Motor Cortical Modulation of Cutaneous Reflex Responses in the Hindlimb of the Intact Cat

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Bretzner, Frédéric and Trevor Drew. Motor cortical modulation of cutaneous reflex responses in the hindlimb of the intact cat. J Neurophysiol 94: 673–687, 2005; doi:10.1152/jn.01247.2004. We have used the technique of spatial facilitation to examine the interactions between the signals conveyed by the corticospinal tract and those of cutaneous afferents in the hindlimb of the intact, walking cat. Microstimulation was applied to 20 cortical sites in the hindlimb representation of the motor cortex and to three different cutaneous nerves innervating the hindpaw in four cats. Conditioning stimuli to the motor cortex induced both facilitation and depression of cutaneous reflexes evoked by stimulation of nerves in the hindlimb contralateral to the cortical stimulation site. Facilitation was most frequently evoked by conditioning stimuli in the range of 10–30 ms before the cutaneous stimulation; depression was normally evoked by shorter and longer conditioning delays. Similar changes were observed after conditioning stimuli to the pyramidal tract, suggesting that the changes were independent of any changes in cortical excitability. Modulation of reflex activity varied according to the muscle under study, the cutaneous nerve used to evoke the reflex and the cortical site used to condition the reflex. Together, these results suggest that there is spatial convergence of corticospinal and cutaneous afferent activity and that this convergence is mediated by distinct subpopulations of spinal interneurons.

INTRODUCTION

Most goal-directed voluntary movements require the integration of feed-forward signals from the cortical structures controlling the movement with the feedback signals generated by the peripheral afferent receptors that are activated by that movement. This is particularly true for locomotion in which cutaneous feedback both provides information on the nature of the substrate (Bouyer and Rossignol 2003; Engberg 1964) and signals unexpected perturbations, particularly during the swing phase (Abraham et al. 1985; Duysens and Loeb 1980; Duysens and Stein 1978; Duysens et al. 1980; Forssberg 1979; Loeb 1993; Pratt et al. 1991; Prochazka et al. 1978; Wand et al. 1980).

The results from electrophysiological experiments in reduced preparations suggest that cutaneous reflex activity is subject to regulation from supraspinal structures, including the motor cortex. For example, in one early series of experiments, Lundberg and Voorhoeve (1962) showed that stimulation of the motor cortex in the anesthetized cat increased the magnitude of the responses evoked in hindlimb flexor and extensor motoneurons by stimulation of flexor reflex afferents, including low-threshold cutaneous afferents. Later experiments confirmed and extended these observations by showing clear spatial convergence of corticospinal and cutaneous afferent inputs onto motoneurons innervating both the forelimbs (Illert et al. 1976, 1977) and the hindlimbs (Fleshman et al. 1988; Pinter et al. 1982). In both limbs, the minimal corticospinal connection to motoneurones is disynaptic, although cutaneous pathways in the hindlimb are more frequently trisynaptic (Burke 1999).

From a functional viewpoint, however, there is very little information on the effects of concurrent activation of the corticospinal and cutaneous pathways. Certainly, there is abundant evidence that cutaneous afferent information modulates cortical neuronal activity (Asanuma 1981) and that, during locomotion, this afferent information is itself modulated according to the dynamics of the step cycle (Chapin and Woodward 1982; Marple-Horvat and Armstrong 1999; Palmer et al. 1985). There is also evidence from experiments using transcranial magnetic stimulation (TMS) in humans that cutaneous afferent stimulation may initiate a transcortical reflex loop that participates in the production of the longer latency responses evoked by cutaneous stimulation both during quiet standing (Nielsen et al. 1997; Wolfe and Hayes 1995) as well as during the swing phase of locomotion (Christensen et al. 1999; Pijnappels et al. 1998). However, there is no information about the capacity of corticospinal afferents to modulate the short-latency responses evoked during locomotion. Moreover, TMS activates a relatively large area of the cortex and leaves open the question of the extent to which more focal stimulation of the cortex may differentially modulate reflex pathways.

In the present manuscript, we address these issues by using the technique of spatial facilitation (Lundberg 1964) to examine the effects of intracortical microstimulation on the reflex responses evoked from different cutaneous nerves of the hindlimb in intact, walking cats.

METHODS

Care and training

Experiments were carried out on four male cats (weight: 4.2–5.5 kg) trained to walk at a comfortable and constant speed (circa 0.35–0.45 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods ≥20 min. These were the same four animals that were used in our previous experiments detailing the contribution of the motor cortex to the structure and the timing of hindlimb locomotion in the cat (Bretzner and Drew 2005). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Surgical procedures

The surgical procedures used in these experiments are detailed elsewhere (Bretzner and Drew 2005). In brief, in three cats (MC24, MC26, and MC27), microwire electrodes (Tri-ML insulated stainless steel: 25 μm diam) attached to a miniature connector (Neuralynx: EIB27) were manually inserted, one at a time, into the posterior bank of the cruciate sulcus that contains the hindlimb representation of the motor cortex (Armstrong and Drew 1984b; Bretzner and Drew 2005; Nieoullon and Rispal-Padel 1976; Widajewicz et al. 1994). In all three cats, the wires were implanted on the right side. Appropriate positioning of the microwires was facilitated by recording neuronal activity and by stimulating through the wires as they 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. In one cat, MC25, a recording chamber was positioned over the right motor cortex (Drew 1988, 1993; Widajewicz et al. 1994). Penicillin (Novopharm: 40000 UI/kg iv) and analgesics (buprenorphine hypochloride: 5 μg/kg) were provided at the beginning and at the end of each surgery and for ≥48 h after surgeries. Antibiotics (cephadroxil: 100–200 mg/day) were administrated daily for the duration of the experiment.

