows one example in which stimulation after the denervation but before the pyramidotomy produced clear short latency responses in the St and TA at a strength of 25 μA. After the pyramidotomy, these responses were lost even at current intensities of 75 μA.

In cat MC24 the lesion was only partial. Nonetheless, there was an increase in the threshold current intensity (between 230 and 1,200%) required to evoke movements at rest in all seven electrodes tested. Similarly, the responses evoked in the St by stimulation during locomotion (at the same intensities as used before the pyramidotomy) were decreased. In three out of seven wires the stimulation no longer evoked responses in St, whereas in the other four out of seven the responses in St were reduced by 45–88% of the magnitude observed before the pyramidotomy.

**DISCUSSION**

Cutaneous denervation of the hindpaw produced locomotor deficits that were rapidly compensated by modifying the level of the background EMG activity in both flexor and extensor muscles. This recovery of function was accompanied by rapid and pronounced increases in the magnitude of the responses that were evoked by cortical stimulation. We suggest that this increase in corticospinal efficacy contributes to the recovery of
The finding that the responses evoked by stimulation of the pyramidal tract were also increased (albeit more modestly) suggests that the increased corticospinal efficacy reflects changes at both the cortical and the spinal levels.

**Functional recovery after the denervation**

The overall changes that we observed after the denervation were very similar to those detailed by Bouyer and Rossignol (2003a) after a bilateral cutaneous denervation. As in their study, the major deficit that we observed was a transient inability to flex the leg sufficiently to prevent the paw dragging on the treadmill surface at the onset of swing. The cats rapidly compensated for this deficit by modulating the level of activity in different flexor and extensor muscles throughout the limb. Similar rapid changes in the level of EMG activity have been described after other interventions designed to examine locomotor plasticity, notably after motor neurectomy of either ankle flexors (Carrier et al. 1997) or ankle extensors (Bouyer et al. 2001; Misiaszek and Pearson 2002; Pearson and Misiaszek 2000; Pearson et al. 1999; Whelan and Pearson 1997).

In our study, as in that of Bouyer and Rossignol (2003a), a major contribution to the recovery of function was made by increased activity in the St and changes in the activity of this muscle were seen in all cats. This increased activity would serve to increase the flexion of the knee and raise the paw away from the treadmill belt. Changes in the amplitude of the other flexor muscles would serve to increase the flexion of the hip or the ankle. It should also be emphasized, however, that cat MC24 recovered despite any lack of overt increased activity in the major muscles that we recorded. This suggests that in some cases, the ability to simply restore a pattern similar to that observed in the control situation is sufficient to compensate for the deficit. Finally, it should also be noted that because the lesion that we used was unilateral there were also compensatory changes in the other hindlimb, consisting of an increase in the level of the extensor muscles, such as the VL. We suggest that this increase in extensor muscle activity would serve to elevate the level of the pelvis during the swing phase of the denervated limb and thus also contribute to the compensation.

**Unilateral denervation increased corticospinal efficacy**

The major finding from these experiments was that the unilateral denervation led to a rapid increase in the magnitude of the responses evoked by stimulation of the motor cortex. We consider these increased responses as being indicative of an overall change in the efficacy of the corticospinal projection. Several factors support this suggestion and argue against the results being produced by other, unrelated, changes, such as movement of the cortical wires.

First, we recorded control activity over a period of several months before the denervation and the magnitude of the evoked responses changed very little over this period (see e.g., Figs. 2 and 5). Second, changes in corticospinal efficacy were observed from stimulation of nearly all of the implanted electrodes and in all of these cases the change in corticospinal efficacy began shortly after the denervation. (Fig. 5). Third, we...
successfully antidromically activated several neurons recorded from these electrodes by stimulating the pyramidal tract both before and after the denervation (although never the same neuron). Fourth, there were no changes in the impedance of the electrodes over time. The contention that changes in corticospinal efficacy are independent of any changes in the properties or location of the microwire electrodes is fully in agreement with previous studies that have also argued that such electrodes have limited movement over extended periods of time (Armstrong and Drew 1985; Palmer 1985, 1990).

