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Corticospinal control of antagonistic muscles in the cat

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Abstract

We recently suggested that movement-related inter-joint muscle synergies are recruited by selected excitation and selected release from inhibition of cortical points. Here we asked whether a similar cortical mechanism operates in the functional linking of antagonistic muscles. To this end experiments were done on ketamine-anesthetized cats. Intracortical microstimulation (ICMS) and intramuscular electromyographic recordings were used to find and characterize wrist, elbow and shoulder antagonistic motor cortical points. Simultaneous ICMS applied at two cortical points, each evoking activity in one of a pair of antagonistic muscles, produced co-contraction of antagonistic muscle pairs. However, we found an obvious asymmetry in the strength of reciprocal inhibition; it was always significantly stronger on physiological extensors than flexors. Following intravenous injection of a single bolus of strychnine, a cortical point at which only a physiological flexor was previously activated also elicited simultaneous activation of its antagonist. This demonstrates that antagonistic corticospinal neurons are closely grouped, or intermingled. To test whether releasing a cortical point from inhibition allows it to be functionally linked with an antagonistic cortical point, one of three $GABA_A$ receptor antagonists, bicuculline, gabazine or picrotoxin, was injected iontophoretically at one cortical point while stimulation was applied to an antagonistic cortical point. This coupling always resulted in co-contraction of the represented antagonistic muscles. Thus, antagonistic motor cortical points are linked by excitatory intracortical connections held in check by local GABAergic inhibition, with reciprocal inhibition occurring at the spinal level. Importantly, the asymmetry of cortically mediated reciprocal inhibition would appear significantly to bias muscle maps obtained by ICMS in favor of physiological flexors.

Introduction

During voluntary movements and postural adjustments co-contraction of antagonistic muscles is a key mechanism for increasing joint stiffness and damping, thereby increasing mechanical stability (Humphrey & Reed, 1983; Milner *et al*., 1995; Milner & Cloutier, 1998). The fundamental neural mechanism controlling antagonistic muscles is reciprocal inhibition as defined by Sherrington (1913) – relaxation of the antagonist muscle during activity of the agonist. Reciprocal inhibition is mediated, at least in part, by a disynaptic circuit in the spinal cord that is subject to several supraspinal as well as segmental modulatory mechanisms (reviewed by Jankowska, 1992). In the cat, interneurons of reciprocal inhibition can be activated directly by corticospinal terminals and indirectly by C3–C4 propriospinal neurons (Alstermark & Lundberg, 1992). Here we use the term reciprocal inhibition in the functional sense defined by Sherrington. Synergies between antagonists include simple patterns of reciprocal activation, co-contractions, triphasic activation patterns and complex synergies such as during finger movements (Hoffman & Strick, 1986; Doemges & Rack, 1992; Schieber, 1995). Fist clenching is a common example of a synergy involving co-contraction of wrist extensor muscles with the finger flexors. Co-contractions of antagonists occur in other tasks such as writing, painting and wrestling and can occur at various times during a movement. For example, during a ball catching, flexor and extensor muscles at the wrist and elbow are co-activated in an anticipatory manner shortly before impact of the ball with the hand and reflexively thereafter (Lacquaniti & Maioli, 1987). Milner (2002) has shown that agonist–antagonist co-activation is used to stabilize the wrist in response to different types of destabilizing loads. Interestingly, co-contraction of antagonists can occur in early or late parts of a movement. Moreover, the level of co-contraction can be constant or declining to counteract load instabilities. It has also been suggested that co-contraction of antagonists is involved in the initial phase of motor learning (Humphrey & Reed, 1983; Milner & Cloutier, 1993) and in task-dependent adaptations of limb impedance (Burdet *et al*., 2001).

The existence of neural mechanisms involved in the differential control of antagonistic muscles is apparent from the above considerations of coordination patterns and the reported task-dependent differences in the strength of reciprocal inhibition (see Lavoie *et al*., 1997). However, little is known about the cortical mechanisms controlling antagonistic muscles. In a previous study we showed that motor cortical points controlling antagonistic muscles are connected by intrinsic horizontal collaterals and suggested that they are likely to be part of the neural substrates involved in the complex coordinations of antagonistic muscles described above (Capaday *et al*., 1998). The nature of these connections, such as whether excitatory or inhibitory, and their physiological role was not determined in that study. More

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recently we showed that motor cortical points controlling muscles acting at different joints can be functionally linked by focalized release from inhibition, demonstrating that they are coupled by excitatory connections held in check by local inhibitory neurons (Schneider *et al*., 2002; Capaday, 2004). Here we asked whether a similar mechanism operates between cortical points controlling antagonistic muscles. In the course of the experiments, however, we found it difficult to locate cortical points controlling physiological extensor muscles. Furthermore, we noted an obvious asymmetry of cortically evoked reciprocal inhibition, being much stronger on physiological extensors than flexors. We thus reasoned that the difficulty in finding cortical points controlling physiological extensors may be due to a masking of their response to the corticospinal volley by the cortically evoked, spinally mediated, reciprocal inhibition. Consequently, we reduced the efficacy of spinal inhibition by intravenous (i.v.) injections of strychnine and found that responses in physiological extensors were readily evoked from the same cortical point that previously evoked only a flexor response. Thus, corticospinal neurons controlling flexors and extensors appear to be closely grouped or even intermingled. The asymmetry of cortically evoked, spinally mediated, reciprocal inhibition would appear significantly to bias muscle maps obtained by intracortical microstimulation in favor of physiological flexors.

Materials and methods

Experiments were performed on 13 adult cats (12 males and one female) weighting between 3.5 and 5.5 kg. The study was approved by the Comité de protection des animaux de l'Université Laval and conformed to the procedures outlined in the Guide for the Care and Use of Laboratory Animals published by the Canadian Council for Animal Protection.

Animal preparation

Details on surgical procedures, electrophysiological methods and homeostatic measures used in the present study can be found in previous reports from this laboratory (e.g. Capaday *et al*., 1998; Schneider *et al*., 2001, 2002; Ethier *et al*., 2006). Briefly, the animals were anesthetized with an intramuscular injection of ketamine (33 mg/kg) and xylazine (1 mg/kg). Once the surgical procedures terminated, a perfusion pump was connected to a cannula in the femoral vein and a steady flow of anesthetic (10–30 mg/h ketamine, depending on the animal) was delivered throughout the experiment. The animal's temperature was maintained near 37 °C by a heating blanket wrapped around the animal's trunk and by an overhead heat lamp. The blood pressure was maintained at about 100 mmHg. A long skin incision was made to expose the muscles of the left forelimb and shoulder. A pair of multi-stranded, stainless steel wires, separated by approximately 1.5 cm, was inserted in the following pairs of antagonistic muscles: the flexor digitorum profondus (FDP), the extensor digitorium communis (EDC), the palmarus longus and flexor carpi radialis (PL/FCR), the extensor carpi radialis (ECR), the brachialis (Br) and the lateral head of the triceps (TriLat), the clavobrachialis (ClBr) and the latissimus dorsi (LD). Recordings were also made in the spinodeltoid (SpD), the brachioradialis (Brad) and pectoralis minor (PM). The electromyographic (EMG) signals were amplified by a factor of 1000, high pass filtered at 20 Hz, rectified, and low pass filtered at 1000Hz.