One to 2 wk after recovery from the initial surgery, the cats were anesthetized with isoflurane (2–3% with oxygen) to complete the surgical procedures. In all cats (MC24–MC27), multiple pairs of Teflon-insulated, braided stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record electromyographic (EMG) activity during locomotion. These muscles included physiological flexor and extensor muscles acting around all of the major joints of the hindlimb contralateral to the motor cortex stimulated (see Bretzner and Drew 2005 for a list of these muscles and their major functions). Microwires were also implanted in the right pyramidal tract at P7 (Drew 1993) to allow comparison with the responses evoked by cortical stimulation. In addition, in all cats (MC24–MC27), cuff electrodes (Julien and Rossignol 1982) were implanted around the saphenous, superficial peroneal and tibial posterior nerves of the left and right hindlimb (Fig. 1). The superficial peroneal nerve innervates primarily the dorsal surface of the paw while the tibial posterior nerve innervates the ventral surface. The saphenous nerve innervates the skin on the medial side of the lower hindlimb as well as part of the dorsal surface of the paw (see Crouch 1969). Table 1 summarizes the number of cortical and pyramidal sites stimulated with each cutaneous nerve in all four cats.

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 tested the integrity and threshold of all chronically implanted electrodes. For stimulation of the cortex and of the pyramidal tract, we used trains of stimuli (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms) adjusted to produce a small response in the left (contralateral) semitendinosus, St, when the stimulus was applied during locomotion at the onset of swing (defined as a threshold response). In all experiments the stimulation of the motor cortical electrodes was delayed 50 ms with respect to the onset of activity in the anterior head of the sartorius, Srt. For stimulation of the cuff electrodes implanted around the nerves of the left hindlimb (contralateral to the motor cortex), we used single pulses (pulse duration 0.2 ms) and also adjusted the intensity to produce a small response in the St at the same delay of 50 ms (with respect to Srt).

A similar procedure was used for stimulation of the nerves in the right hindlimb, so that stimulation occurred at the onset of swing in the left hindlimb. In this case, the evoked EMG responses represent crossed cutaneous reflexes. In some cases, stimulation of the nerves in the right hindlimb were ineffective in eliciting responses in the left St; in these cases, we adjusted the stimulus intensity to evoke responses in either the Srt or the tibialis anterior (TA). In the case of the experiments performed in the cat with the recording chamber, the electrode was advanced to layer V (as verified by antidromic activation of neurons by stimulation of the pyramidal tract) and threshold was then verified as for the implanted cortical wires.

The threshold for each nerve and cortical site was verified at the beginning of each experimental session. Once the current strength was appropriately adjusted, the stimulation of the cortex/pyramid and the cutaneous nerve were combined, with the cutaneous stimulation (test stimulus = T) always being delayed with respect to the onset of the cortical stimulation (conditioning stimulus = C). The cortical stimulation thus served to condition the later cutaneous reflex. The electrical stimuli to the cutaneous nerves were applied at different intervals (C-T interval) following the conditioning stimulus, ranging from 0 to 70 ms (Fig. 2, A and B). The test stimuli were interleaved so that we initially tested C-T intervals of 0, 10, 20 . . . 70 ms and then 5, 15, 25 . . . 65 ms. This assured that any changes in response magnitude were not simply related to general changes in excitability. Note that at

![](https://via.placeholder.com/150)

**FIG. 1.** A: view of medial side of the distal hindlimb illustrating the 3 nerves that were stimulated in this study together with the location of the cuff electrodes used to stimulate these nerves. B: the approximate area of innervation of each of the 3 nerves is indicated by the shaded regions. Saph, saphenous nerve; SP, superficial peroneal nerve; Tib, tibial posterior nerve. Adapted from Crouch (1969).

**TABLE 1.** Cutaneous nerve stimulated

<table>
<thead>
<tr>
<th>Cutaneous nerve stimulated</th>
<th>Left Cutaneous Reflexes</th>
<th>Right Cutaneous Reflexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial Peroneal</td>
<td>Tibial Posterior</td>
</tr>
<tr>
<td></td>
<td>Saphenous</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC24</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>MC25</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>MC26</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MC27</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>12</td>
</tr>
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</table>

**A. Stimulation of the right motor cortex**

<table>
<thead>
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<th>Cat</th>
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<th>Tibial Posterior</th>
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</thead>
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<td>MC24</td>
<td>—</td>
<td>5</td>
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<tr>
<td>MC25</td>
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<tr>
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<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

**B. Stimulation of the right pyramidal tract**

<table>
<thead>
<tr>
<th>Cat</th>
<th>Superficial Peroneal</th>
<th>Tibial Posterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC24</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>MC25</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>MC26</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MC27</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Number of experiments in which cortical or pyramidal tract stimulation was combined with stimulation of different cutaneous nerves.
a C-T interval of 0 ms both the motor cortical and the cutaneous stimulus were delayed 50 ms with respect to the onset of swing. Ten to 25 repetitions were applied at each delay. In some cases, control responses evoked by the cortical, pyramidal or cutaneous nerve stimulation alone were recorded during the experiment (see DISCUSSION) to ensure that the small additional delay (with respect to Srt onset) of the cutaneous stimulus had no effect on the results. Because of the time required to perform each series of experiments, we normally performed a maximum of two series of stimuli in any one experimental session; in a few exceptional cases three series were performed.

