Exp Brain Res (2002) 146:86–94
DOI 10.1007/s00221-002-1137-2

RESEARCH ARTICLE

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Neural mechanisms involved in the functional linking of motor cortical points

Received: 19 December 2001 / Accepted: 15 April 2001 / Published online: 23 July 2002

Abstract We sought to understand the basic neural processes involved in the functional linking of motor cortical points. We asked which of the two basic neural mechanisms, excitation or inhibition, is required to functionally link motor cortical points. In the ketamine-anaesthetized cat, a microstimulation electrode was positioned at a point (control point) that was identified by the following three characteristics of the EMG responses: the muscle(s) activated at threshold, any additional muscles recruited by supra-threshold stimulation, and their relative latency. A second distinct point (test point) producing activation of a muscle at a different joint was then identified. At this test cortical point the $GABA_A$ receptor antagonist bicuculline was ejected iontophoretically, while stimulating the control point near threshold. A combined response was elicited consisting of the response normally elicited at the control point plus that elicited at the test point. Thus, an artificial muscle synergy was produced following disinhibition of the test point. This was never the case when glutamate was ejected at the test point, even when supra-threshold stimuli were used at the control point. Therefore, simply increasing the excitability of a cortical point was not sufficient to release the muscle(s) represented at that point into a muscle synergy. Kynurenate, a broadly acting excitatory amino acid receptor antagonist, ejected at the bicuculline point reversed the effect of bicuculline. This shows that the release phenomenon was mediated synaptically and was not due to spread of the stimulating current. We suggest that release from inhibition may be one of the neural mechanisms involved in functionally linking motor cortical points. This functional linking may be part of the ensemble of motor cortical mechanisms involved in recruitment of muscle synergies.

Keywords Disinhibition · Intracortical inhibition · Bicuculline · Motor synergies · Multi-joint movements · Motor cortex · GABAergic inhibition

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Introduction

Studies involving the superposition of somatotopic maps of the motor cortex obtained by microstimulation and morphological connectivity maps obtained by tracer injections in physiologically identified sites have shown that motor cortical zones controlling the various forelimb muscles are strongly interconnected by intrinsic horizontal collaterals (Capaday et al. 1998; Huntley and Jones 1991; Keller 1993; Tokuno and Tanji 1993). These intracortical connections have been suggested to be an anatomical substrate of muscle synergies involved in the coordination of forelimb movements (Huntley and Jones 1991). In support of this, results of microstimulation experiments show that, even at threshold, a widespread pattern of forelimb muscle activation is elicited (Armstrong and Drew 1985; Capaday et al. 1998; Donoghue et al. 1992; Park et al. 2001; Schneider et al. 2001). Furthermore, the intrinsic motor cortical connectivity may have its actions reinforced by the extensive intra-spinal branching of corticospinal axons (Shinoda et al. 1976; Tantisira et al. 1996). Recently McKiernan et al. (1998) have shown that about 50% of corticospinal neurons project to both proximal (e.g., shoulder, elbow) and distal motoneurons (e.g., wrist and hand), some controlling muscles at three different forelimb segments.

How the intrinsic connectivity of the motor cortex is used dynamically during natural movements and whether it is the basis of muscle synergies remains to be elucidated. What is known is that experiments involving single-unit recordings, brain imaging, or magnetic brain stimulation have shown that movements of the arm or

hand engage large areas of the motor cortex (Amassian et al. 1995; Sanes et al. 1995; Schieber and Hibbard 1993). This suggests that during natural movements different motor cortical loci must be functionally linked. The question we sought to answer was: What are the basic neural mechanisms that allow cortical points to interact with each other? As Penfield and Rasmussen (1950) have elegantly put it, *"It is a far cry from the gross movements produced by cortical stimulation to the skilled voluntary performance of the hand of man or monkey. Our problem is to discover, if we can, how this cortical mechanism is utilized in the composition of such performance."* Our approach in this study was to consider a simplified version of the problem, the functional linking of two cortical points. We use the term 'cortical point' – after the expression used by Leyton and Sherrington (1917) – to mean a spatial coordinate at which microstimulation activates a muscle(s). We asked: How might two cortical points be functionally linked? For example, if one point is activated artificially by microstimulation will excitation of the second point be sufficient to functionally link them? In the event, we found that disinhibition is required to functionally link two cortical points; excitation on its own seems inadequate. An abstract summarizing the present data was published (Lavallée et al. 1999).

Materials and methods

The data reported herein were obtained from experiments on 16 cats weighing between 2.5 and 4.0 kg. The methods used were approved by the local ethics committee 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.

