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RESEARCH ARTICLE

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Intracortical connections between motor cortical zones controlling antagonistic muscles in the cat: a combined anatomical and physiological study

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Abstract Experiments were done on nine cats anaesthetized with pentobarbitone to determine whether motor cortical zones controlling antagonistic muscles are synaptically interconnected. Motor cortical zones controlling wrist flexors, or extensors, were identified by microstimulation and intramuscular electromyographic recordings (microstimulation: 11 pulses at 333 pulses/s, current 10–40 μA). The position of each zone of interest was marked by a small ink spot on the surface of the cortex and on a scaled drawing of the cortical surface (cruciate region). Following the identification of wrist flexor and extensor zones the anterograde tracer biocytin was injected into one, or two, wrist extensor zones at three depths (400, 800 and 1500 μm) from the cortical surface. A small injection of horseradish peroxidase (HRP) – producing a dark brown spot of approximately 300–500 μm – was made in layer II–III of one or more wrist flexor zones. Similar HRP injections were made in the deep layers of wrist extensor zones that were not labelled by biocytin. The HRP injections served to mark the position of potential targets of biocytin-labelled fibres. In some experiments the biocytin was injected into a wrist flexor zone and HRP was deposited in one or more wrist extensor zones. Biocytin-labelled fibres (blue) were found throughout the expanse of the forelimb representation zone, as has been previously reported. More specifically, in all animals biocytin-labelled fibres were found in identified cortical zones controlling the same muscle(s) as well as in zones controlling an antagonist(s). Club-like swellings, indicative of synaptic boutons, were observed on these fibres. The density of labelled fibres was greater in the upper cortical layers (II–III), but a large number of terminals were also present in the lower cortical layers (V–VI). We conclude that there exist intracortical circuits linking motor cortical zones controlling antagonistic muscles. Elucidating the nature and function of these circuits is likely to be important for understanding the mode of operation of the motor cortex.

Key words Motor cortex · Microstimulation · Intracortical horizontal connections · Reciprocal inhibition · Biocytin · HRP

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Introduction

Classic studies of the intrinsic organization of the motor cortex using nerve fibre degeneration and Golgi staining of single neurons are well summarized by Gatter and Powell (1978). It was clearly appreciated by that time that different topographical areas of the motor cortex are interconnected by horizontal fibres extending for several millimetres. Gatter and Powell (1978) suggested that the density of connections was greatest in a radius of about 300 μm and decreased over the next 2–3 mm. They concluded that "no group of cells can be functionally independent of other cells within the adjoining few millimeters of cortex". They referred to this zone of intracortical connectivity, about 2 mm wide by 5 mm long, as an ellipsis oriented in the anterior-posterior (A-P) plane. More recent refinements involved the superposition of topographical maps of the motor cortex obtained by microstimulation and morphological maps obtained by tracer injections in physiologically identified sites (Huntley and Jones 1991; Keller 1993; Tokuno and Tanji 1993). These studies have shown that motor cortical zones controlling the various forelimb segments are strongly interconnected by horizontal intrinsic collaterals. These intracortical connections have been suggested to be an anatomical substrate of muscle synergies involved in coordinated multijoint movements (see also Allum et al. 1982; Kwan et al. 1987). In fact, this idea was already clearly expressed by Leyton and Sherrington (1917), who thought that the

"mutual associations" between separate motor cortical zones must be a key to the "synthetic powers" of the motor cortex (see also Graham-Brown and Sherrington 1912).

Over the past few years our studies of the cortical control of antagonistic muscles during functional motor tasks (Lavoie et al. 1995, 1997; Capaday et al. 1996; Capaday 1997) have led us to ask whether motor cortical zones that control antagonistic muscles are intrinsically interconnected. For example, how is a motor cortical zone at which microstimulation produces wrist flexion (Wf) synaptically connected with a zone at which microstimulation produces wrist extension (We)? Although one may surmise from the work of Huntley and Jones (1991) and Keller (1993) that such connections may exist, these studies were not done at the fine scale required to answer this question. For example, technical issues such as problems involved in aligning anatomical reconstructions with two-dimensional microstimulation maps limit spatial resolution. Additionally, zones controlling antagonistic muscles were not specifically identified in those studies. Two motor cortical zones each controlling one of a pair of antagonistic muscles may be either completely unconnected, connected by reciprocal (or non-reciprocal) inhibitory circuits, connected by reciprocal (or non-reciprocal) excitatory circuits, or linked by some combination of excitatory and inhibitory circuits. Based on our current knowledge of how antagonistic α-motoneuron pools are linked in the spinal cord (e.g. Baldissera et al. 1981), we expect that the answer to this question will be rather detailed and intricate. Therefore, the results described in this paper are a first glimpse at the issue.

There are reasons going back to the studies of Graham-Brown and Sherrington (1912) for expecting some degree of moment-to-moment variation of the responses obtained by stimulating two antagonistic motor cortical zones. They showed that when a given "cortical point" was stimulated the type of response obtained from a pair of antagonistic muscles is dependent on a variety of factors, including afferent input, and prior stimulation history at that point or at the antagonistic cortical point. We have, indeed, observed some of these phenomena in the present experiments. Nonetheless, with low-intensity microstimulation delivered at low repetition rates it was possible to clearly identify by electromyographic (EMG) recordings antagonistic as well as synergistic motor cortical zones and to show that these are interconnected by using the fast anterograde marker biocytin (Smith 1992). In the Discussion, we suggest that these results must be an important clue to the mode of operation of the motor cortex, and in particular to the mechanisms underlying the control of antagonistic muscles. These connections may also be a substrate of cortical reorganization following damage to the motor cortex or to its inputs. A summary of these results has been published in abstract form (Bertrand et al. 1996).