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.

Data analysis

Data were analyzed as previously described (Bretzner and Drew 2005; Rho et al. 1999). The responses evoked by the stimuli were computer-rectified and averaged and displayed on a monitor. The average activity from a similar time period taken from unstimulated cycles was superimposed on this display (Bretzner and Drew 2005; Drew and Rossignol 1984). The onset and offset of the responses were determined manually using the interval of confidence (P < 0.01) of
the SE 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 each stimulus, we initially examined the averaged responses and determined the latency of the onset and offset of those responses that were greater or less than the interval of confidence for each of the recorded muscles. We used these values to calculate the integrated value of the traces within this window for each individual stimulus. We then calculated the net amplitude of the responses by subtracting the values obtained from the unstimulated cycles from those in which stimulation was applied. These values were averaged to produce a mean response together with the SD and the SE.

To determine the effect of the conditioning stimuli, we initially calculated the magnitude of the responses evoked by stimulation of the motor cortex (or pyramidal tract) and the nerve alone. These values were then added algebraically and an interval of confidence (P < 0.01) for the SE of the mean was calculated from these values. This control level of activity is plotted as a gray shaded region on the graphs of Figs. 2–6. Mean values that were greater than the interval of confidence were considered to be evidence of a facilitation of the response and those that were less, of a depression.

**Histology**

At the end of the experimental sessions, the animals were deeply anesthetized with sodium pentobarbitol (Somnotol) and perfused per cardium. The brain was removed and sectioned in the parasagittal plane before being stained with cresyl violet.

**RESULTS**

The effects of cortical and pyramidal tract conditioning stimuli on the magnitude of cutaneous reflexes evoked from different cutaneous nerves was investigated in four cats (Table 1). Conditioning of reflex responses evoked from the left hindlimb by the motor cortex was studied in depth for three cutaneous nerves in cat MC25; supplementary data were obtained from two other cats. Conditioning reflex responses in the right hindlimb were obtained in four cats, primarily for the saphenous and superficial peroneal nerves. In addition, conditioning stimuli to the pyramidal tract were studied for all 3 nerves from both limbs. All of the cortical sites stimulated were within the caudal bank of medial aspect of the cruciate sulcus and produced twitch responses in hindlimb muscles; these sites are detailed in Bretzner and Drew (2005).

*Cortical modulation of cutaneous reflexes in the left hindlimb*

Figure 2 illustrates a representative example of the effects of a conditioning stimulus delivered at a site in the right motor cortex on the reflex effects evoked in the left St by stimulation of the left saphenous nerve. Just suprathreshold stimulation of both the motor cortical site and of the saphenous nerve evoked relatively small responses in the St (Fig. 2, A and C). Simultaneous stimulation of the motor cortex and the saphenous nerve (C-T interval = 0 ms) evoked a response that was slightly smaller than the algebraic sum of the responses to the individual stimuli (Fig. 2, C and E). However, the combination of both stimuli at C-T intervals of ≥10 ms evoked a response that was more than twice the magnitude (216%) of the algebraic sum of the responses evoked individually. Longer C-T intervals produced progressively smaller responses, and at a C-T interval of 40 ms, the response was almost abolished. Note that the response evoked by stimulation of the saphenous nerve alone at a delay of 70 ms after the onset of activity in the St (i.e., 20 ms after the motor cortical stimulation would have been given; Fig. 2D, bottom) is similar to the control response (Fig. 2C), demonstrating that the enhanced responses were not simply due to the small change in the relative phase of application of the stimulus. Although the cortical response at this same time (Fig. 2D, top) was slightly greater than in the initial control recording (Fig. 2C, top), the combined response did not exceed the limits of the interval of confidence based on the initial measures (Fig. 2E).

Figure 3, A–D, illustrates the results from stimulation of a different cortical site in the same cat on the reflex effects evoked by the left saphenous nerve on selected flexor and extensor muscles. As in Fig. 2, the strength of each stimulus was adjusted to produce just suprathreshold responses in the St when delivered alone. No noticeable change in amplitude was observed when the motor cortex and the saphenous nerve were stimulated simultaneously (C-T interval = 0 ms). However at intervals of 15–35 ms, reflex amplitude was increased in the St, and at intervals of 20–35 ms, there was also a pronounced facilitation of the reflex responses evoked in the ankle extensor, gastrocnemius lateralis (GL). No changes were observed in the amplitude of the TA.

