Facilitation From Ventral Premotor Cortex of Primary Motor Cortex Outputs to Macaque Hand Muscles

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1Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, London WC1N 3BG, United Kingdom; and 2University Paris-VII and Institut National de la Santé et de la Recherche Médicale U.483, Paris, France

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Cerri, G., H. Shimazu, M. A. Maier, and R. N. Lemon. Facilitation from ventral premotor cortex of primary motor cortex outputs to macaque hand muscles. J Neurophysiol 90: 832–842, 2003; 10.1152/jn.01026.2002. We demonstrate that in the macaque monkey there is robust, short-latency facilitation by ventral premotor cortex (area F5) of motor outputs from primary motor cortex (M1) to contralateral intrinsic hand muscles. Experiments were carried out on two adult macaques under light sedation (ketamine plus medetomidine HCl). Facilitation of hand muscle electromyograms (EMG) was tested using arrays of fine intracortical microwires implanted, respectively, in the wrist/digit motor representations of F5 and M1, which were identified by previous mapping with intracortical microstimulation. Single pulses (70–200 μA) delivered to F5 microwires never evoked any EMG responses, but small responses were occasionally seen with double pulses (interval: 3 ms) at high intensity. However, both single- and double-pulse stimulation of F5 could facilitate the EMG responses evoked from M1 by single shocks. The facilitation was large (up to 4-fold with single and 12-fold with double F5 shocks) and occurred with an early onset, with significant effects at intervals of only 1–2 ms between conditioning F5 and test M1 stimuli. A number of possible pathways could be responsible for these effects, although it is argued that the most likely mechanism would be the facilitation, by corticortical inputs from F5, of corticospinal I wave activity evoked from M1. This facilitatory action could be of considerable importance for the coupling of grasp-related neurons in F5 and M1 during visuomotor tasks.

INTRODUCTION

The ventral premotor area (PMv or F5) is thought to play an important role in visuomotor control of the hand. It is part of a corticocortical circuit that is essential for the development of object-specific grasp (Fig. 1A) (Geyer et al. 2000; Jeannerod et al. 1995; Rizzolatti et al. 1998). There are dense corticocortical projections to F5 from regions of posterior parietal cortex that process visual inputs related to grasp (Murata et al. 2000; Sakata et al. 1995; Taira et al. 1990). These projections originate from parietal area AIP and terminate in the region of F5 that lies in the bank of the inferior arcuate sulcus (Luppino et al. 1999; Rizzolatti et al. 1998; Tanne-Gariépy et al. 2002). Single-unit activity within this same arcuate bank region of F5 is related to grasp (Murata et al. 1997; Rizzolatti and Luppino 2001), and visually guided grasp of objects is temporarily impaired after injection of the GABA agonist, muscimol, into this same region (Fogassi et al. 2001).

How then does area F5 influence movement of the hand and fingers? This issue is of considerable importance in understanding how visuomotor activity in F5 is transformed into motor commands that ultimately determine the kinematics and dynamics of hand shaping that is appropriate for the object to be grasped. It is known that intracortical microstimulation in area F5 evokes discrete wrist and finger movements (Gentilucci et al. 1988; Godschalk et al. 1995; Hepp-Reymond et al. 1994). The most direct pathway that might mediate these effects is the corticospinal projection (Dum and Strick 1991). A part of that projection does indeed arise from the F5 arcuate bank region, but it is rather weak in the macaque, and, critically, it does not project to the motor nuclei in the cervical enlargement that innervate the wrist and finger muscles. Thus He et al. (1993) demonstrated that a cluster of F5 corticospinal neurons that were labeled from the upper cervical segments (C2–C4 segments) were not labeled when the injection was made in the cervical enlargement (C7–T1). This result was confirmed by Galea and Darian-Smith (1994).