Methods

General considerations and procedures

To answer the question of whether identified motor cortical zones are intrinsically interconnected the following general procedures were followed. First, we identified by microstimulation in the forelimb area of the cat motor cortex separate zones controlling wrist flexors and wrist extensors. Perusal of several published maps of the cat motor cortex (e.g. Armstrong and Drew 1984; Tatton and Bedigham 1985) revealed that these muscles are well represented over large enough cortical distances (1–3 mm) to make the experiments feasible. The effects of microstimulation at a given site were determined by intramuscular EMG recordings, rather than the usual methods of muscle palpation and visual inspection of joint movement. Schmidt and McIntosh (1990) have shown that microstimulation mapping can be misleading unless the EMG activity of the responding muscles is recorded. For example, inhibition of a muscle may be interpreted as excitation if muscle palpation or visual observation of joint movement are used as the sole criteria. The surface of the cortex at which intracortical microstimulation produced a low-threshold wrist muscle response was marked by a small ink spot and on a scaled map of the cortical surface. Once at least two separate synergistic cortical zones (e.g. We-We) and at least one antagonistic zone were identified (e.g. Wf) the fast anterograde tracer biocytin was iontophoretically injected into one, or more, of the identified zone(s). To mark the potential target sites at which biocytin-labelled terminals might be found a small quantity of horseradish peroxidase (HRP) was iontophoretically injected at the other identified sites at measured depths from the cortical surface.

Surgical procedures

The experiments were done on nine adult male cats weighting between 2.5 and 3.5 kg. The experimental procedures were approved by the local ethics committee. The animals were first anaesthetized with an intraperitoneal injection of pentobarbitone (40 mg/kg). A tube was inserted into the trachea, and the left femoral artery and vein 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 trunk and by an overhead radiant heat lamp. The anaesthesia was maintained by small injections of pentobarbitone (5–25 mg) via the venous cannula, as needed. Ten millilitres 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 mm Hg. 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 about 1.5 cm, was inserted in each of the following muscles: 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) and the deltoid group (Gilbert 1984). To minimize cross-talk in the EMG recordings (Matthews 1959; Capaday 1995) 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 perpendic-

ular 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 oedema and brain pulsations, respectively. Finally, a craniotomy was done to expose the sensorimotor region. 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 mineral oil preheated to a temperature of 37°C.

Microstimulation and recording

Stainless steel microelectrodes ranging in impedance from 0.5 to 1 MΩ were used to microstimulate the motor cortex. Trains of stimuli (33-ms trains of 200 μs square pulses, at a rate of 333 pulses/s) were delivered through 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 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 and averaged in real time.

Iontophoresis of biocytin and HRP

In most animals biocytin (2% in 0.5 M potassium acetate) was ejected iontophoretically at three different depths (400, 800 and 1500 μm) at a single identified site, usually a wrist extensor zone (Fig. 1). In some animals biocytin was deposited in two separate synergistic motor cortical zones, as above. The tips of the biocytin pipettes were about 5 μm in diameter. At each depth biocytin was ejected for 30 min by a positive current of 300 nA (1 s "on", 1 s "off") superimposed on a 100 nA positive DC current (Bourassa et al. 1995). HRP was deposited iontophoretically at a measured depth to mark the position of identified sites, either synergistic or antagonistic to the biocytin zone. The HRP micropipettes (tip 2–3 μm) were filled with a solution of 4% HRP in 0.2 M NaCl adjusted to a pH of 7.4 with TRIS buffer (TB). Positive currents of 100–150 nA (1 s "on", 1 s "off"; 15 min duration) superimposed on a 100-nA positive DC current were used to eject the HRP. This method produced a dark brown spot of approximately 300–500 μm (Fig. 1). To distinguish between an HRP-marked synergistic versus antagonistic zone, the deposits were made at different measured depths (e.g. deep layers versus superficial layers).

Histological procedures

At least 4 h after the injection of biocytin the animal was perfused via the intracardiac route with 1 l of cold saline followed by 2 l solution of 4% paraformaldehyde and 0.5% glutaraldehyde in a phosphate buffer (PB: 0.1 M, pH 7.4). Following the perfusion a block of cortex including the cruciate area and the coronal gyrus was removed from the brain and placed in 100 ml of the above solution for at least 24 h. The fixed block was then cut into sections of 80 mm in the sagittal plane using a manual Vibroslice and placed in small wells containing cold PB. The sections were rinsed twice for 20 min in PB and then placed in a solution of 3% hydrogen peroxide for a further 20 min. This served to eliminate any endogenous peroxidase activity and non-specific labelling. The sections were then rinsed for 10 min three times in PB and TB (0.05 M, pH 7.4) while being agitated. Following the rinses the sections were reacted with a solution containing diaminobenzidine (DAB) for 5–10 min to reveal, with a brown coloration, the HRP injection sites. The sections were thereafter washed for 30 min in PB and then incubated overnight in the ABC kit (Vector Laboratories, Burlingame, Calif.). The following day the sections were washed in PB for 10 min and three times for 10 min in TB. The sections were then reacted for 10–15 min with a solution of Ni-DAB containing 0.3% hydrogen peroxide to reveal the biocytin-labelled neural elements with a dark blue colour. Finally, the sections were washed in PB buffer followed by TB, for 25 min in each case. The sections were mounted in a numerically ordered arrangement (medial to lateral) on Chromealum-coated slides, air dried, and a coverslip was affixed to the slide with Permount.

The sections were observed with a light microscope to which a camera lucida was attached. Biocytin-labelled terminals (blue) within a radius of about 300–500 μm from the centre of an HRP-marked site (brown) were drawn using the camera lucida.