As illustrated in the graphs of Fig. 3D, the cortical facilitation of reflex effects in the St reached 400% of the algebraic sum of the two stimuli applied independently and facilitation of the response in the GL reached almost 300% of control. Similar effects on reflex activity in these three muscles were observed from conditioning stimuli to the pyramidal tract in the same cat, MC25 (Fig. 3E). As for the single cortical site, the pyramidal tract stimulation evoked facilitation of the responses evoked in the St and the GL and was without effect on the TA. In this example, the facilitation of the St was relatively less than that produced by stimulation of the cortical site illustrated in Fig. 3D while facilitation of the GL was substantially greater. Very similar responses were also observed for stimulation of cortical sites and the pyramidal tract in other cats.

*Cortical modulation of left superficial peroneal and tibial posterior reflexes in the left hindlimb*

Cortical stimulation also modified the responses evoked from other cutaneous nerves of the left, contralateral, limb. For example, Fig. 4, A–D, shows that stimulation of the same cortical site as illustrated in Fig. 3, A–D, also modulated the responses evoked from the superficial peroneal nerve. However, in this case, the reflex responses evoked in both the St and the GL were depressed at C-T intervals from 0 to 15 ms and weakly facilitated at conditioning intervals of 25 to 35 ms. The reflex responses in the TA were weakly depressed at all conditioning delays tested. Similar effects were seen from other sites in this cat. Figure 4E, for example, illustrates that stimulation of a different cortical site from the same cat facilitates the St and the biceps femoris (BF) while the response in the TA was depressed as in Fig. 4D. Comparable effects on the reflex responses evoked in these muscles, and the others studies, were seen after stimulation of the cortex and the pyramidal tract in all cats.

Cortical stimulation preceding the stimulation of the left, contralateral, tibial posterior nerve also facilitated the reflex.
responses. However, in contrast to stimulation of the other cutaneous nerves, stimulation of the tibial posterior nerve at just suprathreshold intensities in cat MC25 frequently evoked both short- and long-latency duration reflexes in several of the recorded muscles. In the example illustrated in Figs. 5, A–C, stimulation of the tibial posterior nerve at low intensity evoked either small (St) or no (TA and GL) short-latency responses but evoked large longer latency responses in both the St and the GL. Conditioning stimuli to the motor cortex had differential effects on these two responses. This is most clearly observed with a C-T interval of 25 ms for the St (Fig. 5A) in which it can be clearly observed that the short latency response (1st arrow)
Cortical modulation of Superficial Peroneal nerve reflexes

is strongly facilitated (see Fig. 5D) while the longer-latency response is almost abolished (2nd arrow). Similarly the longer-latency response in the GL was also abolished at similar delays. Similar effects on the long-latency responses were observed in all nine sites in cat MC25 in which the effects of conditioning stimuli on the tibial posterior nerve were tested and were observed to stimulation of both the motor cortex and the pyramidal tract.

The effects of the conditioning stimuli on the short-latency responses evoked by the tibial posterior nerve were more variable than those seen with the other two nerves. However, one of the more frequent observations was a depression of the reflex response at short conditioning delays. This is illustrated for a different site in the same cat in Fig. 5E. Conditioning stimuli at short delays produce a depression in all three muscles. However, in contrast to the example of Fig. 5D, there is no subsequent facilitation of the TA while there was facilitation of the St and GL.

Cortical modulation of the reflex effects evoked by stimulation of the cutaneous nerves in the right hindlimb

We also examined whether conditioning stimulation of the motor cortex and pyramidal tract had any effect on the crossed cutaneous reflex responses that were evoked in the left hindlimb (contralateral to the motor cortical stimulation) by stimulation of cutaneous nerves in the right hindlimb. In this case the stimuli were triggered to arrive at the onset of the swing
phase of the left limb, i.e., the stimuli were applied during stance of the stimulated, right, limb. As illustrated in Fig. 6A, the crossed responses were normally not modulated or, if they were, depression was the normal effect as illustrated for the Srt. In a few cases only, facilitatory responses were occasionally observed in the St (Fig. 6B).

**Synthesis**

As is evident from the preceding paragraphs, there is clear evidence that conditioning stimuli applied either to the motor cortex or to the pyramidal tract modulates the reflex responses evoked by stimulation of the cutaneous nerves contralateral to the site of cortical stimulation. However, the magnitude and the time course of that modulation varied quite extensively.

Figure 7 summarizes the results obtained by stimulation of different cortical sites in cat MC25 on the reflex responses evoked in four different muscles by the three cutaneous nerves of the left hindlimb that we studied and serves to illustrate several points. First, the magnitude of the modulation of any given reflex evoked from any one cortical site could vary quite widely. For example, while some cortical sites produced a modulation of >300% of the reflex responses evoked by the saphenous nerves, others had much less influence on the magnitude. Second, the cortical sites that produced the greatest
modulation of the reflexes evoked by the saphenous nerve did not necessarily produce the largest modulation of the reflexes evoked from the other nerves. This is quite clear when inspecting the responses evoked in the St; the sites evoking the largest responses in the saphenous reflex (thick black line and dotted mauve line) were not those evoking the largest responses in response to stimulation of the superficial peroneal nerve. Third, in the most general terms, the nature of the responses evoked in any one muscle in response to all three cutaneous nerves was very similar. The reflex responses evoked in St and GL, for example, were generally facilitated by stimulation of most cortical sites at C-T intervals of 10–30 ms. Conversely, stimulation of very few cortical sites facilitated the responses evoked in the Srt, regardless of the nerve stimulated despite the fact that clear reflex responses were frequently evoked in the Srt by the cutaneous stimulation alone. The situation was slightly different in the TA in which only the reflex responses evoked by the superficial peroneal and tibial posterior nerves were facilitated. This is probably because the just suprathreshold stimulation of the saphenous (determined on the basis of responses in the St) rarely produced clear reflex responses in the TA.