Summary of experimental procedures

After exposing the cruciate area of the cat's cerebral cortex, the electromyographic (EMG) response elicited by microstimulation of a given cortical point was identified according to the following criteria: (1) the muscle(s) activated at threshold, (2) any additional muscles recruited by suprathreshold stimulation, and (3) their relative timing. After identifying a first cortical point, its 3-D Cartesian coordinates were noted. The stimulating microelectrode was then moved to find a second cortical point at which microstimulation produced activation of a muscle acting at a different joint (e.g., wrist vs elbow). This insured that we could clearly differentiate control responses from those induced experimentally as explained in what follows. After identifying this second cortical point and noting its 3-D Cartesian coordinates, the stimulating electrode was either left in place or brought back to the first identified point. At one of the identified points (test point) a multi-barreled iontophoretic pipette was placed. The microstimulation electrode was placed at the other identified cortical point (control point). The basic experimental procedure, as illustrated in Fig. 1, consisted of microstimulating the control point while either exciting or disinhibiting the test point by iontophoretic drug ejections. Glutamate was used to increase local excitability, whereas bicuculline, a $GABA_A$ receptor antagonist, was used to produce local disinhibition. The methods and procedures used in the present study followed closely those used in previous studies from this laboratory (Capaday et al. 1998; Schneider et al. 2001).

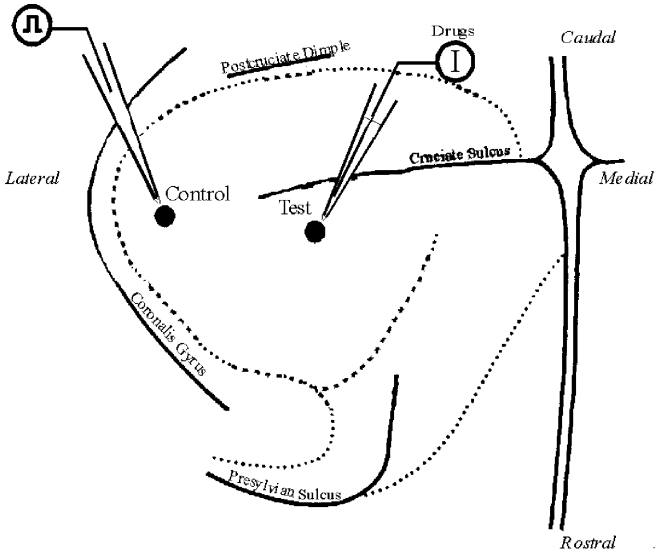

Fig. 1 Schematic of the basic experimental arrangement in the right motor cortex of the cat. The distance between control and test points in this illustration is not to scale. Methodological details are given in the text

Surgical procedures

The animals were first anaesthetized with an intramuscular (IM) injection of ketamine (40 mg/kg) and xylazine (1 mg/kg). A tube was inserted into the trachea, and the left femoral artery and vein were cannulated to measure blood pressure and for injection of physiological solutions and supplementary doses of anaesthetic, respectively. Body temperature was measured by a rectal probe. The animal's temperature was maintained near 37°C by a heating blanket wrapped around the animal's trunk and by an overhead radiant heat lamp. During surgery the anaesthesia was maintained by small injections of ketamine-xylazine via the venous cannula, as needed. Ten milliliters of a pH-balanced solution of 5% glucose in physiological saline was given to the animal every 2–3 h. The blood pressure was maintained at about 100 mmHg. In no case was there a need to use a plasma volume expander, or noradrenaline, to maintain a normal blood pressure.

A long skin incision was made to expose the muscles of the left forelimb and shoulder. The muscles of interest were separated from each other by blunt dissection. A pair of multi-stranded, stainless steel EMG electrodes, separated by approximately 1.5 cm, was inserted in each of the following muscles: the flexor digitorium profundus (FDP), the flexor carpi ulnaris (FCU) and palmaris longus (PL), extensor carpi radialis longus and brevis (ECRl, ECRb), the lateral head of the triceps (L.Tri), the brachialis (Br), the medial head of the biceps (Bi), the teres major (Tm), the latissimus dorsi (Ld) and the spinodeltoid (Spd). To minimize cross-talk in the EMG recordings and prevent desiccation, the muscles were profusely covered by mineral oil. To ensure that the wires were inserted into the appropriate muscles electric stimuli of 1 ms duration (4–10 V) were delivered via the wires and the resulting limb movement observed. The skin incision was closed with wound clips. The wires were attached to spring-loaded connectors from which the signals were led to optically isolated preamplifiers.

Following insertion of the EMG wires, the animal was transferred to the stereotaxic frame in which its head was fixed and its body laid on a cushion. The forelimbs were free in space hanging perpendicular to the ground. A solution of dexamethasone (2 mg per animal) was injected through the venous cannula and followed by a cysternectomy at the level of the foramen magnum. These procedures served to reduce brain edema and brain pulsations, respectively. Finally, a craniotomy was done to expose

Table 1 Combinations of control and test points at which bicuculline (*Bic.*) and kynurenate (*Kyn.*) were applied

Control point	Bicuculline test point				
	ECR	FCR	Br	Spd	Ld
ECR[a]			Bic. *n*=8/8 Kyn. *n*=4/4	Bic. *n*=6/6 Kyn. *n*=3/3	
FCR					
Br	Bic. *n*=3/3				
Spd	Bic. *n*=1/1	Bic. *n*=0/1	Bic. *n*=1/1		
Ld	Bic. *n*=2/3				

[a] Interpret to mean, e.g., that the ECR as a control point was paired with the Br as a test point eight times and in all cases (*n*=8/8) an artificial muscle synergy was created following application of bicuculline. In all cases kynurenate reversed the effects of bicuculline

the sensorimotor region of the cortex. The underlying dura was cut under microscopic guidance and removed to expose the cruciate sulcus and surrounding coronal gyrus. The surface of the exposed cortex was immediately covered by a mixture of heavy mineral oil thickened with Vaseline and preheated to a temperature of 37°C.