An alternative pathway through which F5 could influence hand movements would be via the dense and numerous corticocortical connections from F5 to the primary motor cortex (M1) (Ghosh et al. 1987; Godschalk et al. 1984; Jeannerod et al. 1995; Lu et al. 1994; Matelli et al. 1986; Muakassa and Strick 1979). Tokuno and Nambu (2000) showed recently that pyramidal tract neurons in M1 respond at short latency to stimulation in the bank of the inferior arcuate sulcus. M1 gives rise to a very large number of corticospinal projections to the cervical enlargement (Dum and Strick 1991; Galea and Darian Smith 1994; He et al. 1993) including many direct cortico-motoneuronal projections to motoneurons innervating wrist and finger muscles (Armand et al. 1997; Fetz and Cheney 1980; Kuypers 1981; Lemon et al. 1986; Maier et al. 2002).

We investigated whether we could influence M1 outputs to intrinsic hand muscles through activation of the bank region of F5, and we report here robust, short-latency effects of conditioning stimulation of F5 on EMG responses evoked in hand muscles by single shocks to M1. Although other pathways may be involved, one possible mechanism to explain these results is...
a fast cortico-cortical pathway from F5 to M1 that facilitates the corticospinal outflow from M1 to hand motoneurons.

This work has been published previously in abstract form (Cerri et al. 2002).

METHODS

The study was performed on two adult, purpose-bred monkeys (M. fascicularis; CS13 and CS14, weights: 3.1 and 4.4 kg, respectively). Animal care and use was in accordance with the UK Animals (Scientific Procedures) Act 1986.

Identification of hand representations in F5 and M1

Two approaches were used to locate accurately these representations: magnetic resonance imaging (MRI) and repetitive intracortical microstimulation (rICMS).

MRI. This was used to determine the precise sulcal geometry of the central and arcuate sulci to allow accurate placement of recording chambers, microelectrode penetrations for ICMS mapping, and final placement of intracortical microwire electrodes. Scans were carried out under deep general anesthesia; for detailed, methods see Baker et al. (1999).
2.5% in a 1:1 O2-N2O mixture). A craniotomy was made and a stainless steel chamber (18 mm ID) was mounted over the lateral surface of the precentral gyrus, giving access to the F5 and M1 hand areas. The orientation of the chamber was guided by the MRI scan and set to allow electrode penetrations in the depths of the central and inferior arcuate sulci. After the surgery, an antibiotic (tetracycline LA 20 mg/kg, Pfizer, Sandwich, Kent, UK) and an analgesic (buprenorphine hydrochloride 5–10 μg/kg im; Veteregic, Reckitt and Colman, York, UK) were administered.

Over periods ranging from 3 to 6 wk, rICMS was used to map the motor representations of F5 and M1. For this, the monkey was lightly sedated with a mixture of ketamine and medetomidine HCl (Dormitor, Pfizer). This preparation allows investigation of the motor system under conditions in which the level of motoneuronal excitability is reasonably stable, although cortical stimulation thresholds may be somewhat elevated by the use of ketamine (Olivier et al. 2002). The doses used were 3.6 mg/kg ketamine and 0.044 mg/kg Dormitor, both given intramuscularly. Small additional doses (1–2 mg/kg) of ketamine were given every 15–30 min so as to provide a very low level of ongoing muscle activity.

rICMS was delivered through a glass-insulated platinum-iridium microelectrode with a low tip impedance (0.3–0.5 MΩ at 1 kHz). Trains of 20 rICMS 0.2-ms biphasic current pulses at 300 Hz were delivered at a rate of 0.5 Hz from a Neurolog NL800 stimulus isolator (Digitimer) with a search intensity of ≤40 μA. Biphasic pulses were used to minimize electrode polarization. rICMS was tested every 250 μm along each penetration to a depth of ≤7.5 mm from the point of electrode entry. Mapping continued until a number of contiguous tracks within each area were made from which digit movements could be evoked with low-threshold rICMS (<10 μA for M1, <28 μA for F5; see Fig. 1).