Results

Physiological identification of injection sites

This study focused on the wrist extensors (ECRl and ECRb) and their antagonists the wrist flexors (PL and FCU). Motor cortical zones – operationally defined as a site at which microstimulation produces clear and reproducible activation of a muscle, or a group of synergistic muscles – were identified according to the following criteria: (1) the threshold and latency of the response, (2) its constant character from stimulus to stimulus, (3) the greater variability of co-activated muscles for suprathreshold stimuli, (4) inhibition of ongoing activity in the antagonist(s), (5) decrease of the response by stretch of the antagonist(s), (6) the relative size of the response, (7) a clear corresponding joint movement for stimuli above threshold. In fact, the EMG recordings were sensitive enough to detect activation of a few motor units in a muscle without any apparent joint movement. Thus, for example, a site was designated as a primary wrist extension zone if a wrist extensor muscle was activated at the lowest stimulus intensity, the response was repeatable from stimulus to stimulus (i.e. robust), a clear extension of the wrist was produced by suprathreshold stimuli, its latency was the shortest when other muscles where recruited at higher stimulus intensities (e.g. activation of ECR and Br) and the size of the response was greatest at or just above threshold.

An example of a zone at which microstimulation (18 μA) elicited a response in the ECR is shown in Fig. 2. Note that no other muscle at the wrist or elbow was activated. Usually only one of a group of synergists (e.g. ECR vs ECU) was activated at threshold, as reported by previous authors (Asanuma and Sakata 1967; Strick and Preston 1982). Note also that despite the tonic activity in the Br, no response was evoked in that muscle. Activation of the Br occurred at higher stimulus intensities (22 μA) and the response had a longer latency than that of the ECR. Co-activation of wrist extensors and elbow flexors is a common synergy represented in the cat motor cortex, but primary wrist or elbow zones can be clearly distinguished by the above criteria.

Finally, reciprocal inhibition was also an important criterion by which to identify a motor cortical zone. In the example shown in Fig. 3 the ECR alone was activated by a stimulus of 30 μA. When the wrist flexors were maintained stretched by the experimenter, they became tonically active (i.e. a tonic stretch reflex). This complete-

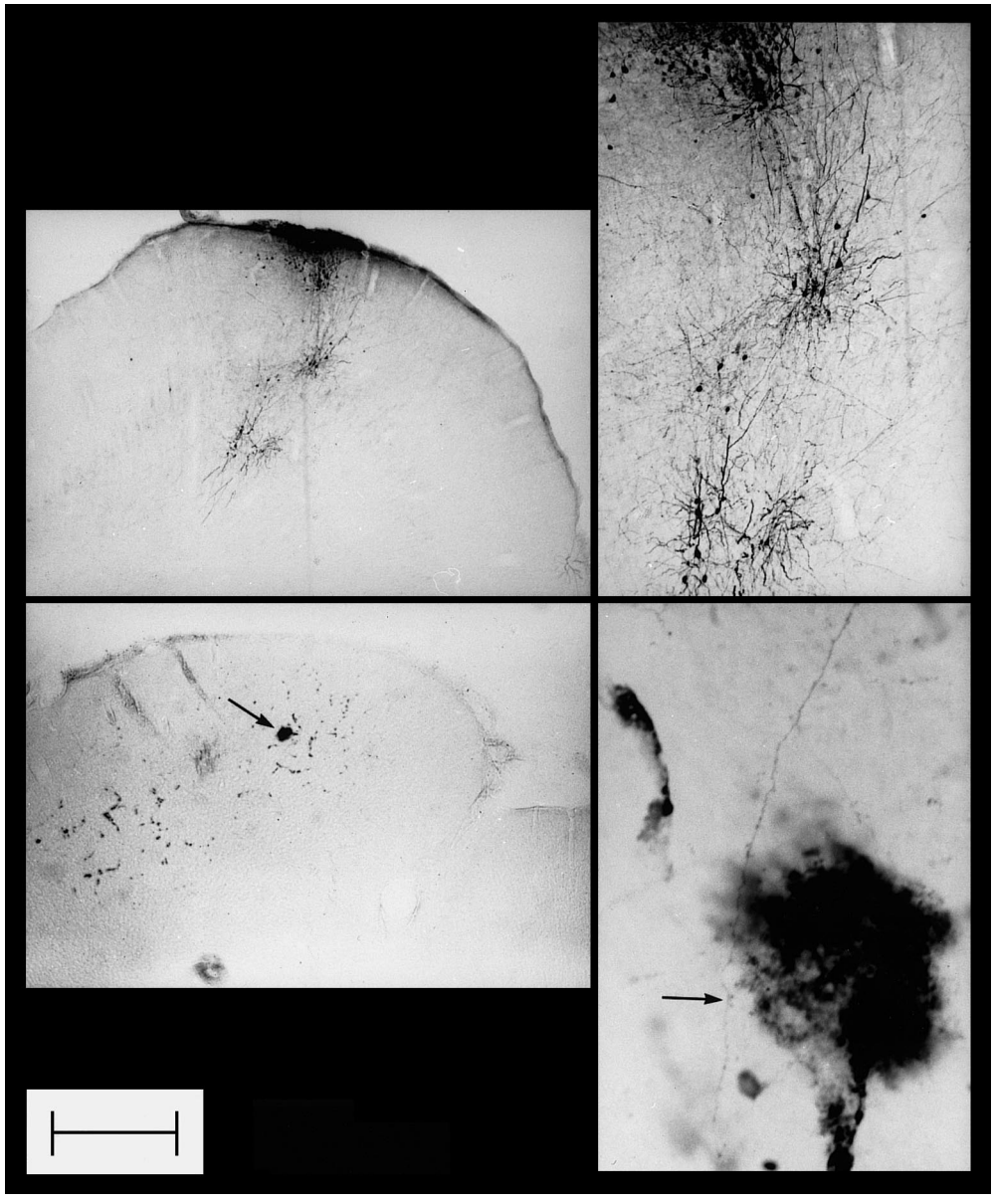


Fig. 1 Photomicrographs of parasagittal sections through the motor cortex in the region of the coronal gyrus showing examples of a biocytin (*upper*) and an HRP (lower) injection site. The figure in the *upper left* shows Golgi-like labelled cells at three different depths in the grey matter (scale bar represents 760 μm). Layer V is just above the deepest injection site. In the adjacent figure at the *upper right* biocytin-labelled axons can be seen coursing out of the injection site (scale bar represents 190 μm). The *lower left* and *lower right* photomicrographs show an HRP site (*arrow*) at low magnification (scale bar represents 760 μm) and high magnification (scale bar represents 47.5 μm) respectively. In the latter photomicrograph note the biocytin-labelled fibre in the vicinity of the dense core at the centre of the HRP deposit and the club-like extensions along the fibre (*arrow*), indicative of axon terminals