Once the surgical procedures were terminated, a perfusion pump was connected to the venous cannula and a steady flow of ketamine (10–30 mg/h, depending on the animal) was delivered throughout the experiment.

Microstimulation and recording

Stainless steel microelectrodes ranging in impedance from 500 KΩ to 1 MΩ were used to microstimulate the motor cortex. To identify a cortical point, trains of stimuli (33-ms trains of 200-μs square pulses, at a rate of 333 pulses/s) were delivered by a constant current source every 1.5 s as the microelectrode was slowly advanced through the motor cortex. The current intensities used ranged between 10 and 40 μA. The EMG signals were amplified, typically by a factor of 1000, high-pass filtered at 20 Hz, rectified, and low-pass filtered at 1 kHz. Selected EMG signals were monitored on a four-channel oscilloscope and the stimulating current on a separate oscilloscope. Responses of interest were digitized at 2 kHz and averaged in real time. During online sampling, the stimulus was delivered at random every 1.5–4 s. In the experiments involving pairing of a control and test point, the stimulus intensity at the control point was set at between 1.1 and 1.3 times the motor threshold, so as to give a response to each stimulus. EMG responses were obtained just before drug ejection, every 20 s during the 1st min after the start of drug ejection, then once per minute for up to15 min after beginning ejection. When bicuculline was used, we continued testing every 5 min for up to 1 h after ejection was stopped.

Iontophoresis

Each barrel of the pipette contained, respectively, 1 M saline, 10 mM bicuculline methobromide, 100 mM glutamate and 50 mM kynurenate, dissolved in distilled water. All drugs were obtained from Tocris Cookson (Ballwin Mo., USA). The barrels of the micropipette were connected to the head stage of the iontophoresis unit by Ag/AgCl wires. The tip of each barrel was between 2 and 4 μm. Bicuculline, a specific $GABA_A$ receptor antagonist was used to disinhibit a cortical point. Glutamate was used to increase the overall excitability of a cortical point. Kynurenic acid, a broadly acting excitatory amino acid receptor antagonist, was used to reduce local excitatory synaptic transmission. Drugs were ejected with 50–150 nA of DC current, positive for bicuculline and negative for the others. A retaining 100 nA DC current of opposite polarity was used to prevent unwanted diffusion from the pipette. Recordings from the saline-filled barrel were used to monitor the effect of the drugs on the neural activity at the point of ejection. At the stimulation point, neural activity was monitored by periodically switching the input stage of the microelectrode amplifier from the stimulate to the record position.

Results

The results section is divided into three parts. In the first section we present evidence that disinhibition of a cortical point allows it to be recruited in synergy with a microstimulated cortical point. We use the term ‘synergy’ as a verbal expedient to mean the close temporal association of elicited muscle activities. In the second part of the results section we show that a local increase in excitability produced by ejection of glutamate does not release a cortical point into a synergy. In the last part of the results section, we show that the release of a cortical point into a synergy depends on synaptic connections between the microstimulated point and the disinhibited point.

Effects of bicuculline

When bicuculline was ejected at an identified test point, microstimulation of the identified control point elicited a combined response consisting of the response normally elicited at the control point plus that elicited at the test point (Table 1). Out of 23 paired control-test points in 16 animals, only two pairings failed to show this effect and both were in the same animal. The result was thus repeatedly obtained in the other 15 animals. In the example shown in Fig. 2, microstimulation of the control point at 10 μA elicited a response in the ECR. At the test point, microstimulation elicited a response in the brachialis. Within 40 s after the start of bicuculline ejection at the test point, stimulation (10 μA) of the control point alone elicited an ECR response as well as a brachialis response. In other words, disinhibiting a cortical point while stimulation was applied to another cortical point artificially created a muscle synergy. The elicited EMG activity produced clearly visible forelimb movements (e.g., elbow flexion and wrist extension for Fig. 2). The latency of the EMG response released from the test point was longer (Table 2) than that of the response at the control point (mean latency difference 6.9 ms, SD 4.6 ms). Based on linear regression analysis, the latency difference