Although the cortical modulation of the reflex responses evoked in any one given muscle by each of the three cutaneous nerves tested were broadly similar, there were, nonetheless, some consistent differences in the pattern of activation. These are summarized in Fig. 8, which illustrates only those responses that exceeded the limits of the interval of confidence of the algebraic sum of the two independent stimuli. Considering data from all sites in all cats, it can be seen that cortical stimulation (□) normally depressed the responses evoked in the St by stimulation of the saphenous nerve at C-T intervals of 0–10 ms and again at intervals ≥40 ms but that there was facilitation at intervals of 15–35 ms (Fig. 8, top left). Facilitation, at some conditioning interval, was observed at 70% of the sites stimulated and averaged 190% of the control (Table 2). Depression of the response was observed at 60% of the sites and averaged 29% of control. Stimulation of the superficial peroneal and the tibial posterior nerves also induced a mix of depression and facilitation with the former producing greater depression at longer delays and the latter greater depression at earlier delays. This same pattern of response was also seen in the other three muscles illustrated. For example, the responses evoked in the Srt, TA, and GL by stimulation of the tibial posterior nerve were all depressed by conditioning stimuli applied at C-T intervals of <15–30 ms. Conversely, the pattern of modulation of the reflex responses evoked in the TA and the GL by the superficial peroneal nerve resembles that observed in the St, consisting of facilitation at intervals of 10–30 ms and depression at longer conditioning delays.

Conditioning stimuli to the pyramidal tract tended to show similar effects to those obtained from stimulation of individual cortical sites. For example, inspection of Fig. 8, illustrates that the pattern of facilitation and depression after pyramidal tract stimulation (□) were generally similar to those observed from cortical stimuli (□), particularly in the case of the responses evoked by the saphenous nerve. The crossed reflex responses evoked in the muscles of the left hindlimb (—) were generally depressed.
That these differences in the nature of the modulation of the responses for the three nerves are not simply the result of population averaging is illustrated in Fig. 9 for a site in cat MC25 in which cortical stimulation at a single site was applied during stimulation of each of the three nerves in the left hindlimb. Inspection of this figure clearly shows that responses of the same type as described with respect to Fig. 8 were also observed in individual cases. In particular, the figure emphasizes the depression of the reflex response evoked by the superficial peroneal nerve at long C-T intervals, but the depression of the response evoked by the tibial posterior only at short intervals.

**DISCUSSION**

Conditioning stimuli to the motor cortex and the pyramidal tract produced strong modulation of the short-latency reflex effects evoked by stimulation of several cutaneous nerves of
the hindlimb in the cat at the onset of the swing phase of locomotion. This modulation consisted of both facilitation and depression of reflex responses and varied according to the muscle recorded and the nerve and cortical site stimulated. The results suggest a convergence of cortical and cutaneous afferent activity on distinct interneuronal populations in the spinal cord.

**General considerations**

We have concentrated in this report specifically on the effects of spatial convergence between the corticospinal tract and cutaneous afferents rather than detailing the responses evoked by each structure individually. Nonetheless, it should be emphasized that the reflex effects evoked in these cats by...
CORTICAL MODULATION OF HINDLIMB REFLEX ACTIVITY

TABLE 2. Relative increase in the magnitude of the reflex responses

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Left Cutaneous reflexes</th>
<th>Right Cutaneous reflexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Facilitation</td>
<td>Depression</td>
</tr>
<tr>
<td>A. Saphenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St</td>
<td>189.5 ± 77.3</td>
<td>29.0 ± 20.1</td>
</tr>
<tr>
<td>TA</td>
<td>397.6 ± 576.0</td>
<td>46.6 ± 26.1</td>
</tr>
<tr>
<td>EDL</td>
<td>320.2 ± 389.7</td>
<td>53.0 ± 19.4</td>
</tr>
<tr>
<td>Srt</td>
<td>152.7 ± 38.2</td>
<td>17.8 —</td>
</tr>
<tr>
<td>EDB</td>
<td>1753.0 —</td>
<td>33.6 ± 21.6</td>
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<tr>
<td>GL</td>
<td>275.5 ± 98.7</td>
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<td>GM</td>
<td>442.1 ± 252.6</td>
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<td>VL</td>
<td>51.3 ± 348.8</td>
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<tr>
<td>BF</td>
<td>154.4 —</td>
<td>3.3 —</td>
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<tr>
<td>Sol</td>
<td>1055 ± 2087</td>
<td>26.1 ± 13.2</td>
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<td>FDL</td>
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<td>B. Superficial peroneal</td>
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</tr>
<tr>
<td>St</td>
<td>228.7 ± 56.8</td>
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<td>26.1 ± 13.2</td>
</tr>
<tr>
<td>FDL</td>
<td>228.7 ± 56.8</td>
<td>29.6 ± 19.6</td>
</tr>
<tr>
<td>C. Tibial posterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St</td>
<td>1055 ± 2087</td>
<td>26.1 ± 13.2</td>
</tr>
<tr>
<td>TA</td>
<td>198.6 ± 63.7</td>
<td>14.9 ± 20.2</td>
</tr>
<tr>
<td>EDL</td>
<td>246.4 ± 75.5</td>
<td>19.9 ± 16.7</td>
</tr>
<tr>
<td>Srt</td>
<td>137.7 ± 15.3</td>
<td>52.2 ± 15.2</td>
</tr>
<tr>
<td>EDB</td>
<td>277.1 ± 139.3</td>
<td>36.2 ± 14.0</td>
</tr>
<tr>
<td>GL</td>
<td>188.9 —</td>
<td>10.0 —</td>
</tr>
<tr>
<td>GM</td>
<td>1163.0 —</td>
<td>30.6 ± 20.8</td>
</tr>
<tr>
<td>VL</td>
<td>1033.3 ± 1527</td>
<td>33.5 —</td>
</tr>
<tr>
<td>BF</td>
<td>895.7 —</td>
<td>10.0 —</td>
</tr>
<tr>
<td>Sol</td>
<td>1055 ± 2087</td>
<td>26.1 ± 13.2</td>
</tr>
<tr>
<td>FDL</td>
<td>228.7 ± 56.8</td>
<td>29.6 ± 19.6</td>
</tr>
</tbody>
</table>