Microstimulation

Stainless steel microelectrodes ranging in impedance from 0.5 to 1 MΩ were used to microstimulate the motor cortex (area 4γ). Constant current stimulus trains of 50-ms duration were delivered randomly at intervals between 2.5 and 7 s in layer V of the right motor cortex. The duration of single stimulus pulses was 200 μs and the stimulus rate was 333 Hz. Two microelectrodes were used to explore the motor cortex and locate antagonistic motor cortical points. The horizontal interelectrode spacing between the antagonistic motor cortical points we studied was between 1.75 and 4 mm. Threshold values for eliciting an EMG response were typically between 10 and 20 μA. Selected motor cortical points were stimulated at different current intensities to characterize their motor output. Stimulus currents never exceeded 80 μA. The same range of intensities was also used in different combinations to stimulate two cortical points simultaneously. This allowed us to compare responses to separate and simultaneous activation of two antagonistic motor cortical points.

Analysis of evoked EMG activity

The microstimulation evoked EMG activity was sampled at 2 kHz by a Power 1401 interface controlled by Signal (Cambridge Electronic Design, Cambridge, UK). The duration of the sampled sweeps was 800 ms, including a 100-ms prestimulus period. Typically eight sweeps were sampled, stored on hard disk and averaged in real-time. The integral of the rectified evoked EMG activity was determined for each muscle. Any background EMG activity preceding the stimulus was subtracted. These EMG integrals were used to quantify reciprocal inhibition and to compare EMG activity evoked by separate and simultaneous microstimulation of two antagonistic cortical points. Additionally, reciprocal inhibition of ongoing activity was quantified by taking the difference between the mean value of the EMG activity prior to the cortical stimulus (i.e. the background activity) and the mean value during the period of inhibition (Capaday *et al*., 1990). Measured in this way, the amount of inhibition is a linear function of the background level of motor activity, with a zero *y*-intercept (Capaday *et al*., 1990).

Local disinhibition of cortical points

Local GABAergic synaptic transmission was reduced at one of two antagonistic motor cortical points by iontophoretic ejection of $GABA_A$ antagonists. We used two $GABA_A$ competitive antagonists, bicuculline methochloride (BIC) and gabazine. Picrotoxin, a $GABA_A$ non-competitive antagonist, was also used. Bicuculline methochloride (Tocris) and gabazine (SR-95531, Tocris) were used at a concentration of 10 mM and picrotoxin (Tocris) was used at a concentration of 5 mM. All drugs were dissolved in distilled water (pH 6.6, 4 and 3.7 for bicuculline, gabazine and picrotoxin, respectively) and ejected from micropipettes having tip diameters of about 2–3 μm with positive DC current ranging between 80 and 150 nA. A retaining 100-nA DC current of opposite polarity was used to prevent unwanted diffusion from the pipette. Gabazine is a specific $GABA_A$ receptor antagonist; whereas bicuculline may also block calcium-activated potassium SK channels (Seutin & Johnson, 1999; Pflieger *et al*., 2002). However, the results obtained with both drugs and with picrotoxin were similar as has been previously reported (e.g. Holdefer *et al*., 2005; Ethier *et al*., 2006). The effects of the $GABA_A$ antagonists appeared within a minute, or less, of the start of iontophoretic ejection. Total ejection time never exceeded 3 min. Bicuculline and picrotoxin effects proved to be reversible after about 1 h (Schneider *et al*., 2002), whereas those of gabazine where protracted, lasting several hours (Ethier *et al*., 2006).

Experimental procedures

After determining the stimulus response characteristics of a pair of antagonistic cortical points separately, the two points were microstimulated simultaneously over the same range of current intensities used to characterize each point on its own. The 3-D Cartesian coordinates of the two motor cortical points was then noted and one of the microelectrodes was removed and replaced by an iontophoretic micropipette. Thus, one cortical point was microstimulated while the other was disinhibited by iontophoretic ejection of a $GABA_A$ antagonist. To reduce the efficacy of inhibitory synapses in the spinal cord, the glycine receptor antagonist strychnine was used. As strychnine has no effect in the cortex (Cooper *et al*., 2003) and sectioning of the pyramidal tract abolishes responses to microstimulation over the range of current intensities used in the present experiment (Armstrong & Drew, 1985), we reasoned that the effects of strychnine would be due to its actions in the spinal cord. Consequently, in three animals, a bolus of strychnine (0.1 mg/kg) was injected i.v. following the dose determined by Tribble *et al*. (1983). This paper provides a detailed dose–response study of the effects of strychnine on IPSPs subserving reciprocal inhibition. Responses to microstimulation were measured every 30 s following injection of strychnine, for up to 5–10 min. In one animal three boluses were injected more than 3 h apart to test the effects of microstimulation at different cortical points.

Results

The results are presented in three parts. In the first section we describe the microstimulation-evoked response characteristics. We show that, in the vast majority of cases, cortically evoked activity in a muscle is accompanied by inhibition of its antagonist. However, there was a paucity of responses evocable from physiological extensor muscles at the elbow, wrist and digits. Consequently, finding two cortical points each evoking activity in one of a pair of antagonists was difficult. Notwithstanding the difficulty in finding such antagonistic cortical points, their simultaneous microstimulation evoked a co-contraction of the antagonistic muscle pair. Similarly, in the second section, we show that separate antagonistic motor cortical points can be functionally linked to evoke co-contraction of antagonistic muscles when one of the points is released from inhibition and microstimulation applied at the other. However, the coupling of antagonistic motor cortical points by either method resulted in a markedly asymmetric pattern of reciprocal inhibition, being stronger on physiological extensors than physiological flexors. We reasoned that this may explain the paucity of responses obtainable from physiological extensors, as these may be masked by strong inhibition at the spinal level. To this end, inhibition in spinal networks was reduced by i.v. injections of strychnine. In the third section we report that in this condition, stimulation of a cortical point which previously activated a physiological flexor also evoked a response in physiological extensors, an unmasking effect.