ly inhibited the response of the ECR and the stimulus had to be increased to 40 μA to activate it, but the response was clearly smaller than at 30 μA. Furthermore, activation of the ECR was clearly accompanied by an inhibition of the ongoing activity in the flexors. Upon release of the flexors, their activity stopped and the response of the ECR increased markedly (Fig. 3). On the basis of the above operational definitions this site was, therefore, designated as a primary We zone.

Anatomical results

Shown in Fig. 4 are two scatterplots of the position and distance of the HRP sites (synergistic or antagonistic site) relative to the biocytin injection site at the origin of the graphs. The left scatterplot is for biocytin injection sites placed in a We zone; the one on the right is for biocytin injections sites placed in a Wf zone. A different symbol is used for each animal and the response obtained by microstimulation at each site is marked by the symbols Ag or At indicating, respectively, a response of the same muscle as at the origin of the plot or of its antagonist(s). In all cases, regardless of the distance or position of the target zone relative to the biocytin injection site, labelled fibres (blue) were found in synergistic and antagonistic motor cortical zones (Figs. 1, 5, 6).

In the example shown in Fig. 5, biocytin was injected into a We zone at three different depths (400, 800, 1400 μm) from the cortical surface. HRP was deposited in layer V of a separate We zone, 1.61 mm anterior and 1.22 mm posterior to the biocytin injection site. Biocytin-labelled fibres within a 500 μm radius from the centre of the HRP deposit were drawn with a camera luc-

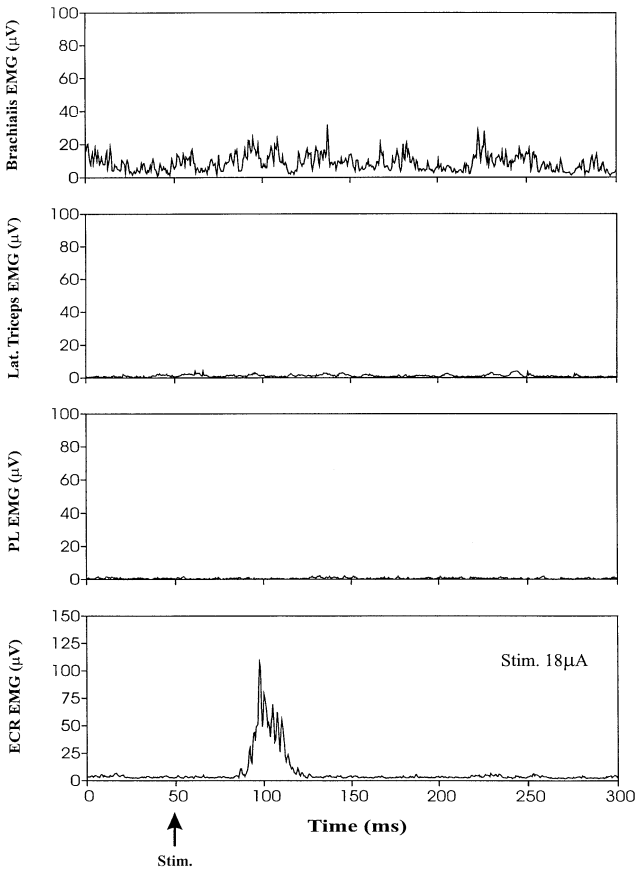
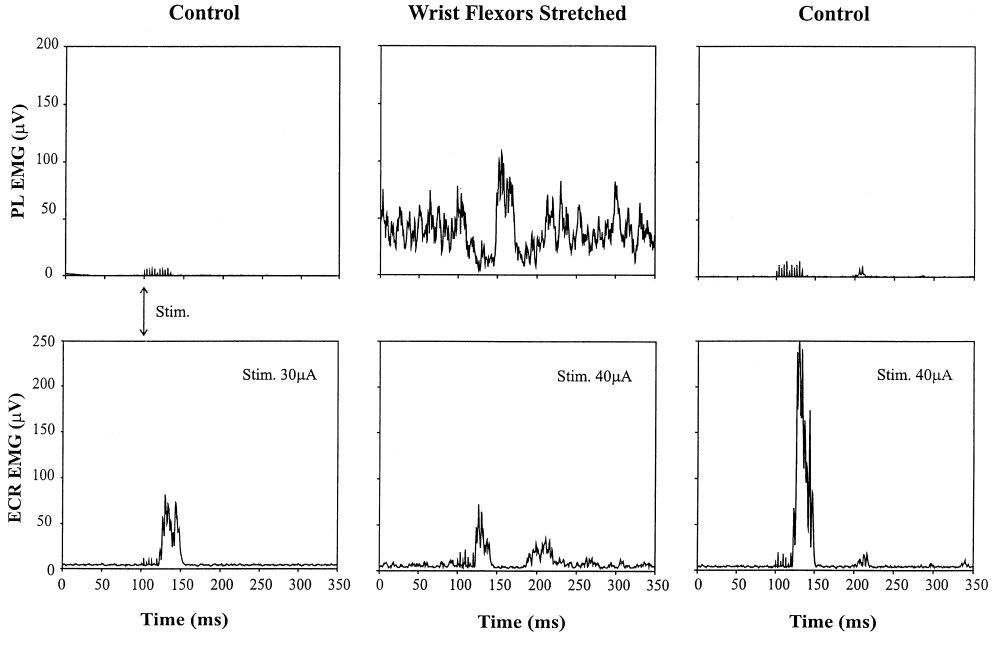