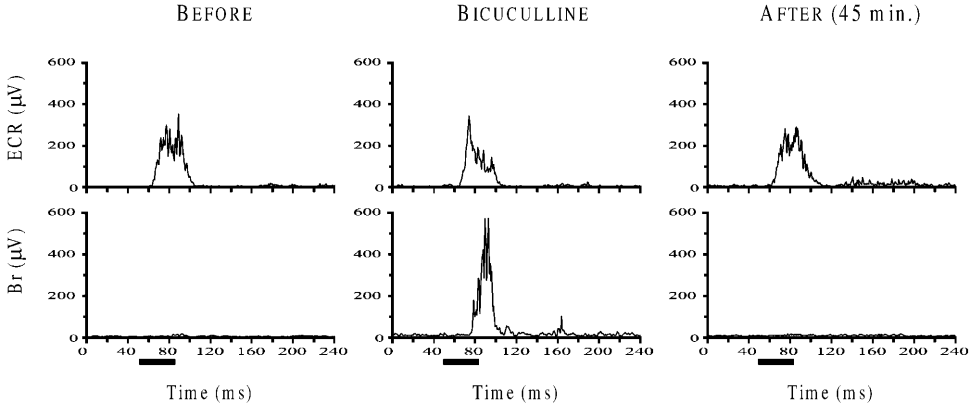

Fig. 2 Effects of bicuculline ejection in a brachialis (*Br*) muscle test cortical point. The stimulus current to the control ECR point was 10 μA (1.2 × threshold) and was delivered starting at 50 ms. The *dark bar* under the time axes indicates the onset and duration of the stimulus train in this and all similar figures. The recording shown of the combined ECR-brachialis response was obtained 2 min after beginning of the bicuculline ejection. Reversal of the response took some 45 min after stopping the iontophoretic current. The distance between the control and test points was 2,400 μm. In this and all other figures each EMG record is the average of eight consecutive responses

Table 2 Statistical summary of the experiments. Each experiment involving kynurenate was done on a different animal

	Distance from control to test points (mm)	Difference in EMG latency (ms)	Time to effect (min)	
			Bicuculline	Kynurenate
Mean	2.44 (*n*=23)	6.9	2.18	4.2
SD	0.94	4.6	1.36	2.08
Paired points (*n*)	23	23	21	7
Range (min to max)	1.17–4.85 mm	1–16	30 s to 5 min	2–7

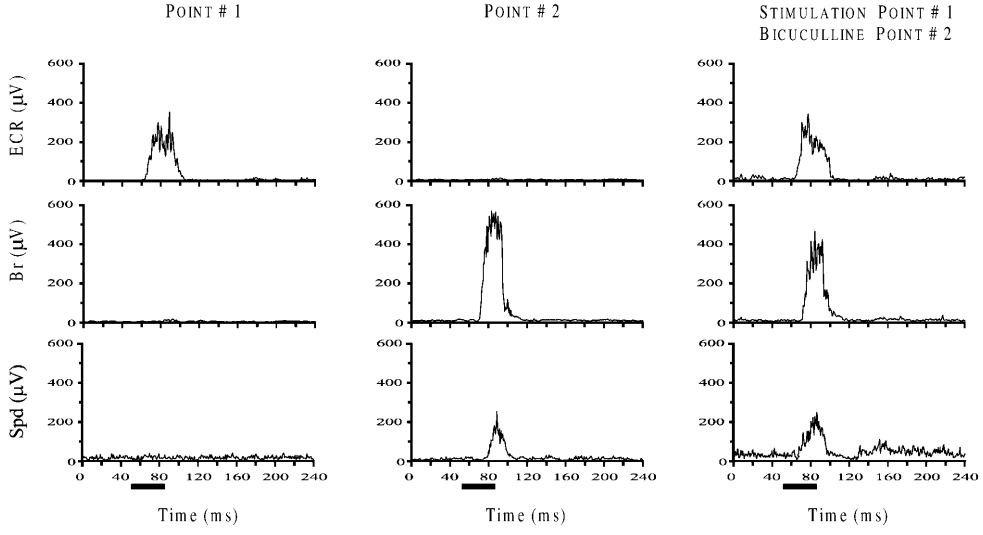

Fig. 3 Effects of bicuculline ejection in a motor cortical point at which the brachialis and spinodeltoid (*Spd*) were represented (*point 2*). The stimulus current to the control ECR point (*point 1*) was 20 μA (1.25 × threshold) and was delivered starting at 50 ms. The distance between the control and test points was 2,350 μm

between control and test responses increased with distance by 4.5 ms/mm (slope SE ± 0.94, r^2=0.6, P<0.001).