Microstimulation-evoked response characteristics

The most often observed microstimulation-evoked response pattern was activation of a muscle and inhibition of its antagonist when the latter was tonically active. This was true whether a single muscle was recruited, or a small set of synergistic muscles acting at the same or a different joint. In the example shown in Fig. 1A, the ClBr was activated by a stimulus of 20 μA. When the LD was stretched by flexing the arm, it became tonically active (i.e. a tonic stretch reflex). Combination of LD stretching and microstimulation at 20 μA applied to the ClBr cortical point produced a clear reduction of the ClBr-evoked EMG response (compare Fig. 1A, top left and right panels) by approximately half compared with control (2300 vs. 1200 μV.ms). Moreover, the evoked EMG response of the ClBr was accompanied by an inhibition of the ongoing tonic activity in the LD (Fig. 1A, bottom right panel). These reductions of evoked and ongoing EMG activity are the signature of reciprocal inhibition. Figure 1B illustrates the reduction of the microstimulation-evoked EMG activity of a muscle when its antagonist was tonically stretched (59 observations pooled across muscles). The evoked responses were reduced by 817 μV.ms (SEM = 286 μV.ms), or 36.7% of control during stretch of the antagonistic muscle. A paired *t*-test indicates a statistically significant difference between the two conditions ($P < 0.0001$). The stretch-evoked tonic activity, as in Fig. 1A, was reduced on average by 15.1 μV (SEM = 2.7 μV), or 52% of the mean background activity when microstimulation evoked an excitatory response in its antagonist (Fig. 1C). A paired *t*-test indicates that the difference is highly statistically significant ($P \leq 0.0001$, $n = 60$ observations pooled across muscles).

We specifically looked for points at which microstimulation evoked co-contraction of antagonistic muscles at threshold (T) or at up to $2.5 \times T$ in nine animals. Co-contraction of antagonistic muscles evoked by stimulation of a single cortical point was rare. The EDC and FDP were co-activated at 10/81 cortical points (12%) and at 7/81 for the ECR and PL/FCR (8.6%). The triceps were never co-activated with either the biceps or brachialis (0/71 cortical points). The shoulder muscles ClBr and LD were co-activated at 21/71 cortical points, or 30% of points. In nearly all cases co-contraction was evoked near threshold.

Of the 81 cortical points investigated, we found ten pairs of antagonistic motor cortical points. These ten pairs of points were used to determine the effects of their separate vs. simultaneous microstimulation. Figure 2 illustrates an example of separate and simultaneous microstimulation of two antagonistic cortical points. Simultaneous microstimulation elicited co-contraction of the two antagonistic muscles ClBr and LD. This was observed for all ten pairs of antagonistic motor cortical points studied. The distance between the antagonistic motor cortical points of the ten pairs studied was between 1.75 and 4 mm (mean = 2.63 mm, SD = 0.74 mm). However, as can be seen in Fig. 2, the LD response was markedly inhibited whereas that of the ClBr was not. Taking all observations into account, the strength of cortically evoked reciprocal inhibition between antagonistic muscles was found to be markedly asymmetric, as summarized in Table 1. Using paired *t*-tests, we compared evoked EMG responses obtained by simultaneous microstimulation of two antagonistic motor cortical points with those evoked by separate stimuli. We separated antagonistic muscles at different joints. For the wrist joint, the physiological flexors ECR and EDC were pooled together and likewise for the physiological extensors FCR and FDP. The strength of reciprocal inhibition is significantly stronger on the physiological extensors FCR and FDP than on physiological flexors ECR and EDC (Table 1). In fact, there was no inhibition of the wrist/finger physiological flexors when a wrist/finger physiological extensor points were stimulated. On the contrary it appears that there was a statistically significant increase of the wrist/finger flexor responses. At the shoulder, cortically evoked reciprocal inhibition was stronger on the LD than on the ClBr, but the effect was not as marked as for the wrist and fingers (Table 1). We found no cortical points from which the triceps could be activated, and thus no data were available for the elbow. Nonetheless, this observation suggests that the