Cortical modulation of left and right cutaneous reflexes during locomotion in four cats MC24–MC27. For each nerve, the table provides information on the mean ± SD facilitation and depression in the reflex response produced by different nerves of the left and right hindlimbs (expressed as a percentage of the algebraic sum of the control values) as well as the percentage of cortical sites that produced either a facilitation or depression. St, semitendinosus; TA, tibialis anterior; EDL, extensor digitorum longus; Srt, sartorius; EDB, extensor digitorum brevis; GL, gastrocnemius lateralis; GM, gastrocremius medialis; VL, vastus lateralis; BF, biceps femoris; Sol, soleus; FDL, flexor digitorum longus.

FIG. 9. Modulation of the reflex responses evoked by the 3 left cutaneous nerves by conditioning stimuli to a single cortical site.
stimulation of the cutaneous nerves ipsi- and contralateral to the EMG recordings in the left hindlimb were identical to those reported in other studies in intact walking cats, in which stimulation of low threshold cutaneous afferents during swing generally facilitates flexor muscle activity and stimulation during stance produces inhibition of extensors (Abraham et al. 1985; Duy sens and Loeb 1980; Duy sens et al. 1980; Loeb 1993; Pratt et al. 1991; Rossignol et al. 1988). The effects produced by stimulation in the same cortical sites as used in this report have been detailed in a recent manuscript (Bretzner and Drew 2005) and are analogous in their properties to those that have been described in more detail in the forelimb (Armstrong and Drew 1985; Rho et al. 1999). As such, the effects of the spatial convergence that we describe in this report provide a firm basis for discussion of the effects of cortical and cutaneous convergence.

Cortical modulation of cutaneous reflexes during locomotion

Conditioning stimuli to many cortical sites produced modulation of the responses evoked in muscles by stimulation of one or more of the cutaneous nerves of the same limb. This was particularly true for the responses evoked in the St muscle in which substantial facilitation of the reflex responses were observed in more than 60% of the experiments (irrespective of the nerve stimulated; Table 2). Moreover, similar results to stimulation of the saphenous nerve were observed in all cats. The extent and magnitude of this spatial convergence is compatible with the results from experiments in the anesthetized preparation in which spatial convergence onto flexor and extensor motoneurones (including St) was observed from stimulation of the pyramidal tract and the sural nerve (Lundberg and Voorhoeve 1962; Lundberg et al. 1962; Pinter et al. 1982) or of the superficial peroneal nerve (Fles hman et al. 1988). In the current experiments, facilitation was most frequently observed at C-T intervals of $15 - 20\text{ ms}$, although facilitatory responses in some cases could be obtained at shorter delays. This is similar to the intervals used by Lundberg and Voorhoeve (1962) and by Fles hman et al. (1988).

Stimulation in most effective sites also produced depression of the responses, in both flexor and extensor muscles, at conditioning delays both shorter and longer than those that produced facilitation. Such a depression of reflex responses was not described in the aforementioned experiments in anesthetized cats. The reason for this is not clear, but it might be related to the more complex and phase-dependent changes in excitability that are to be expected in an intact waking cat.

Because, in our experiments, stimulation was applied during locomotion, the experimental protocol resulted in the cutane ous stimuli being applied at progressively later times during the swing phase. We therefore have to consider the possibility that apparent changes the amplitude of the reflex responses are simply a result of a phase-dependent modulation of the amplitude of the cutaneous reflexes. We controlled for this in several experiments by stimulating the cutaneous nerve alone at the different conditioning intervals. As illustrated in Fig. 2, D and E, the small change in delay, with respect to the onset of activity in the Srt, did not result in any appreciable change in the amplitude of the cutaneous reflex. This was true for all 12 sites in which this was tested, and it seems most likely that the results obtained were truly due to spatial convergence of the supraspinal and peripheral inputs.