Fig. 2 Example of the specificity of responses in a given zone. Intramuscular electromyographic (EMG) recordings from two antagonistic elbow muscles (*upper traces*) and from two antagonistic wrist muscles (*lower traces*; *PL* palmaris longus, *ECR* extensor carpi radialis) following microstimulation of the motor cortex at 18 μA. Note that only the ECR was activated at this stimulus intensity, despite tonic activity in the brachialis. Each trace is the average of eight responses at the same stimulus intensity

ida. A two-dimensional representation of the biocytin-labelled fibres found in this block of cortex is shown superimposed on a parasagittal section in the middle of the HRP injection site. The fibre density is highest in the upper cortical layers, but fibres are present in all cortical layers. Fibres within the boundary of the HRP site are also shown in the more enlarged view in Fig. 5B. Club-like extensions on these fibres, indicative of synaptic boutons, can be seen even at this relatively low magnification. The responses obtained following microstimulation at each of these two We zones are also shown in Fig. 5C. These two injection sites were characterized as primary We zones by the criteria given in the preceding section. Note that in Figs. 5 and 6 the fibre density appears much greater than it actually is because they are two-dimensional representations of a three-dimensional block of tissue.

Similarly, when antagonistic motor cortical zones marked by an HRP deposit were examined, biocytin-labelled fibres were also found. In the example shown in Fig. 6, biocytin was injected into a We zone at three different depths (400, 800, 1400 μm) and HRP in the deep layers of a Wf zone. The HRP site was 2.15 mm lateral and 1.79 mm posterior to the biocytin site. The EMG re-

Fig. 3 Intramuscular EMG recordings from two antagonistic wrist muscles (PL and ECR) following microstimulation of the motor cortex at a site identified as a primary ECR zone. Stimulus delivery (indicated by an *arrow* in the left panels) occurred at 100 ms in each case. Small stimulus artefacts can be seen at that time in the left and right panels. At 30 μA (left panels), the ECR muscle was activated alone. When its antagonist the PL was tonically activated (centre panels) by muscle stretch, the ECR response was completely suppressed. Increasing the stimulus strength to 40 μA partially restored the ECR response (centre panels). Note that during tonic activity of the PL, the evoked ECR response was associated with simultaneous inhibition of the PL (centre panels). Release of the PL stopped the tonic activity in the PL and the 40 μA stimulus now produced a larger ECR response (right panels). Each *trace* is the average of 16 responses



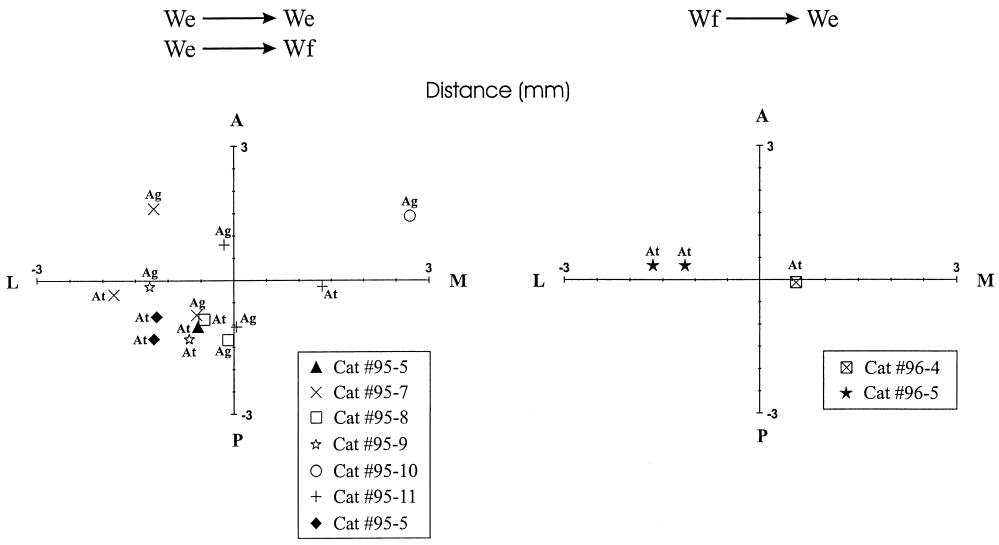


Fig. 4 Scatterplots of the position and distance of the HRP sites relative to the biocytin injection sites (origin of the graphs). Biocytin was injected either in a wrist extensor cortical zone (*left*) or in a few cases in a wrist flexor cortical zone (*right*). HRP deposits were made in identified agonistic (*Ag*) or antagonistic (*At*) cortical zones. Each *symbol* corresponds to a different animal. Several HRP deposits were sometimes made in the same animal. Each *tick* on the axes corresponds to 0.5 mm. *L* lateral, *M* medial, *A* anterior, *P* posterior