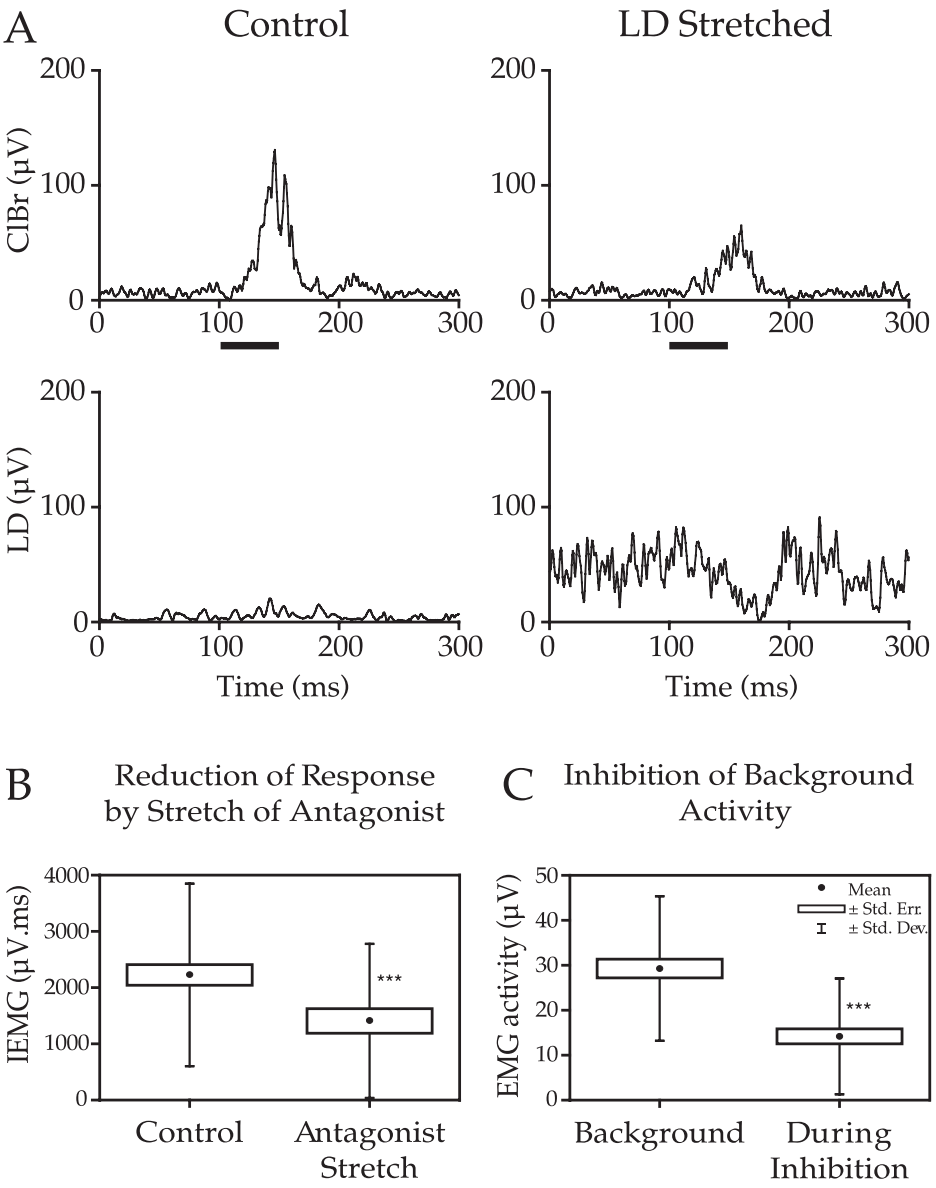

FIG. 1. (A) Example of intramuscular EMG recordings from two antagonistic muscles (ClBr and LD) following microstimulation of the motor cortex. The dark bar under the time axes indicates the onset and the duration of the stimulus train. Microstimulation of a cortical point at 20 μA activated the ClBr alone (left panels). When LD (ClBr antagonist) was tonically activated by muscle stretch (right panels), the ClBr response was decreased. Note also that the evoked ClBr response was associated with simultaneous inhibition of the LD. Each trace is an average of eight consecutive responses. (B) Box and whisker plot of evoked IEMG activity when cortical points were stimulated (Control) and when their antagonist was tonically activated by stretch. A paired *t*-test indicated a highly statistically significant difference between the two conditions as denoted by *** (59 paired values, $P < 0.0001$). (C) The tonic activity in the stretched antagonistic muscle was also markedly reduced by cortically mediated reciprocal inhibition following microstimulation of the antagonistic cortical point. The ongoing activity was on average inhibited by 52% of its mean level measured over a 100-ms time segment before the stimulus. The inhibition of stretched evoked tonic activity was highly statistically significant ($P < 0.0001$).

cortically evoked, spinally mediated, reciprocal inhibition of the triceps must be very strong, as will be taken up further below.

Antagonistic motor cortical points are linked by excitatory connections

$GABA_A$ antagonists were iontophoretically ejected at an identified motor cortical point and intracortical microstimulation (ICMS) applied to an antagonistic cortical point. In this condition ICMS applied at one point evoked a co-contraction of the antagonistic muscles represented at each point. This result was obtained for all such paired points ($n = 7$ pairs). The distance between the antagonistic cortical points studied in this way was 2–4 mm (mean = 2.85, SD = 0.7). In the example shown in Fig. 3, ICMS at one point elicited a response in the ClBr (Fig. 3, left panel). At the second point, ICMS elicited a response in the LD. When bicuculline was ejected at the latter point, ICMS of the ClBr point elicited a co-contraction of the ClBr and LD (Fig. 3, middle panel). As previously reported (Schneider *et al.*, 2002), the muscle synergy created by disinhibition did not of necessity depend on augmentation of the background EMG. Figure 4 illustrates a similar effect for digit muscles obtained following ejection of gabazine. ICMS of 18 μA at one point elicited a response in the FDP and ICMS of 24 μA at the

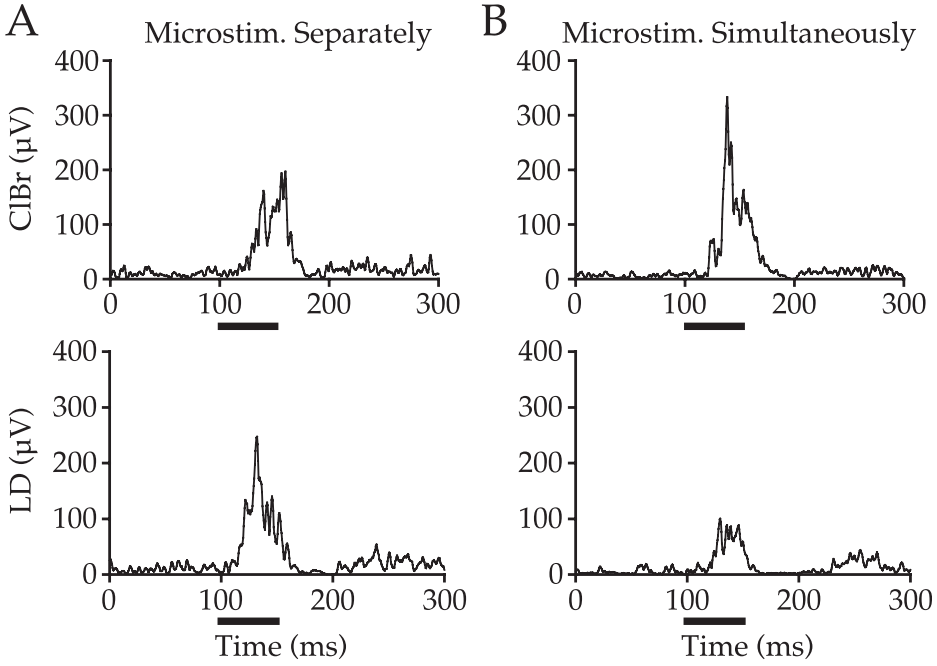

FIG. 2. Example of the asymmetry of reciprocal inhibition between the shoulder muscles LD and ClBr. (A) Microstimulation of this cortical point at 50 μA activated the ClBr alone (upper panel) and microstimulation of another cortical point at 50 μA activated the LD alone (lower panel). (B) When the two points were simultaneously stimulated the LD was markedly inhibited (control = 4337 vs. 1833 μV.ms), whereas the ClBr was not (4118 vs. 5417 μV.ms).