The mean latency of the bicuculline effect was 2.18 min (SD 1.36 min), but could occur within seconds (Table 2). On average the effects of bicuculline were completely reversed some 45 min (range 40–60 min) after iontophoresis was stopped (Fig. 2). In no case did the artificially created muscle synergy persist beyond the action of bicuculline. That is, pairing of microstimulation and disinhibition did not induce long-lasting changes of the cortical circuitry. Often stimulation of a cortical point, even at threshold, elicits responses from muscles acting at two or more joints (Armstrong and Drew 1985; Capaday et al. 1998). Figure 3 shows an example of such a point at which stimulation elicited a response in the Br and the Spd. When disinhibition of this point was paired with stimulation of an ECR control point, responses were evoked in the ECR as well as in the Br and Spd (Fig. 3). Thus, disinhibition of a cortical point recruits all muscles

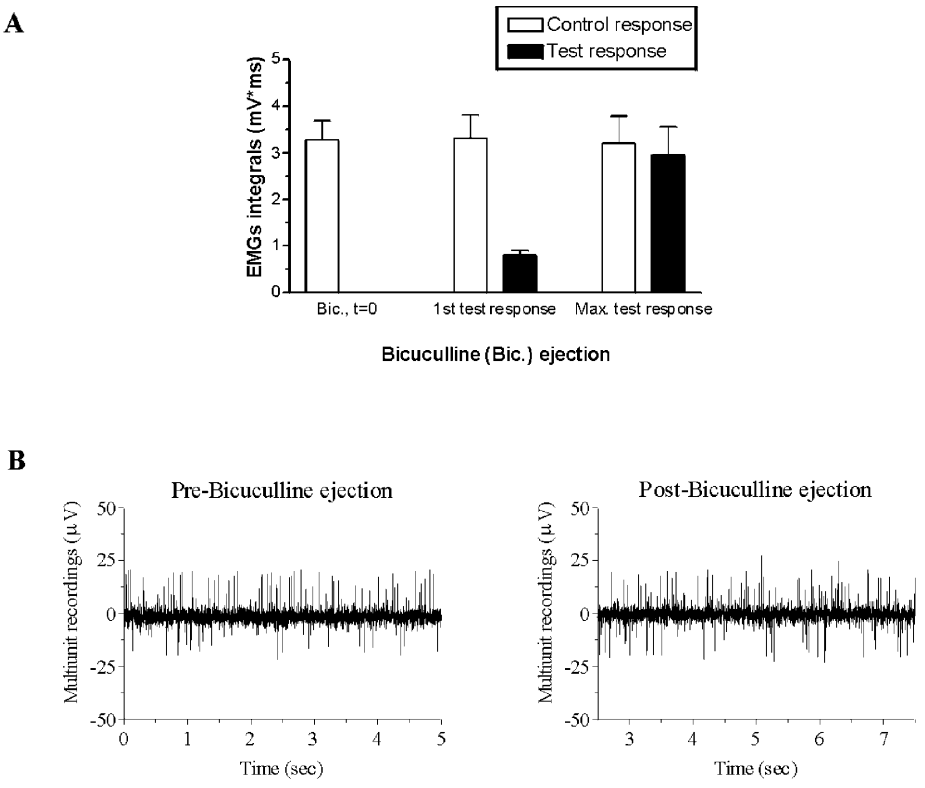

Fig. 4A, B Summary showing the relative constancy of the control responses as a function of time (**A**), indicating that bicuculline did not diffuse to any significant extent from the test to the control point. Each histogram bar represents the average (+1 SD unit above the mean) from the 21 pairings of control and test points. An example of multi-unit recordings from a control point before and 4 min after bicuculline ejection is shown in **B**

represented at that point. The recruitment of a muscle represented at the disinhibited test point did not depend on an increase in background activity in that muscle (Figs. 2, 3, and 6), although this often occurred several minutes after bicuculline ejection. In no case was a muscle(s) other than that represented at the disinhibited test point recruited into a synergy.

An artificial muscle synergy could be created regardless of whether a proximal or distal muscle(s) was represented at the control or test points, respectively (Table 1). For example, if a cortical point controlling a shoulder muscle was used as a control point and bicuculline was ejected at a wrist point, a proximo-distal muscle synergy could be created. As previously stated, in only two cases was there a failure to recruit the test muscle following bicuculline ejection (Table 1). In neither case was the failure due to the distance between control and test points, since the distances involved (2.33 mm and 2.3 mm, respectively) were in the range (1.17–4.85 mm) within which the release phenomenon was observed (Table 2).

Over the distances involved between control and test points (1.0–4.85 mm) and the durations of drug ejections (typically 1–5 min) we found no evidence of drug diffusion from the test to the control point. This can be seen, for example, in Figs. 2, 3, 5, and 6, where the size of the control responses remains approximately constant for the duration of the experiment. This is also shown more explicitly in the histogram of Fig. 4A, where the response integrals of all control and test responses were respectively pooled. As can be seen, the size of the control response remains stable over the course of bicuculline ejection at the test point. This is in contrast to events at the test point where the size of the response after bicuculline ejection increases with time up to a maximum. Multi-unit recordings taken from control points at various times before and after drug ejections showed no obvious changes in the low-level, random spike activity (Fig. 4B). Taken together our results are consistent with those reported by Jacobs and Donoghue (1991) based on autoradiographic measurements of drug diffusion after prolonged iontophoretic ejection.