Specificity according to the nerve stimulated

Conditioning stimuli at any one given cortical site frequently modulated reflex amplitude in a number of muscles, including both flexor and extensor muscles. This is not unexpected given the relatively widespread effects that are produced by stimulation of each structure independently. Stimulation of cutaneous nerves during the swing phase of locomotion invariably produces an organized pattern of reflex activity throughout the limb involving primarily flexor muscles but also some extensor muscles (Rossignol et al. 1988). Similarly, stimulation of many cortical sites during the swing phase of locomotion may produce evoked responses in multiple hindlimb muscles, again including both flexors and extensors (Bretzner and Drew 2005). It is, therefore, to be expected that conditioning cortical stimuli will modulate reflex effects in multiple muscles. What is perhaps more unexpected is that the conditioning stimuli did not modulate the reflex responses in all of the muscles facilitated by the cutaneous stimulation and that the effects evoked in any one experiment frequently produced facilitation of one muscle but depression of another (see, e.g., Fig. 4D).

The first of these observations may be related to the fact that we examined the effects of conditioning stimuli at only one phase of the step cycle (the onset of swing). Although stimulation of both the motor cortex and the cutaneous nerves at this time of the step cycle is optimal for activation of the St, it is less efficient in producing reflex responses in some other flexor muscle. For example, cortically evoked responses in both TA and Srt are maximal in mid-swing and responses in muscles such as the extensor digitorum brevis are optimal at the end of swing (Bretzner and Drew 2005). It is possible that conditioning stimuli applied at longer delays after the onset of the Srt would be more effective in modulating reflex activity in muscles such as the TA.

The fact that responses in some muscles were facilitated and those in other muscles were depressed or unchanged may speak to the specificity of the terminations of cortical and cutaneous pathways on different interneuronal networks in the spinal cord. These differential effects on the different muscles, particularly facilitation versus depression, infer that the site of the spatial convergence is specific to interneuronal populations regulating the activity of different synergistic groups of muscles.

Specificity according to the muscle recorded

Conditioning stimuli applied to the motor cortex produced modulation of the reflex responses evoked by each of the three nerves that we tested. The nature of the cortical modulation of the reflex responses was broadly similar for each nerve although there were some consistent differences, as seen in Fig. 8. For example, while the reflex responses evoked by the superficial peroneal nerve were generally facilitated at conditioning delays $\approx 25 - 30\text{ ms}$ and depressed by greater conditioning delays, the inverse was seen for the reflex responses evoked by the tibial posterior nerve which were depressed at conditioning delays $\approx 30\text{ ms}$ and facilitated at longer delays. Indeed, it is possible that one should consider the pattern of
modulation of the responses evoked by the tibial posterior nerve (innervating the plantar surface of the foot) to be the reciprocal of those evoked by stimulation of the superficial peroneal nerve (innervating the dorsum of the paw), at least in the St. The modulation of the responses evoked by the saphenous nerve was different again from both the superficial peroneal and the tibial posterior nerves, consisting primarily of a facilitation at medium delays. That these responses are not purely a population effect is illustrated by Fig. 9, which clearly shows the same effect from stimulation of a single cortical site. Evidence of a specificity according to the site of origin of the reflex response is also provided by the results obtained from stimulation of the cutaneous nerves of the right hindlimb. In this case, the crossed reflex responses were almost always depressed by cortical stimulation, irrespective of the conditioning delay.

These findings again argue for a certain degree of specificity in the interneuronal populations as the modulation evoked in a given muscle from a given cortical site differs according to the cutaneous nerve that is stimulated. In the case of the superficial peroneal nerve and the tibial posterior nerve, there is the added possibility that the cortical stimulation acts through reciprocal excitatory and inhibitory pathways.

Specificity according to the cortical site stimulated

The magnitude and temporal profile of the effect of the conditioning stimulation also depended on the cortical site that was stimulated. This can be clearly observed in the summary presented in Fig. 7 for the responses evoked from different cortical sites in cat MC25. While stimulation in some sites strongly facilitated the responses in a given muscle (e.g., St), stimulation in other sites was less effective. Moreover, the magnitude of the modulation evoked in a given muscle from a given cortical site showed wide variability. This variability is almost certainly related to the differences in the termination patterns of the corticospinal neurons activated by the cortical stimulation. As shown in our recent study of the effects of cortical stimulation on EMG activity during locomotion (Bretzner and Drew 2005), some cortical sites produced their strongest effects on knee flexors such as the St, whereas others more strongly activated hip flexors such as the Srt, or ankle flexors, such as the TA. Corticospinal afferents from different regions of the hindlimb representation of the motor cortex, therefore have differential terminations on the interneuronal populations regulating the activity of muscles acting around different joints of the hindlimb. This diversity in the pattern of the responses evoked from different cortical sites undoubtedly contributes to the diversity in the modulation of the reflex patterns observed in the present experiments.

Nonetheless, although the magnitude of the facilitation evoked from different sites varied, the general characteristics, and particularly the temporal profile, was similar. Moreover, the characteristics of the modulation evoked by stimulation of the pyramidal tract was also similar to that evoked from the majority of the cortical sites (Figs. 3 and 8). This suggests that the electrical stimulation of the pyramidal tract provides an indication of the net activity of the corticospinal projection to the lumbar spinal cord in modulating reflex activity. In this respect, the results are similar to those obtained from examining the EMG responses evoked by pyramidal tract stimulation (Bretzner and Drew 2005).