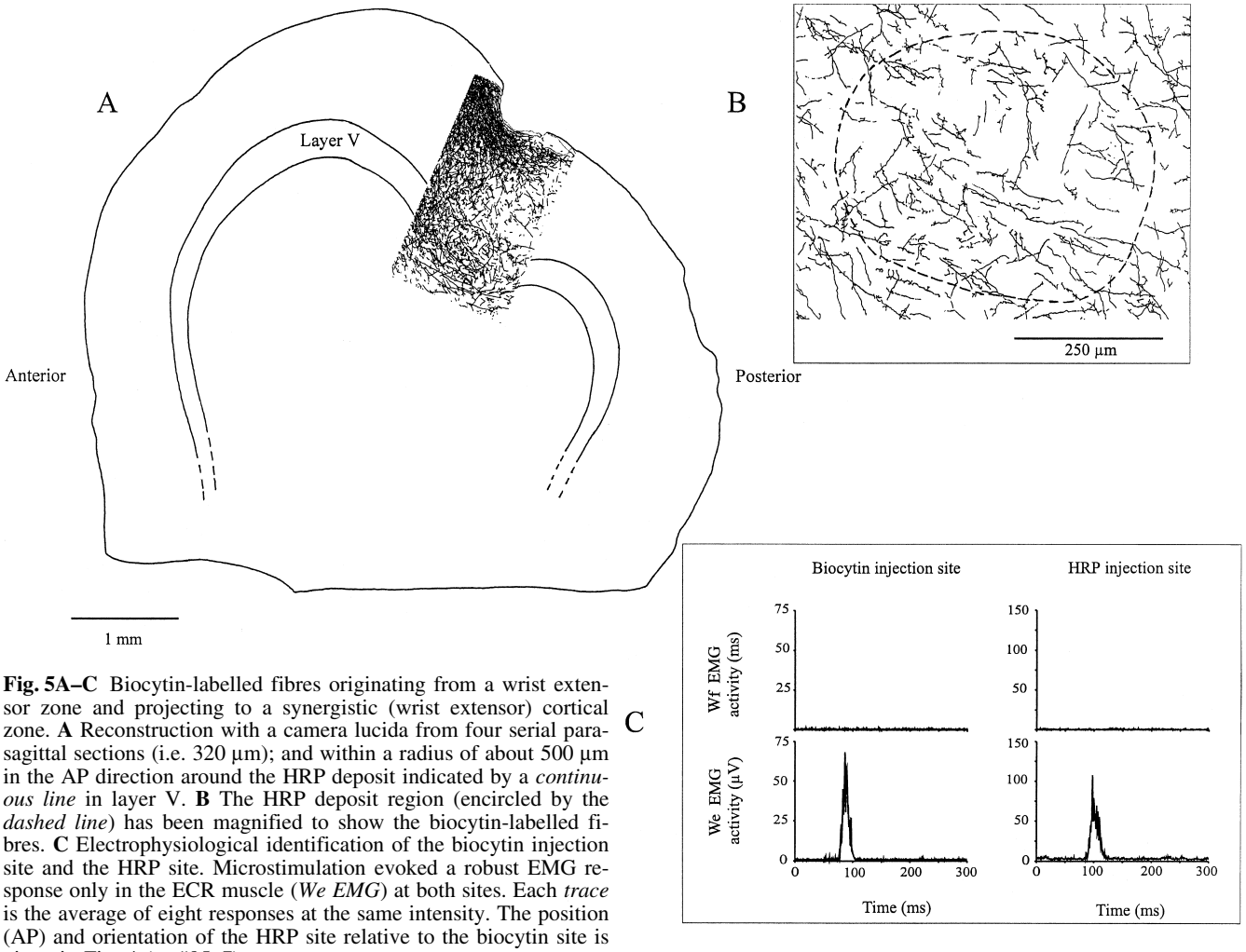


Fig. 5A–C Biocytin-labelled fibres originating from a wrist extensor zone and projecting to a synergistic (wrist extensor) cortical zone. **A** Reconstruction with a camera lucida from four serial parasagittal sections (i.e. 320 µm); and within a radius of about 500 µm in the AP direction around the HRP deposit indicated by a *continuous line* in layer V. **B** The HRP deposit region (encircled by the *dashed line*) has been magnified to show the biocytin-labelled fibres. **C** Electrophysiological identification of the biocytin injection site and the HRP site. Microstimulation evoked a robust EMG response only in the ECR muscle (*We EMG*) at both sites. Each *trace* is the average of eight responses at the same intensity. The position (AP) and orientation of the HRP site relative to the biocytin site is given in Fig. 4 (cat#95–7)