Effects of glutamate

When glutamate was ejected at a test point, stimulation of the control point did not result in the release of the muscle(s) represented at the test point into a synergy. In none of 20 paired control-test points did glutamate result in the release of the muscle(s) represented at the test point upon stimulation of the control point. In the example shown in Fig. 5, glutamate was ejected continuously for up to 5 min at the same point at which bicuculline was ejected (Fig. 2). The only response elicited by stimulation of the control point is an EMG burst in the ECR, i.e., the muscle represented at the control point. This result holds true whether bicuculline was the first drug ejected at the test point and one waited for its effects to stop, or when glutamate was the first drug ejected. Multi-unit recordings taken from the glutamate ejection site showed the expected increased levels of neural activity and this was

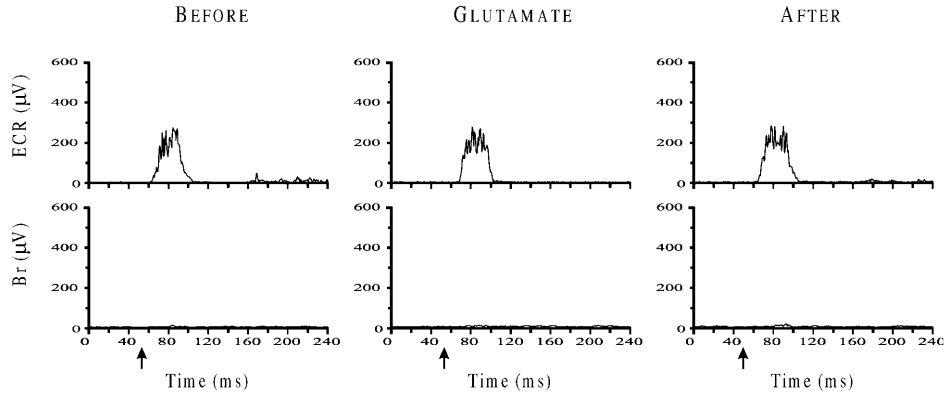

Fig. 5 Effects of glutamate ejection in a brachialis test point (same as in Fig. 2). The stimulus current at the control ECR point was 10.5 μA (1.2 × threshold) and was delivered starting at 50 ms. In this example glutamate was ejected for about 5 min. In contrast to the effect of bicuculline, glutamate did not release the muscle(s) represented at the test point into a muscle synergy. The distance between the control and test points was 2,400 μm

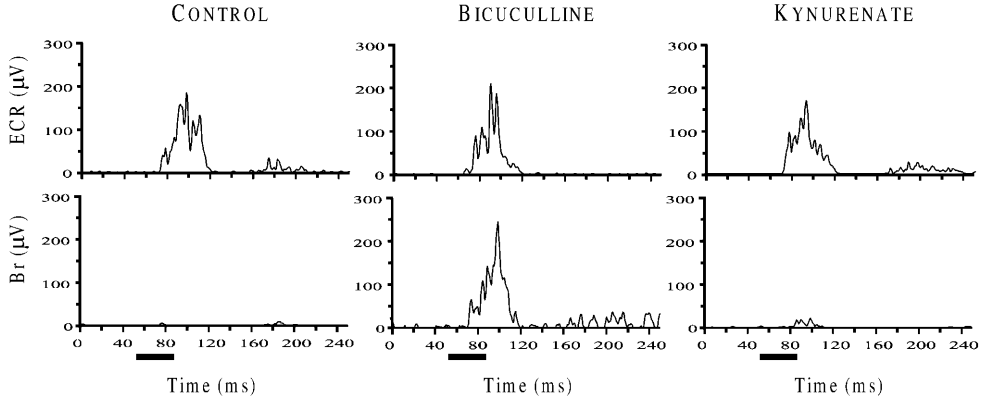

Fig. 6 Example showing how kynurenate reversed the effects induced by bicuculline. The control ECR point was stimulated at 20 μA (1.3 × threshold), starting at 50 ms bicuculline was ejected for 8 min at the test point (brachialis). Kynurenate was ejected immediately after and reversed the effect of bicuculline (i.e., release of the Br) within 4 min. The distance between the control and test points was 3,550 μm

particularly clear in layer V, where the large action potentials of corticospinal cells were prominent.

Effects of kynurenate

Were the effects induced by microstimulation of the control point on the test point following bicuculline ejection synaptically mediated? An alternative possibility is that the effects were due to the spread of current from the control point acting directly on a hyper-excitable test point. The results using kynurenate at the test point (n=7) provide strong evidence that the effects were synaptically mediated. Following ejection of bicuculline and the artificial creation of a muscle synergy, kynurenate was ejected at the test point (Table 1). Within 2–6 min after ejection of kynurenate, the effects of bicuculline were strongly reduced or completely reversed (Table 2). In the example shown in Fig. 6, kynurenate nearly completely abolished within 4 min the brachialis activity released by bicuculline at the test point.

Discussion

Three new observations were made in the course of our experiments. First, we have shown that the release of a muscle(s) represented at a test motor cortical point into a synergy with the one(s) represented at a microstimulated cortical point occurs when the test point is disinhibited. In contrast, increasing the excitability of a test motor cortical point, on its own, does not result in the release of muscle(s) therein represented into a synergy. Third, by inhibiting excitatory synaptic transmission at the test point, we have shown that the effects of the microstimulated point on the test point were mediated by synaptic connections. Based on these observations we suggest that during natural motor activities the functional linking of

motor cortical points involves not only excitation of selected points, but also their disinhibition.