Mechanisms

There are two major mechanisms that may explain the modulation of the evoked responses by the conditioning stimuli.

The first is that the cutaneous stimuli might modulate the activity of the cortical neurons, thus modulating cortical excitability, and by this means alter the descending corticospinal volley. This mechanism has been suggested to be, at least in part, responsible for the changes in the long-latency cutaneous reflexes evoked by stimulation of cutaneous nerves during human locomotion (Christensen et al. 1999, 2000; Pijnappels et al. 1998). However, we feel that this is unlikely to be a major contributing factor to the results obtained in this study as we obtained similar effects from stimulation of both the motor cortex and of the pyramidal tract. As argued by Nielsen et al. (1997), the magnitude of the descending volley evoked by stimulation of the corticospinal tract should be independent of any changes in corticospinal efficacy. Although our conclusion is different from that of Nielsen and his collaborators, it should be emphasized that we examined the effect of conditioning only on short-latency responses while they studied longer-latency reflexes. In addition, their conclusions pertained mostly to the earliest parts of the response evoked by TMS, i.e., that part which is most likely to be produced by monosynaptic cortical activation, and for which there is no correlate in cats.

The other major mechanism, and the one that we favor, is that the modulation is the result of changes in excitability of the spinal interneuronal pathways onto which cutaneous and corticospinal pathways converge. Spatial facilitation in such interneuronal pathways would explain all of the observed changes in modulation. Such spatial convergence may be direct, i.e., both afferents converging directly onto the same interneurones or indirect, i.e., each afferent contacting different interneuronal populations that then converge at a later stage. The difference in the temporal profile of the responses argues against the spatial facilitation being a simple function of nonlinear motoneuronal properties (Brownstone et al. 1994) as this might be expected to affect convergent inputs to a given muscle in a similar manner; clearly, this was not always the case. Last, there is also the possibility that the modulation of the cutaneous reflexes might be influenced via cortical modulation of presynaptic afferent depolarization (PAD) (Andersen et al. 1964; Fetz 1968; Lundberg 1964; Rudomin et al. 2004). Such modulation may be particularly important in explaining the depression of the responses observed with small C-T intervals. Again, our experiments cannot address this issue.

Regardless of the exact mechanism, the arguments in the preceding sections and paragraphs strongly suggest a very strong degree of specificity in the organization of this spatial facilitation. Clearly, the nature of the modulation differs according to the muscle under study, the cutaneous nerve that is used to produce the cutaneous reflex and the exact cortical site that is stimulated. Within each of these categories, different patterns of modulation can be observed if any one factor (muscle, nerve, or cortical site) is changed. This suggests that there must be a highly specific and fractionated organization of interneuronal pools (and/or PAD pathways) to produce the range of modulatory patterns observed in these experiments. In
this respect, our suggestions strongly resemble those made by others who have argued for highly differentiated and specialized interneuronal pathways in the mediation of the reflexes produced by low- and high-threshold cutaneous reflexes (Burke 1999; Moschovakis et al. 1991; Pratt et al. 1991; Schmidt et al. 1989; Schouenberg et al. 2002).

Functional considerations and conclusions

These experiments suggest that activity in pyramidal tract neurons may modulate the activity in different reflex pathways in a very specific manner. From a functional point of view, it is probable that this convergence provides a means of modifying the magnitude of a reflex depending on circumstance. In our experiments, stimulation was applied as swing was initiated and the foot was lifted from the treadmill. This is a critical transition phase of the step cycle and a time when discharge activity in many cortical neurons is maximal (Armstrong and Drew 1984a; Drew 1993), as are the cutaneous reflex responses in some muscles, and particularly the St. The large facilitation of the reflex activity evoked in the St by stimulation of the saphenous and superficial peroneal nerve would serve to increase knee flexion. This would move the limb away from any natural stimulus that would activate these nerves, especially in the case of activation of the superficial peroneal nerve innervating the dorsum. Similarly, facilitation of the responses in GL would also act to move the paw away from the stimulus by extending the ankle or counteracting any tendency toward ankle flexion (Wand et al. 1980). Suppression of the reflex responses in Srt and TA would likewise prevent the limb and paw being moved forward where they would risk contacting the stimulus again. As such, the cortical activation reinforces the most natural course of action when contacting an obstacle at swing onset. The almost reciprocal effects of pyramidal tract stimulation on the responses evoked by the tibial posterior nerve serve to mostly depress the reflex responses that are evoked by this nerve. Reflex responses evoked by stimulation of the plantar surface of the paw at swing onset may not be desirable (or likely under natural circumstances) as they would tend to produce a plantarflexion of the paw.

Finally, although not tested in these experiments, it is possible that the major importance of this convergence might be to modulate reflex activity according to context. For example, pyramidal tract neurons increase their discharge frequency during behaviors that require modulation of gait and/or fine control over limb trajectory (Beloozerova and Sirota 1993; Drew 1988, 1993). In addition to contributing to the modified patterns of muscle activity that these tasks require, this increased discharge would also serve to further modulate reflex excitability and might serve to enhance reflexes that serve to promote or stabilize the movement and depress those that serve to destabilize it.

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