TABLE 1. Average integrated EMG responses to stimulation of antagonistic cortical points separately and simultaneously

Joint and muscles	IEMG during separate stimulation (μV.ms)	IEMG during simultaneous stimulation (μV.ms)	Paired *t*-test value (and number of observations)
Wrist			
ECR/EDC	1340 ± 300	2085 ± 317	0.0040** (10)
PL/FDP	3585 ± 697	1333 ± 494	0.0116** (14)
Shoulder			
LD	2710 ± 359	1623 ± 130	0.0059** (10)
ClBr	3213 ± 322	3096 ± 489	0.7831** (10)

The values are means ± SEM obtained from all cortical points studied. Significant differences are marked with asterisks. Note that for the ECR/EDC, while the *t*-test indicates a significant difference between separate and simultaneous stimulation, the response during simultaneous stimulation is larger, indicating that reciprocal inhibition was ineffective.

other point elicited a response in the EDC (Fig. 4, left and middle panels). Following gabazine ejection at the FDP point, ICMS of the EDC point elicited a co-contraction of the EDC and FDP (Fig. 4, right panel). Qualitatively similar results were obtained with picrotoxin ejections. However, the effects of picrotoxin proved to be weaker than those of bicuculline and gabazine (i.e. the responses resulting from the picrotoxin disinhibited point were smaller). The latency difference between the microstimulation evoked responses and those released from the disinhibited cortical points was between 2 and 8 ms (mean = 5.3 ms, SD = 3.1 ms). In summary, local disinhibition of a cortical point while stimulation was applied to an antagonistic cortical point produced a co-contraction of antagonistic muscles. The asymmetry of reciprocal inhibition noted for simultaneous microstimulation of wrist points was also observed in the present condition. The average evoked EMG response in physiological wrist flexors was about the same before or during ejection of bicuculline or gabazine in an antagonistic cortical point (2316 vs. 2748 μV.ms, $n = 3$ pairs of points). When the $GABA_A$ antagonists where applied at a wrist extensor point, however, the average EMG response to stimulation of a wrist flexor point was markedly decreased (6792 vs. 270 μV.ms, $n = 1$ pair of points). No evidence of reciprocal inhibition was observed between the shoulder muscles LD and ClBr during their functional coupling by disinhibition. In two such experiments bicuculline was ejected at an LD cortical point and in one at a ClBr point.

Strychnine injection reveals responses in physiological extensors

As mentioned above it was very difficult to obtain responses from physiological extensors under the heretofore conditions of the experiments. However, following i.v. injection of strychnine (0.1 mg/kg) responses of physiological extensors were unmasked. Our observations are based on stimulation of five points in three animals. In all cases the appearance of evoked responses in physiological extensors was observed near threshold stimuli. In the example shown in Fig. 5A, stimulation of the cortical point at 20 μA (1.4 × threshold stimulus) elicited a response in the EDC, ECR, Br and SpD muscles. Within a minute of strychnine injection, stimulation of the same cortical point at the same intensity elicited a larger response in these muscles as well as in their antagonists FDP, PL, TriLat and ClBr, respectively (Fig. 5B). Additionally, a response appeared in the LD muscle in co-contraction with its antagonist the ClBr (Fig. 5B). A particularly striking effect of strychnine injection was the appearance of the very rarely evocable TriLat response. Thus, points that elicited a Br response prior to strychnine injection evoked a co-contraction of the Br and its antagonist the TriLat after strychnine. Close inspection of the EMG responses of the FDP, PL and TriLat after strychnine injection revealed the continued, albeit diminished, action of reciprocal inhibition. There was a reduction of

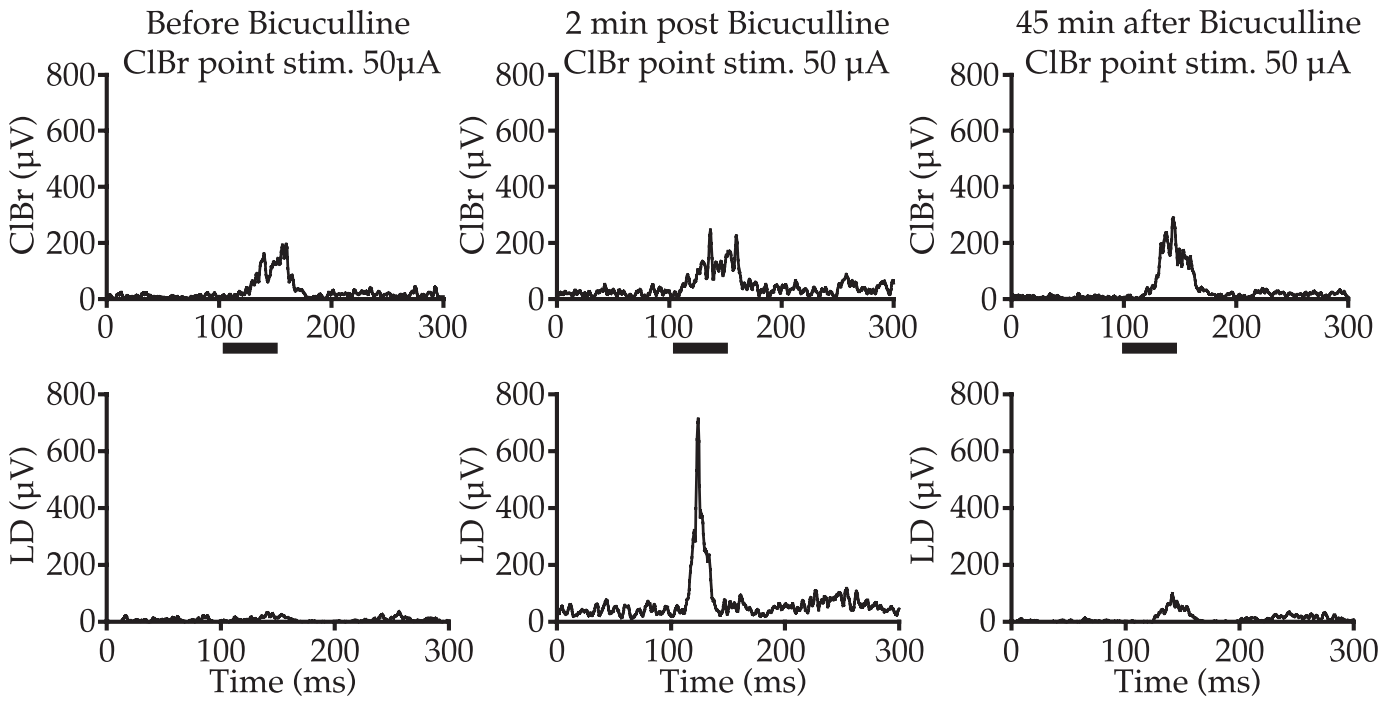

FIG. 3. Example of the effects of disinhibition of a cortical point. Bicuculline was ejected at an LD cortical point and microstimulation applied at a cortical point eliciting a ClBr response. Stimulation of the ClBr point on its own elicited a response in that muscle only (left panels). Following iontophoretic ejection of bicuculline, microstimulation of the ClBr cortical point elicited a co-contraction of the ClBr and LD (central panels). The effect was reversed some 45 min after stopping the iontophoretic current (right panels). The distance between the two cortical points was 2.9 mm.