In the discussion that follows, we first consider the methodological issues and limitations of the present experiments and end with a discussion of the neural mechanisms and functional implications of our observations.

Methodological issues

The nature and dynamics of electrochemical events leading to drug ejection from a narrow micropipette tip are complex (Lalley 1999; Purves 1981). In particular, retaining currents (as used here to prevent leakage) can deplete the test drug from the tip of micropipette, creating a 'dead-space' that considerably delays drug release (Lalley 1999; Purves 1981). Additionally, drug concentration decreases steeply with distance (Lalley 1999). Enzymatic breakdown and uptake by neurons and glial cells may further reduce the local drug concentration, and physical barriers such as neurites and blood vessels add to this effect. In short, the time of effect of a drug ejected iontophoretically bears no relation to the dynamics of neural circuit activity. Thus, the observed time of action of bicuculline is unrelated to the dynamics of cortical inhibitory circuits.

It may be argued that glutamate equally excited excitatory and inhibitory neurons at the point of application and thus did not produce an increase in excitability. Recordings at the point of glutamate ejection clearly revealed an increase in neural activity, consistent with the well-known action of glutamate in the neocortex (Krnjevic 1981). Despite this, stimulation of a control point did not recruit the muscle(s) represented at a glutamate-excited test point into a synergy. In no instance did we observe what may be termed a 'depolarization block' resulting from excessive application of glutamate. The initial effect of bicuculline was similar to that produced by glutamate, viz. an increased level of desynchronized neural activity. Although we did not attempt to quantify which drug produced a greater increase, the recordings of neural activity were not remarkably different, as also reported by Tremere et al. (2001). It is important to note that the creation of an artificial muscle synergy during disinhibition of a cortical point occurred in the period of increased desynchronized neural activity, well before paroxysmal bursts appeared. We conclude that the functional linking between the control and test motor cortical points was specifically the result of disinhibition of the test motor point. The attenuation of the effects of bicuculline by kynurenate demonstrates that the effect of the control point on the test point was synaptically mediated. This is consistent with the fact that the cortical points paired in our experiments (Table 1) are known to be interconnected by intrinsic long-range collaterals (Keller 1993). Furthermore, these muscles can all act as synergists during natural movements.

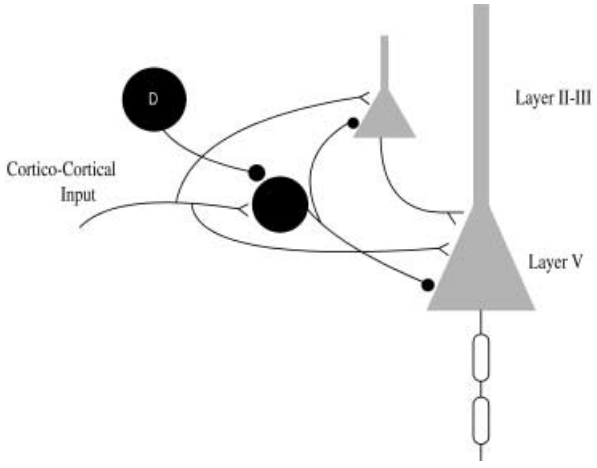

Fig. 7 Neural circuit suggested to explain the present experimental observations. Inhibitory interneurons are shown in *black* and pyramidal cells in *grey*. The inhibitory interneuron labeled *D* is hypothesized to inhibit the local inhibitory neuron upon which cortico-cortical afferents impinge. The cortical layers refer to the positions of pyramidal neurons not those of the inhibitory neurons, or to the positions of synapses

Neural mechanisms

What is the nature of the neural circuit linking motor cortical points? On the basis of the present observations, we can only suggest a neural circuit in broad outlines (Fig. 7). It is clear that under normal circumstances the activity transmitted from the microstimulated point to the test point is ineffective in evoking a response from that point. We suggest that this is because the input is normally blocked in at least two ways. One site at which the input may be inhibited is at the soma of the corticospinal neurons in layer V (output neurons). This may be because of ongoing GABAergic activity, as has been suggested to occur in the brain (Otis and Mody 1992). Another possibility is that the afferent volley activates inhibitory interneurons that, in turn, synapse onto the corticospinal neurons (Fig. 7). The neural mechanisms we have suggested are broadly similar to those proposed by Sanes and Donoghue (1997) on the basis of experiments dealing with the maintenance and reshaping of motor maps in the rat motor cortex (Jacobs and Donoghue 1991). This work will be discussed in relation to the present observations in the next section.