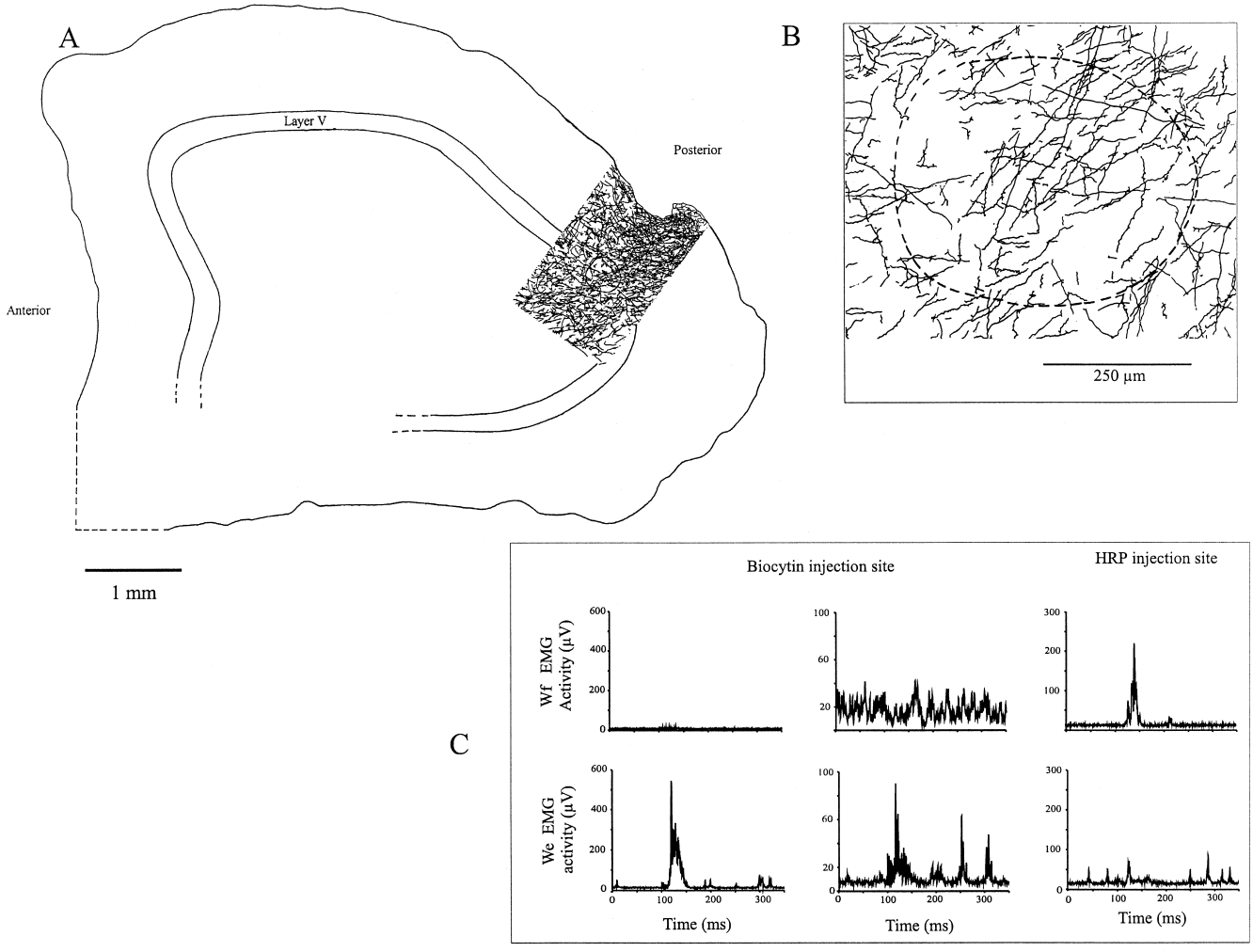


Fig. 6A–C **B**iocytin-labelled fibres originating from a wrist extensor zone and projecting to an antagonistic (wrist flexor) cortical zone. **A** Reconstruction with a camera lucida from four serial parasagittal sections (i.e. 320 µm); and within a radius of about 500 µm in the AP direction around the HRP deposit indicated by a *continuous line* in layer V. **B** The HRP deposit region (encircled by a *dashed line*) has been magnified to show the biocytin-labelled fibres. **C** Electrophysiological identification of the biocytin injection site and the HRP site. In the biocytin injection site (*left*) microstimulation evoked a robust EMG response in wrist extensors (*We EMG*) but not in the antagonists (*Wf EMG*). In this example, as in Fig. 2, the We EMG response decreased when the antagonist Wf was tonically activated, and activation of the We was accompanied by near-simultaneous inhibition of the Wf (*centre*). In the HRP deposit region microstimulation evoked a strong Wf EMG response (*right*). Each trace is the average of eight responses at the same intensity. The HRP site was 2.15 mm lateral and 1.79 mm posterior to the biocytin site

cordings in Fig. 6C clearly show that stimulation of the We zone produced activation of the ECR and inhibition of its antagonist the PL when the latter was active. Stimulation of the Wf zone, into which HRP was deposited, activated the PL. Wf zones were, however, less common than We zones and the responses produced by stimulation at these sites more labile than those of We zones. The method of histological reconstruction was the same as described above. Again, fibres are densest in the upper cortical layers, but clearly present in all layers. In the centre of the HRP deposit club-like extensions on the fibres, indicative of synaptic boutons, were observed in all cases. A high-magnification photomicrograph of a biocytin-labelled fibre near the centre of an HRP deposit in a Wf zone of a different animal is shown in Fig. 1. Note the club-like extensions on the fibre near the centre of the HRP deposit. Terminal field arborizations with terminal boutons were observed within each type of zone, but a large proportion of boutons were observed on the more proximal portion of the fibres (i.e. "boutons de passage"). However, this was not quantified because of the complexity and assumptions required to make such counts. The density of biocytin-labelled fibres decreased as a function of the distance between the source and target sites, regardless of whether these were synergistic or antagonistic motor cortical zones. The density of fibres in target sites was also related to the number of stained somata at the biocytin injection site.

Similar observations were made when biocytin was injected into a primary Wf zone and HRP in a primary We zone (Fig. 4). Thus, motor cortical zones controlling an-

tagonistic wrist muscles are likely to be reciprocally connected.

Discussion

In this study we have shown that motor cortical zones controlling antagonistic wrist muscles are interconnected, as are zones that control synergistic wrist muscles. This study complements those of Huntley and Jones (1991), Tokuno and Tanji (1993) and Keller (1993), which showed that the forelimb area of the motor cortex is a massively interconnected structure. The interconnections demonstrated here between motor cortical zones controlling antagonistic muscles are one of the likely many features of this massive pattern of connections. One important point we wish to make clear is that the horizontal connections described in this study may not only link wrist motor cortical zones, but also zones that control forelimb muscles acting at different joints (e.g. elbow and shoulder). Indeed the intracortical distances involved in the present study (up to about 3 mm) are comparable to the distances involved in the study of the cat motor cortex (also up to about 3 mm) by Keller (1993) and those involved in the study of the monkey motor cortex (up to about 5 mm) by Huntley and Jones (1991). It is thus conceivable that the horizontal fibres analysed in our study innervate along their course motor cortical zones that control muscles acting at other forelimb joints. Elucidating these details will considerably further our understanding of motor cortical function.