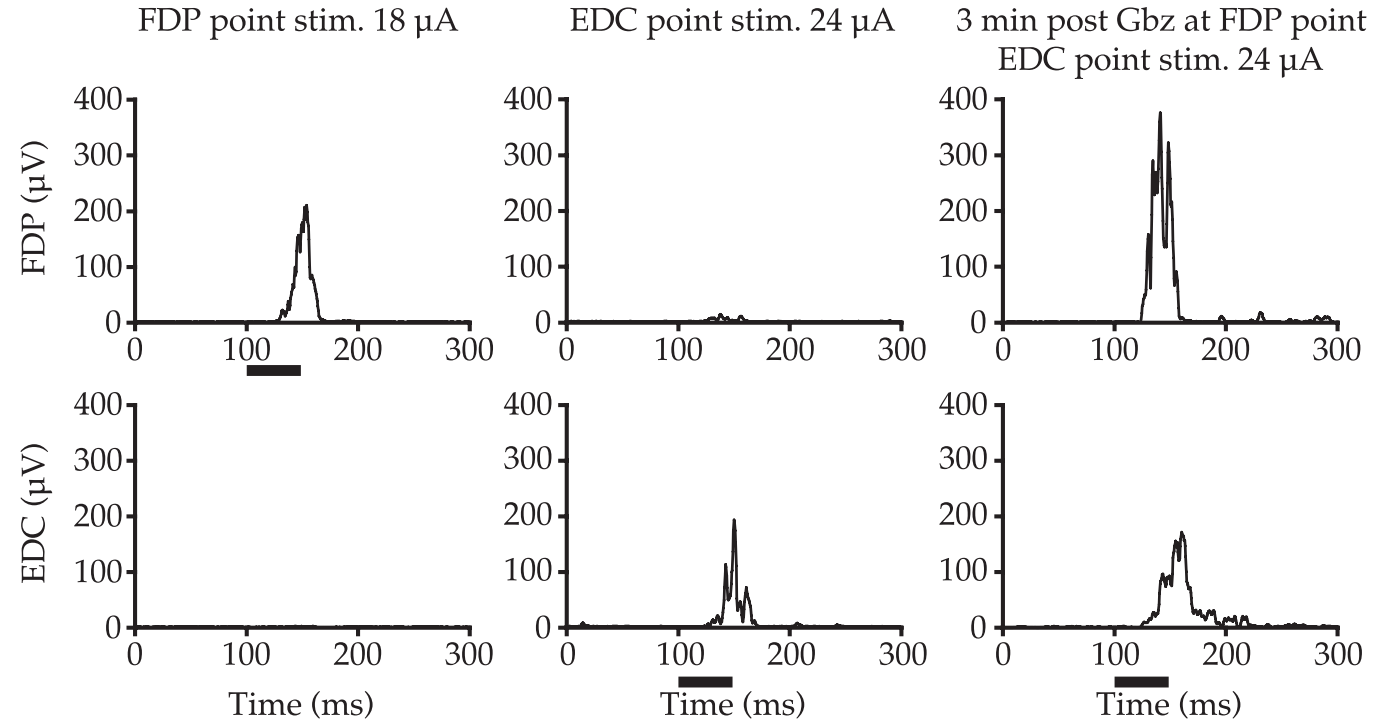

FIG. 4. Another example of the effects of disinhibition of a cortical point. Microstimulation of one point at 18 μA activated the FDP alone (leftmost panels) and microstimulation of another point at 24 μA activated the EDC alone (central panels). Following iontophoretic ejection of gabazine at the FDP point, microstimulation of the EDC point evoked a co-contraction of the EDC and FDP (rightmost panels). The distance between the two cortical points was 2.3 mm.

the background activity on either side of the FDP response temporally coinciding with the evoked activity in the EDC and ECR. For the PL and TriLat the responses appeared curtailed following the peak of activity in their antagonist, the ECR and Br, respectively. These effects are illustrated by thickened grey traces in Fig. 5B.