The observations made during glutamate ejection may provide further clues on the type of neural circuit involved. Glutamate increased the activity of what appeared to be large neurons in layer V (i.e., large spikes were recruited during glutamate ejection). Therefore, we can infer that these were tonically depolarized. In which case, the afferent input elicited by microstimulation should have brought the membrane potential above threshold in at least a fraction of the discharge cycles. The fact that it did not, suggests that the major mechanism involved in blocking the cortico-cortical input may be due to local inhibitory neurons (Fig. 7). Thus a

cortico-cortical input would act, directly or indirectly, to excite the corticospinal neurons and simultaneously on inhibitory interneurons whose net effect is to inhibit the corticospinal output neurons (directly or indirectly). The massive intrinsic connections (Capaday et al. 1998; Huntley and Jones 1991; Keller 1993; Tokuno and Tanji 1993) between various forelimb segments may in fact require such a stabilizing mechanism. Otherwise, activity at a given cortical point would spread well beyond it and recruit muscles that are inappropriate for the intended movement.

The circuit presented in Fig. 7 implies that functional linking occurs when the local inhibitory neurons are themselves inhibited. The question that thus arises is: How during natural activity are the local inhibitory cells disinhibited? In the hippocampus, so-called master inhibitory neurons, which are specialized to innervate other GABAergic interneurons, have been identified (Ascady et al. 1996; Freund and Antal 1988). In the visual cortex, intrinsic connections between GABAergic neurons have also been identified (Tamas et al. 1998). It seems likely, therefore, that in the motor cortex intrinsic circuits for inhibiting GABAergic interneurons exist. The functional linking of cortical points under natural conditions would therefore involve not only excitation of selected points, but also disinhibition of the local inhibitory interneurons (Fig. 7). Whether thalamic or premotor cortical inputs control the disinhibition, remains to be determined.

Functional implications

As stated in the Introduction, our approach was to consider a simplified version of how motor cortical points may be functionally linked during natural movements. To this end, we used an anaesthetized preparation to investigate the neural mechanisms potentially involved. But this does not detract from the value of the results or from their functional implications. The idea that disinhibition of cortical points may be an important mechanism involved in the functional linking of motor cortical points is a testable outcome of the present experiments worthy of further investigation, especially in a behavioural context. It is important to note that this idea does not challenge the fact that the intraspinal branching of corticospinal axons is part of the anatomical substrate of muscle synergies. The issue addressed here is how this anatomical substrate is set into action.

Whether disinhibition is a mechanism of synergy recruitment, per se, or one allowing activation of individual motor cortical points is conceptually difficult to disentangle. Consider that, once a cortical point is released from inhibition, the consequences are that: (1) influences from adjacent points can occur as shown here, and (2) the resulting interaction and spatial pattern of activity is what determines the movement-related muscle synergy. Thus, the functional linking of motor cortical points may be inextricably related to synergy recruitment. What is clear is that the striking and robust phenomenon we have observed here is likely to have a functional role in the operation of the motor cortex. Indeed, independent observations on human subjects using paired magnetic stimuli to the motor cortex suggest that disinhibition is a prominent feature of motor cortical function (Kujirai et al. 1993). At rest, for conditioning-testing intervals of 2–3 ms, a subthreshold conditioning stimulus reduces the size of the test response. When the subject contracts the target muscle, this inhibition is substantially decreased. The interpretation is that localized activity in the motor cortex is accompanied by a reduction of inhibition (Kujirai et al. 1993). During a coordinated gesture, such as pointing, involving a greater area of cortical activation inhibition is further reduced (Devanne et al. 2002). It has also been reported that during the reaction time of a simple movement the decrease in inhibition precedes the increase in cortical excitability (Reynolds and Ashby 1999). It should be noted, however, that there is no direct evidence that the paired-pulse inhibitory phenomenon reported in humans is due to intracortical GABAergic neurons. The present direct experimental results in the cat show that the mechanism is plausible and lend support to the experimental results from humans.

Based on a series of experiments closely related to the present ones, Jacobs and Donoghue (1991) have suggested that GABAergic cells are important for maintaining the spatial features of neocortical sensory and motor maps (see also Capaday et al. 2000). Here we suggest that GABAergic neurons are involved dynamically in functionally linking motor cortical points. There is no contradiction between these two potential roles of GABAergic neurons. In fact, Jacobs and Donoghue conclude that *"These results suggest that the architecture of inhibitory circuits is crucial to dynamic processes and map organization within the cortex*".

Conclusions

With considerable foresight, Leyton and Sherrington (1917) have suggested that 'mutual associations' between separate motor cortical points must be a key to the 'synthetic powers' of the motor cortex. Here we have shown that disinhibition may be one of the neural mechanisms involved in dynamically linking motor cortical points. This functional linking may be part of the ensemble of motor cortical mechanisms involved in recruitment of muscle synergies.

Acknowledgements This work was funded by the NSERC and in part by the CIHR and the Canadian Neurotrauma Foundation. Cyril Schneider was supported by a post-doctoral fellowship from the Canadian Neurotrauma Foundation. Charles Capaday is a senior research scholar of the FRSQ. We thank Drs. E. Fetz and A. Sik for their helpful comments and suggestions on a draft of the manuscript.

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