The motor cortical zones in this study were identified according to well-established physiological criteria such as the response threshold, robustness of the response and reciprocal inhibitory actions. However, when a zone is operationally identified as a We zone, for example, it is not implied that such a zone directly controls only the wrist extensors, or that the activity of neurons in this zone will be related to wrist extension only. Our observations on the effects of microstimulation emphasize the existence of interjoint synergies and these may be a prominent feature of the organization of the motor cortex (see also Armstrong and Drew 1985). Indeed, Drew (1993) made the point that the relationship between the effects of microstimulation and movement-related cell discharge at a cortical site is not one to one, but that there is nonetheless a correspondence. The important point for the purpose at hand is that separate motor cortical zones controlling antagonistic muscles were clearly identified. In particular, reciprocal inhibition was an important aspect of this identification. This shows that there was a clear functional link between these zones according to a classic description of antagonistic muscle actions.

In the following sections we will discuss the nature of these connections, their specificity and their potential functional role.

Nature of the connections

Thalamocortical afferents from the nucleus ventralis lateralis (VL) typically extend for about 1 mm (Asanuma et al. 1974). The connections we have found in the present study extend for several millimetres and are reminiscent of the horizontal intracortical connections described by Gatter and Powell (1978). Additionally, at all sites in which biocytin was injected Golgi-like labelling of neurons was found from which axons could be clearly seen coursing away (Fig. 1). Finally, intrinsic cortical axons are likely to vastly outnumber thalamocortical afferents (Keller 1993). It is therefore concluded that the connections observed between synergistic and antagonistic motor cortical zones in the present study are, for the most part, intrinsic intracortical connections. It is also entirely possible that synergistic as well as antagonistic motor cortical zones may be linked by thalamocortical afferents as well as by cortico-cortical afferents from premotor cortical areas (e.g. Tokuno and Tanji 1993), thus reinforcing the intrinsic motor cortical connections. As shown in Fig. 3 connections were found in all cases, regardless of the relative orientation and distance between motor cortical zones controlling antagonistic wrist muscles. Additionally, since the distances involved in this study fall for the most part within the elliptic AP zone of Gatter and Powell (1978), it may be concluded that some form of specificity is represented by these connections. This elliptic AP zone may be thought of as a "functional cortical block" containing circuitry for the coordination of muscles groups that are represented within it.

To what extent are the results obtained in this study dependent on the labelling of fibres of passage coursing through the identified biocytin injection sites, rather than of fibres originating from neurons in the injection sites? Firstly, biocytin is thought to be taken up mostly by somata and transported anterogradely to the axon terminals (Smith 1992). The density of biocytin-labelled fibres found in target sites was related to the number of labelled somata at the injection site, or to the number of injection sites (i.e. one vs two sites). Nonetheless, it is possible that some fibres of passage may also take up the label. As shown in Fig. 4 the orientation of synergistic and antagonistic motor cortical zones relative to the biocytin injection site differed within each animal and between animals. On this basis it seems unlikely that fibres of passage coursing through the biocytin injection sites account for the observed connections. This would imply that in all nine animals axons destined separately to synergistic and antagonistic cortical zones always coursed together through all the identified biocytin injection sites. In summary, while a contribution of fibres of passage cannot be excluded, the present results are due for the most part to fibres originating from labelled neurons at the identified injection site.

It is not possible to conclude from the present results whether the connections between either synergistic or antagonistic motor cortical zones are excitatory, inhibitory, or some combination of the two, nor whether there are

differences in the connection patterns. This will require electron microscopic analysis combined with intracellular recordings and labelling. But the issue is likely to be complex and not easily resolved in a single study or with a single approach. In particular, task-dependent modulation of these circuits is likely and there is already evidence for this as discussed below.

Possible functional role

The need for neural mechanisms involved in the differential control of antagonistic muscles becomes apparent when considering the variety of ways in which they are activated (Lavoie et al. 1997). Synergies between antagonists include simple patterns of reciprocal activation, co-contractions, complex triphasic activation patterns, and complex synergies such as during finger movements (Hoffman and Strick 1986; Doemges and Rack 1992; Schieber 1995). Fist clenching is a common example of a synergy involving co-contraction of antagonistic wrist muscles linked with activation of the finger flexors. The intracortical connections between motor cortical zones controlling antagonistic muscles are likely to be part of the neural substrates involved in such coordinations. From the complexity of these synergies it may also be surmised that these intracortical circuits may be subject to strong task-dependent modulations. For example, there have been many reports of a dissociation between activity of corticomotoneuronal (CM) cells and the activity of their target muscles (Fetz and Cheney 1980; Mantel and Lemon 1987). In particular, Humphrey and Reed (1983) have shown that some pyramidal tract neurons are active during co-contractions of antagonistic muscles at the wrist, but not during reciprocal patterns of activation. Three types of CM cells controlling wrist muscles have been identified (Lemon 1988; Cheney et al. 1991): cells that excite agonists and simultaneously inhibit antagonists, cells that only excite a group of synergists, and cells that only have an inhibitory action on a group of synergists. The task-dependent coordination of these cell types by modulation of the intracortical circuits between antagonistic motor cortical zones may be an important aspect of synergy selection, worthy of further investigation. In fact, these ideas concerning the coordination of antagonist muscles at the cortical level were clearly expressed by Graham-Brown and Sherrington (1912). Later, Leyton and Sherrington (1917) in referring to reversal of the effects on an antagonistic pair of muscles from the same cortical point – i.e. by prior stimulation history at that point or at adjacent points – concluded that "the functional instability of cortical motor points are indicative of the enormous wealth of mutual associations existing between the separable motor cortical points, and those associations must be a characteristic part of the machinery by which the synthetic powers of that cortex are made possible". The existence of such connections between motor cortical zones controlling antagonistic muscles has been established in the present study.

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