We believe that the changes in corticospinal efficacy that we observed underlie the accompanying changes in the functional recovery of locomotion. This is supported by several lines of evidence. For example, changes in the level of the EMG activity occurred in the first few days after the denervation and progressively increased over the next few weeks before reaching a plateau. In most cats, these changes in EMG activity resulted in the cat compensating for the paw drag within the first 2–3 wk after the denervation (see Fig. 11B). The changes in corticospinal efficacy followed a similar time course with

FIG. 11. A: histological sections from cat MC23 showing the site of the lesion of the pyramidal tract on the right side (arrow: top), and the degenerating axons (black dots) at (from top to bottom) the level of the decussation, the cervical spinal cord, and the lumbar spinal cord. Arrows on the spinal cord sections indicate the location of the degenerating axon in the left, contralateral, dorsolateral funiculus. B: percentage of steps in which a dragging of either the fore- or hindpaw was observed. Values are based on inspection of 50 consecutive steps. C: responses evoked by cortical stimulation at 25 µA at one site before pyramidotomy (top) and at 75 µA after pyramidotomy (bottom).
the responses evoked from most electrodes showing a rapid change in magnitude over the first 2–3 wk and then either reaching a plateau or increasing in magnitude more slowly. Although it is possible that the changes in the magnitude of the evoked responses are simply a function of the increased level of background EMG activity, we feel that this is unlikely. Figure 3B, for example, shows that, after the denervation, there were substantial responses evoked in some flexor muscles during the stance phase of locomotion at a time when these muscles are inactive and presumably hyperpolarized (Jordan 1984). In addition, direct comparison of the gain of the relationship between response magnitude and current intensity as a function of background EMG activity showed no positive relationships (Fig. 7, E and F). The increased responses are thus more likely because of changes in cortical or spinal, interneuronal excitability (see next section).

Further support for the view that these changes in corticospinal efficacy contribute to the recovery of function comes from the two cats in which we performed a pyramidotomy after several months of recovery. Both of these animals showed immediate deficits after the transection, characterized by dragging of the fore- and hindpaws. This is characteristic of the results of damage to the corticospinal system at any level (see Armstrong 1986; Drew et al. 1996). However, in otherwise intact cats, these deficits are transitory and there is complete recovery over a period of several days. In the cats with a unilateral denervation, the deficits in the hindpaw remained over a period of several weeks whereas those in the forepaw quickly disappeared (Fig. 11B). This suggests that after damage of the corticospinal system, the animals were no longer able to fully compensate for the denervation. Similarly, our preliminary results (Bouyer et al. 2000) show that a lesion to the hindlimb representation of the motor cortex before hindpaw denervation delays or prevents recovery. Together, these results support a view that the cortex contributes to this recovery of function.

There are few other studies that have specifically examined changes in corticospinal efficacy after insult to either the peripheral or CNS. Moreover, even those that have examined this issue have mostly studied only the immediate changes that follow a temporary intervention. For example, evidence for changes in corticospinal efficacy have been observed both after anesthesia (Murphy et al. 2003; Rossi et al. 1998) and after ischemic nerve block (Brasil-Neto et al. 1992, 1993; Ridding and Rothwell 1995, 1997; Ziemman et al. 1998a,b, 2001, 2002; McNulty et al. 2002) of a limb. Such changes, however, immediately disappear after removal of the anesthesia or nerve block and are likely to involve different mechanisms from those underlying the long-term changes observed in this study.

The use of multiple, chronically implanted microwires not only allowed us to compare the postdenervation effects in any one electrode to its own control but also provided the opportunity to determine whether there were differences in the changes in corticospinal efficacy elicited from different cortical sites. This, indeed, seemed to be the case. For example, as illustrated in Fig. 6, cortical site D6 in cat MC24 produced significant changes in Srt but not in the other flexor muscles, whereas wire D4 in the same cat produced substantial changes in the activity of the St, TA, and EDB, but not of the Srt. This variability argues against the changes being the result of some homogeneous, global change in activity and argues for specificity in the action exerted from different regions of the motor cortex. This contention is also supported by inspection of Fig. 8, which compares the magnitude of the responses evoked after the denervation with those obtained during the control period. This figure clearly shows that, although some wires produced major increases in response in most muscles (wires 6 and 7, Fig. 8), others produced large responses in the St and the TA but only minimal increases in the responses evoked in the Srt (e.g., wires 1 and 2, Fig. 8). This further supports the view that the responses are not the result of some global increase that produces nonspecific increases in corticospinal efficacy.

**Mechanisms**

There are several possible explanations for the changes in corticospinal efficacy that we observed in these experiments. These can be broadly divided into changes in excitability of the spinal interneuronal networks onto which the motor cortex impinges or changes in excitability of the cortical sites stimulated. We tried to differentiate between these possibilities in these experiments by also studying the effects of the denervation on the effects evoked by stimulation of the pyramidal tract and by stimulation of cutaneous nerves in the contralateral, intact hindlimb.