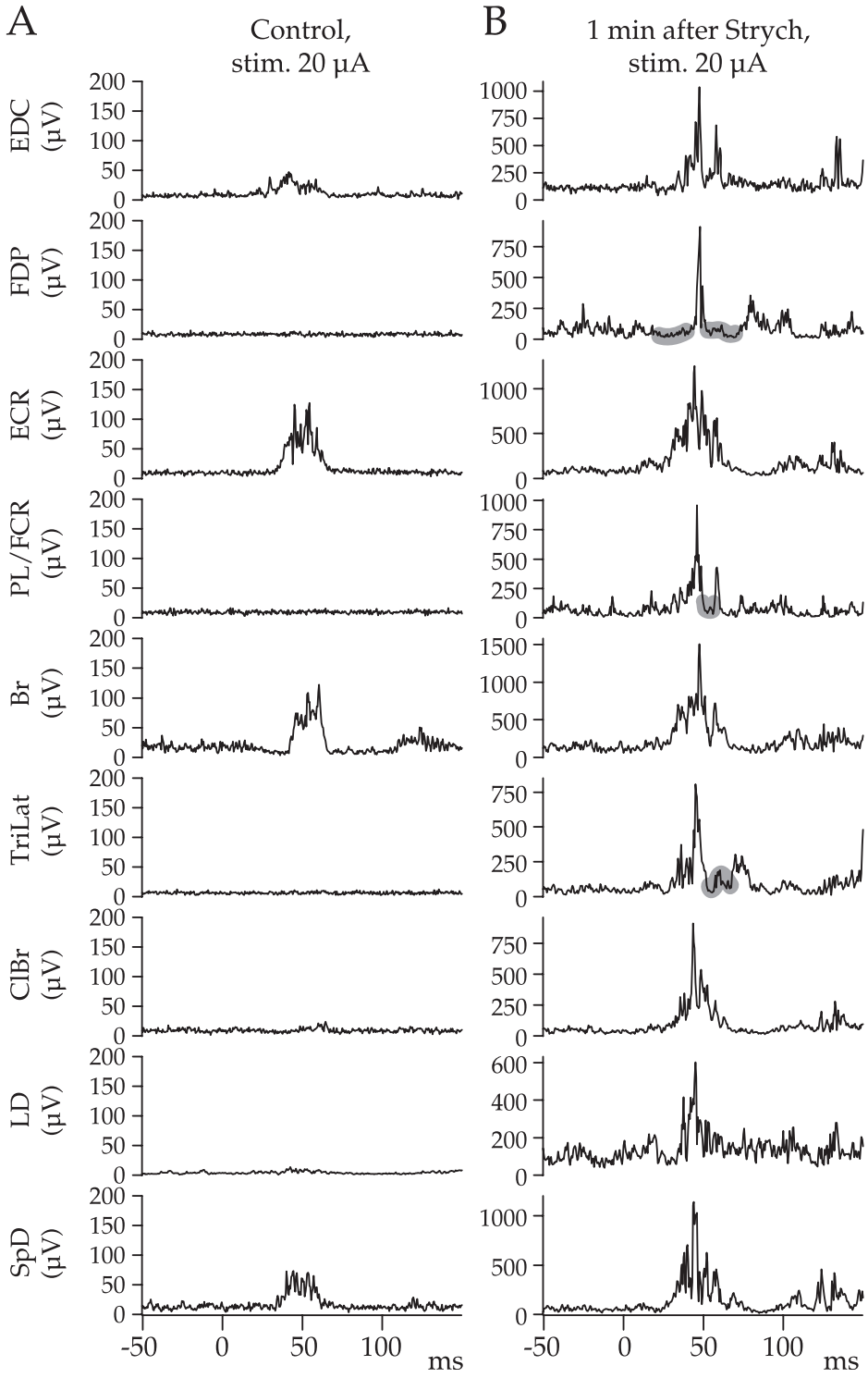

FIG. 5. (A) Stimulation of the cortical point at 20 µA elicited a response in the EDC, ECR, Br and SpD muscles. (B) Within a minute of strychnine injection, stimulation of the same cortical point at the same intensity elicited a larger response in these muscles as well as in their antagonists FDP, PL, TriLat and ClBr, respectively. Additionally, a response appeared in the LD muscle in co-contraction with its antagonist the ClBr. Further details are given in the text.

Discussion

We have reported two main new findings. First, we demonstrated that antagonistic motor cortical points can be functionally linked to produce a co-contraction synergy by focal release of intracortical inhibition at one of the points. Conversely, reciprocal activation implies that cortical points of antagonistic muscles must be held in check by intracortical inhibition. The intrinsic horizontal connections between antagonistic motor cortical points previously demonstrated (Capaday *et al*., 1998) are probably part of the anatomical substrate of this fine interplay. The results presented here demonstrate that antagonistic motor cortical points are functionally linked by a neural circuit similar to that between cortical points representing muscles acting at different joints (Schneider *et al*., 2002). Second, incited by

the paucity of responses evocable from physiological extensors we found that reducing the strength of inhibition in the spinal cord unmasked responses of physiological extensors. This observation demonstrates that microstimulation of a cortical point elicits a volley in corticospinal fibers of both physiological flexors and extensors, suggesting that corticospinal neurons to flexors and extensors are closely grouped, or intermingled. The responses of physiological extensors are masked by the strong cortically mediated reciprocal inhibition of physiological flexors on extensors. Reducing the strength of reciprocal inhibition at the spinal level allows the excitatory component of the corticospinal volley to physiological extensors to evoke activity in them. Strychnine may also release from inhibition excitatory interneurons going to physiological extensor motoneurons and thus further contribute to their activation.

The asymmetry of cortically mediated reciprocal inhibition has important implications for mapping studies of the motor cortex, and their functional interpretation as discussed below. We also discuss the intracortical neural circuitry and mechanisms suggested by our findings, as well as the nature of cortical control of antagonistic muscles.

Neural circuitry and its mechanisms of action

Based on the chart developed by Rank (1975) we estimate that current spread over a radius of about 500 μm from the tip of each microelectrode. The spacing between the antagonistic motor cortical points we studied was always greater than 1.75 mm. Autoradiographic and electrophysiological experiments have shown that bicuculline ejected iontophoretically spreads over a radius of about 500–600 μm from the tip of the micropipette (Jacobs & Donoghue, 1991; Schneider *et al*., 2002; Ethier *et al*., 2006). The minimum distance between the stimulation microelectrode and the iontophoretic pipette was 2 mm, sufficiently far apart that disinhibited points were outside the sphere of microstimulation current. Antagonistic motor cortical points are known to be interconnected by intrinsic axonal collaterals over distances of 3–4 mm (Capaday *et al*., 1998) and neural activity at a cortical point can spread over such distances (Baker *et al*., 1998; Capaday, 2004; Capaday *et al*., 2006). Additionally, Schneider *et al*. (2001) showed that when excitatory synaptic transmission is blocked at the disinhibited cortical point, microstimulation at another cortical point does not result in their functional coupling. We thus conclude that the functional coupling between antagonistic cortical points we have observed is due to synaptic connections between them. Local inhibitory interneurons target all parts of pyramidal cells (see Markram *et al*., 2004; Grillner *et al*., 2005). Schneider *et al*. (2002) suggested that local inhibitory neurons attenuate excitatory cortico-cortical inputs (see their Fig. 7). Inhibition of these local GABAergic neurons (i.e. local disinhibition) would reduce the gating of excitatory cortico-cortical inputs and thus lead to the functional coupling of cortical points representing muscles acting at different joints. We suggest that a similar mechanism operates during co-contraction of antagonistic muscles initiated by the motor cortex. Further support for a cortico-cortical route involved in the coupling of cortical points is based on the latency difference between the direct microstimulation-evoked responses and those released from disinhibited points. In this study we found that the latency difference between these responses was 2–8 ms (mean = 5.3 ms, SD = 3.1 ms), consistent with an intracortical route. If the disinhibited cortical point was activated indirectly via, for example, a cortico-thalamic route, there would be a 15–20-ms latency difference between the evoked responses (e.g. Deschenes & Hu, 1990).

By their nature, the disinhibition experiments were done on spatially distinct antagonistic cortical points. However, as we have found in this study, corticospinal neurons to flexors and extensors may be closely grouped, or intermingled. Nonetheless, their functional coupling during co-contraction may be effected by the local disinhibition mechanism we suggest.