**Chronic implant of cortical microwires**

When rICMS mapping was complete, small arrays of fine low-impedance (~20 kΩ) eligiloy microelectrodes were permanently implanted intracortically at the center of these wrist and digit representations under full anesthesia. Four to five electrodes were implanted in M1 and in F5. Microwire electrodes were mounted in a single planar array of four to five electrodes, with an inter-electrode distance of 1–1.3 mm. Their tips were targeted at the inferior bank of the arcuate sulcus (F5) and rostral bank of the central sulcus (M1) and were fixed 3–5 mm from the pial surface. Electrodes were cemented to small bone screws and connected to a miniature D-connector mounted on the skull. The impedance of the microwires remained constant throughout the experimental period, and there were no continuity leaks between connector and electrode tip.

**PT electrodes**

In one monkey (CS14), two fine varnish-insulated tungsten electrodes were implanted in the medullary pyramid at stereotaxic coordinates A3 and P2 (Oliver et al. 2001). Their location was confirmed during surgery by recording the antidromic field potential from the surface of the motor cortex (threshold: 60 μA).

**Experimental protocol: recording and stimulation**

The same sedative regime described in the preceding text was used to investigate the effects evoked from the implanted microwire electrodes. Experimental sessions were carried out twice per week. Initially, rICMS was delivered through each of the microwires in turn, using the same stimulation protocol described in the preceding text, and we documented the threshold and nature of the movement evoked by rICMS through the microwire electrodes: in both monkeys, the lowest threshold movements were observed in the thumb. Accordingly, in subsequent sessions, EMG activity was recorded from intrinsic thumb (thenar) muscles using either intramuscular wire or surface electrodes and a Neurolog NL820/824 isolated amplifier system. Unrectified EMG and stimulus trigger signals were acquired using a CED 1401plus interface (CED, Cambridge, UK), with a sampling rate of ≥8.8 kHz.

Interactions between stimuli delivered to F5 and M1 were investigated while there was evidence of low-level background EMG activity in the sampled muscles. Monophasic rather than biphasic current pulses were applied to pairs of microwires: this allowed us to define the motor effects due to cathodal versus anodal stimulation through a given electrode. Condition (C), test (T), and combined (C-T) stimuli were interleaved and delivered as follows. Conditioning stimuli were single or double shocks to F5 (duration: 0.2 ms, 70–200 μA); test stimuli were single shocks to M1 (duration: 0.2 ms, 70–200 μA).

**Condition-test intervals** between 0 and 30 ms (F5 before M1) were tested, and the duty cycle was 0.5–1 Hz.

**Histology**

At the end of this study, both monkeys underwent a terminal experiment under full anesthesia (Shimazu et al. 2002), at the end of which they were given an overdose of pentobarbitone sodium (100 mg/kg) and perfused through the heart. Entry points of microwire arrays were confirmed by photography of the fixed cortical tissue (Fig. 1, B and C). The tips of the microwire electrodes were localized by passing DC current (20 μA for 20 s, tip positive) and the sites of cortical (Suzuki and Azuma 1976) and PT stimulating electrodes were confirmed histologically. Frozen parasagittal sections of the cortex (50 μm) were cut, mounted, and Nissl stained. Each section was carefully inspected for electrode tracks and sample sections photographed.

**Analysis**

EMG data for analysis were taken from sessions in which long periods of relatively stable EMG activity were present. EMG was rectified and averaged in relation to condition (C, T, or C-T), with 50 shocks per condition. Facilitation or suppression of EMG was identified by subtraction of the responses to condition and test stimuli, given alone in F5. The conditioned response, peak amplitudes and response areas and latencies were measured from averaged data. For each C-T interval tested, the peak amplitudes of each of the 50 responses per condition were calculated; the 10% largest and smallest responses were then excluded. The average (F5+M1)/M1 ratio (± SE) was calculated for conditioned responses obtained at each C-T interval. A one-way ANOVA was then carried out to confirm a significant relationship between C-T interval and the level of facilitation. Subsequently a Wilcoxon signed-ranks test was carried out for pairs of test (M1 alone) versus conditioned (F5+M1) responses.