Even though the responses evoked by stimulation of the cortical microwires might be influenced by changes in cortical excitability, those evoked by stimulation of the axons of the pyramidal tract would be expected to be largely independent of any such cortical excitability changes. This is similar to the situation in humans in which it has been shown that the responses evoked by transcranial magnetic stimulation (TMS) are influenced by cortical excitability, whereas those evoked by transcranial electrical stimulation, which activates corticospinal axons directly, are largely independent of such changes in excitability (Edgley et al. 1990; Petersen et al. 2003). In our experiments, changes in corticospinal efficacy were clearly observed after pyramidal tract stimulation (although not universally). Such changes suggest that there is some increase in the excitability of the spinal interneuronal pathways onto which these cortical sites impinge. This would be compatible with the results from the experiments of Bouyer and Rossignol (2003b) showing changes in the locomotor capacity of cats spinalized after a denervation. As they have argued, this strongly suggests the existence of long-term, plastic changes in spinal circuits.

We also found that there was no change in the magnitude of the crossed reflex responses evoked by stimulation of the contralateral cutaneous nerves (Fig. 10, A and B). This supports our suggestion made in a preceding section that the changes in efficacy are not the result of any global changes in excitability. This result also suggests that the interneuronal populations that mediate the signals conveyed by the corticospinal tracts are separate from those that are activated by the afferents from the contralateral limb. This is compatible with the results from our previous work (Bretzner and Drew 2005b) showing that conditioning stimuli to the motor cortex generally facilitate the reflex responses from the limb contralateral to the stimulation site (corresponding to the denervated limb in this study) but is without effect or depresses the crossed reflex responses.

Although we argue for some changes in spinal excitability, there are also reasons to believe that changes in corticospinal
excitability also contribute to the observed changes in corticospinal efficacy. For example, the changes in response magnitude evoked by stimulation of the cortical microwires were frequently substantially larger than those evoked from the pyramidal tract. Nevertheless, caution must be exercised in drawing conclusions solely on this basis because it is possible that the populations of corticospinal axons activated by the pyramidal stimulation might not be entirely coincident. Moreover, as we have argued previously (Bretzner and Drew 2005a,b), it seems likely that the effects evoked by the pyramidal tract stimuli might give only a very general idea of the strength and connectivity of the corticospinal projections.

The position that the recovery of function involves changes in corticospinal excitability is, nonetheless, also supported by our finding that lesion of the pyramidal tract or of the hindlimb representation of the motor cortex (Bouyer et al. 2000) subsequent to functional recovery leads to a restoration of many of the initial deficits, suggesting that corticospinal drive is essential for a full recovery (see also Bouyer and Rossignol 2003b).

Given that the changes in functional recovery and corticospinal efficacy occur rapidly after the denervation, it is possible that increases in the discharge frequency of pyramidal tract neurons implicated in regulating hindlimb muscle timing and activity during locomotion (Drew et al. 2002; Widajewicz et al. 1994) contribute at least to the initial changes in corticospinal efficacy. Indeed, such increased activity in the corticospinal (and probably the rubrospinal) pathway will be needed to compensate for the decreased spinal excitability that might be expected after the loss of major portion of the cutaneous input from the hindpaw. This is also compatible with the results from the experiments of Rispal-Padel (Meftah and Rispal-Padel 1994; Rispal-Padel and Meftah 1992) who showed changes in the excitability of thalamocortical and intracortical circuits after conditioning of a forelimb flexion movement.

Nevertheless, the fact that the time course of the overall changes in corticospinal efficacy, evoked from the cortical electrodes, and the changes in corticospinal excitability produced from stimulation of the pyramidal tract followed a very similar time course, makes it impossible to determine whether one mechanism or the other was evoked earlier than the other. Indeed, the results suggest that changes in cortical and spinal excitability may occur simultaneously.

It is also probable that there are concomitant changes in the cortical motor representation of muscles, as has been described after facial nerve section or amputation (see INTRODUCTION). Although we could not address this issue directly because our electrodes were chronically implanted, a change in cortical representation is implicit in the finding that stimulation in a given site, at a given strength, frequently recruited muscles that were not activated by stimulation preceding the denervation (see Ridding and Rothwell 1995, 1997).

Conclusions

To conclude, this study clearly shows that there is a cortical contribution to the mechanisms that underlie functional recovery of locomotion after the loss of cutaneous input from the paw. This serves to emphasize the importance of the corticospinal tract in the regulation of locomotion, demonstrating that the motor cortex not only contributes to voluntary modifications of the gait pattern but also has the capacity to modulate the base locomotor pattern in response to injury. Indeed, it is probable that the motor cortex will contribute to step-by-step modifications of gait under a wide variety of circumstances. The study also serves to emphasize the close interaction and integration of supraspinal and peripheral inputs in the regulation of locomotion. Taken together with the results presented in a previous publication (Bretzner and Drew 2005b), it seems clear that corticospinal and cutaneous pathways act through common interneuronal populations to modulate the activity of the hindlimb musculature during locomotion. Finally, the results serve to emphasize that damage to the peripheral nervous system can produce plastic changes in the corticospinal system that have the capacity to produce major changes in the efficacy of this pathway in modulating motor activity and, probably, reflex activity.

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