Implications for mapping studies of the motor cortex

We have demonstrated that the strength of the cortically mediated reciprocal inhibition is asymmetric between antagonistic muscles at the wrist, elbow and shoulder joints. Such an asymmetry was also reported by Preston *et al*. (1967) based on facilitation and inhibition of monosynaptic reflexes and by Fetz *et al*. (1989) in the post-spike effects of wrist muscle cortico-motoneurons. This is of importance for understanding the nature of motor cortical maps that have been reported and the simplified interpretations derived from these maps, namely that the cat motor cortex excites forelimb physiological flexors and inhibits physiological extensors, or that in the baboon the motor cortex excites forelimb extensors and inhibits flexors (e.g. Preston *et al*., 1967). A direct interpretation of this hypothesis implies that, for example, in baboons the motor cortex would control extension of the forelimb to reach for a food morsel, but that the subsequent flexion movement to bring the morsel to its mouth would be mediated by a different part of the CNS. We suggest that a too literal interpretation of these otherwise sound data does not represent the true nature of motor cortical control. Preston *et al*. (1967) insightfully interpreted the strong cortical inhibition of physiological extensors as part of a mechanism to arrest the tonic anti-gravity activity which occurs during standing postures, but not that motor cortical control has a unidirectional bias. Indeed, recent studies have shown that both types of movements can be elicited by microstimulation of the simian motor cortex and this from the same cortical point (Graziano *et al*., 2002, 2004). We suggest that this can be explained by changes in spinal neural circuit excitability produced by stretch reflex feedback, the associated reciprocal inhibition and the close grouping or intermingling of the corticospinal neurons. That cortical points controlling antagonistic muscles tend to be closely grouped, or intermingled, can be inferred from previous studies going at least as far back Chang *et al.* (1947) (Humphrey & Reed, 1983; Phillips, 1975; Asanuma & Rosen, 1972; Capaday *et al*., 1998). For example, Chang *et al.* (1947) reported a case were a weak stimulus activated the extensor hallucis longus (EHL) and flexor digitorium longus (FDL) equally, but that responses in the FDL were no longer observed when the stimulus was increased. Such an observation may be explained by the strong bias of cortically mediated reciprocal inhibition onto physiological extensors and the intermingling of the two populations of corticospinal neurons. Similarly, the strong bias of reciprocal inhibition onto physiological extensors explains why it is difficult to obtain responses from the triceps brachii muscle group. Activation of an elbow point evokes strong reciprocal inhibition of the triceps group. This bias may be exacerbated by mutual inhibition between antagonistic Ia-interneurons (Baldissera *et al*., 1981).

There are presumably neural mechanisms that, in natural conditions, counteract the bias observed in reduced animal preparations. Certainly in humans voluntary cortical control is not unidirectional, as any joint can be equally flexed or extended. At the spinal level, it has been suggested on the basis of changes in H-reflex amplitude that reciprocal inhibition of the agonist(s) is reduced and conversely that on the antagonist(s) is increased (Day *et al*., 1984; Iles, 1986). Furthermore, the strength of reciprocal inhibition acting on the antagonist has been

shown to be modulated in a task-dependent manner (Lavoie *et al*., 1997). Such changes are due to descending control of the spinal circuits (Lundberg, 1970). We suggest that there also occurs a modulation of intrinsic motor cortical circuits which in turn may change the bias of spinal circuits. For example, some 70% of cerebellar Purkinje cells decrease their discharge rate during antagonist co-contraction and conversely increase their discharge during reciprocal activation (Smith, 1993; Thach *et al*., 1993). Flament & Hore (1986) showed that cerebellar dentate neuron activity is necessary for the generation of agonist and antagonist muscle activity that is appropriate in magnitude and timing to control the dynamic phase of arm movements. The changes in Purkinje cell activity are thought to modulate the circuitry of the motor cortex so as to recruit the required synergy patterns between antagonists (Smith, 1993). Perhaps reduction of inhibition at the selected cortical points is part of the mechanism involved in the cerebellar control of agonists and antagonists via the motor cortex. Regradless, much more needs to be learned on the mechanisms by which the motor cortex controls flexor and extensor muscles. What is clear is that motor cortical maps derived from constrained experiments are a static representation of a dynamically modifiable system and that the state of the spinal circuitry strongly influences the nature of such maps.

On the nature of cortical control of antagonistic muscles

Humphrey & Reed (1983) reported the existence of a distinct region of the macaque motor cortex that elicits co-contraction of antagonistic muscles. We found that co-activation of antagonists occurs rarely at the wrist and never at the elbow, but is more probable at the shoulder, although reciprocal activation is far more prevalent there. The co-activations of antagonistic muscles we observed, however, were often unstable especially at the wrist (i.e. not always repeatable from trial to trial). We cannot therefore confirm the existence of specific co-activation points in our experiments. What we report here is the existence of a cortical mechanism by which antagonistic muscles may be co-contracted and that corticospinal neurons of antagonistic muscles are closely grouped, or intermingled. Thus, many cortical loci may, under appropriate conditions, evoke a co-contraction of antagonistic muscles. Whether antagonistic corticospinal neurons are more intermingled at some cortical loci and more segregated at others remains to be determined. Such intermingling explains, for example, why simultaneous stimulation of two antagonistic wrist points may produce an enhancement of the responses of physiological wrist flexors as reported in Table 1. The reason is that both points may contain corticospinal neurons to physiological wrist flexors, but at one point their activation produces a supra-threshold response, whereas at the other the response is subthreshold. Their simultaneous stimulation would therefore result in response facilitation, a non-linear interaction. The converse would not be observed because of the asymmetry of reciprocal inhibition noted above.

By contrast to the report of Asanuma & Rosen (1972), we found no separate sites for excitation of the agonist and inhibition of an antagonist. The dominant pattern was reciprocal activation of antagonists, with some co-contractions as remarked above. It should be noted that in the experiments of Asanuma & Rosen (1972) only two muscles were studied, the EDC and the PL. In our study up to six pairs of antagonists were studied, making it less likely to miss activation of synergistic muscles. For example, the ECR can be activated in isolation or together with the EDC. Thus, inhibition of the PL may be the result of undetected ECR activation. Moreover, results obtained in the monkey motor cortex, as they may relate to the cat, do not suggest the existence of purely inhibitory zones. Fetz *et al*. (1989) reported that less than 2% of cortico-motoneurons in the monkey motor cortex have an exclusively inhibitory effect on spinal motoneurons.

Conclusion

The predominant effect of motor cortex activation is contraction of a muscle(s) and inhibition of its antagonist(s), with a bias. Responses from physiological flexors are more readily evocable because cortically evoked reciprocal inhibition masks the responses of physiological extensors. The bias of cortically mediated reciprocal inhibition probably also explains why points at which true co-activation of antagonistic forelimb muscles occurs are the exception. Antagonistic shoulder muscles, however, appear to have a greater probability of being co-activated by a cortical stimulus. This observation may be related to the complex architecture of the shoulder, which serves as base of postural support for movements of the forelimb and in its transport. The seminal work of Sherrington (1906) revealed that reciprocal inhibition between antagonistic muscles is a fundamental motor cortical synergy and Smith (1993) emphasized that co-activation of antagonistic muscles is also a fundamental synergy. Indeed, Sherrington (1906) suggested that ‘under certain forms of cerebral action true antagonistic muscles can be thrown synchronously into contraction’. Here we have shown that release from inhibition is part of the neural mechanisms involved in the co-contraction of antagonistic muscles and that corticospinal neurons of antagonistic muscles are closely grouped, or intermingled.

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Abbreviations

EMG, electromyographic; ICMS, intracortical microstimulation.

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