**Results**

**Location of stimulating sites in M1 and F5**

Detailed rICMS mapping was carried out in both monkeys, CS13 and CS14, and results are shown in Fig. 1, B and C, respectively. In monkey CS13, there were 5 tracks in M1 and 13 in F5, and for CS14, 8 tracks in M1 and 5 in F5. Thresholds were considerably lower in M1 (8–10 μA) than in F5 (22–28 μA), and responses from M1 were more robust. In both monkeys, we found at least two to four tracks in each cortical area that yielded digit movements. The surface loci of these penetrations were used to target the intracortical implants, whose final positions are also shown in Fig. 1, B and C.
EFFECTS OF rICMS THROUGH MICROWIRES IN M1. We found that rICMS delivered through at least one pair of microwire electrodes implanted in M1 evoked digit movements; the rICMS protocol is given in METHODS. Thresholds from the fixed microwires were considerably higher than found in the mapping studies. In monkey CS13, the lowest-threshold effect (thumb flexion) was from microwires 2 as cathode (negative, –ve) and 1 as anode (positive, +ve); the threshold was 35 μA. The tips of both electrodes were in the deep cortical laminae (V, VI) in the anterior bank of the central sulcus (see legend to Fig. 1 for details). In monkey CS14, the lowest-threshold effects were evoked from electrode pair 4 (–ve) and 3 (+ve) and evoked thumb abduction (threshold: 50 μA); tips of both electrodes were located in lamina V in the anterior bank (see Fig. 1D). Other electrode combinations gave digit movements at higher thresholds.

EFFECTS OF rICMS THROUGH MICROWIRES IN F5. No motor effects were observed from F5 electrode pairs in monkey CS13 with currents of ±80 μA. In monkey CS14, rICMS between electrodes 10 (–ve) and 7 (+ve) yielded thumb abduction/extension with a threshold of 60 μA. The tip of electrode 10 was located in laminae V/VI in the bank of the inferior limb of the arcuate sulcus (Fig. 1D), about 3 mm from the pial surface; electrode 7 was located in the superficial layers (II and III) deep in the bank, 4.5 mm from the surface (Fig. 1D). All F5 electrodes in both monkeys were located in the inferior bank of the arcuate sulcus.

From the histological analysis, it is unlikely that the cathodal effects in either cortical area resulted from activation of the underlying white matter. This was confirmed by terminal experiments in both monkeys, which single stimuli delivered to the implanted electrodes evoked relatively large indirect or I waves in the corticospinal tract, but only small direct or D waves, which would not be the case if there were significant current spread to the white matter (H. Shimazu, M. A. Maier, P. A. Kirkwood, G. Cerri, and R. N. Lemon, unpublished data).

Powerful conditioning of EMG responses from M1 by F5 stimulation

EMG recordings were taken from the thenar muscles in both monkeys because thumb movements had the lowest threshold responses for rICMS delivered through the intracortical microwires (see preceding text). A total of 21 recording sessions were carried out; typical results are shown in Fig. 2. Single 70-μA shocks applied to M1 evoked robust short-latency responses (Fig. 2B). In contrast, neither single nor double shocks delivered to F5 evoked any consistent EMG responses (see Fig. 2A). However, while F5 stimulation alone was ineffective in eliciting EMG responses, it had a striking facilitatory action when paired with M1 stimulation (Fig. 2C). The conditioned response was more than double the size of the test response. In this example, the separation between the second F5 and the M1 shock was 3 ms. The powerful conditioning effect is revealed by subtracting the test from the conditioned response (Fig. 2D). In all of the conditioning experiments, we selected an M1 test shock intensity that was clearly submaximal for the EMG response.
keys, application of single monophasic stimuli to M1 electrode pairs evoked clear EMG responses in thenar muscles; the thresholds were 50 \( \mu \text{A} \) in CS13 (electrode 2–ve, 1+ve) and 120 \( \mu \text{A} \) in CS14 (electrode 4–ve and 3+ve). Providing that there was some ongoing background EMG present, these responses were seen with high probability but with variable latency and amplitude. This can be seen in the superimposed unrectified EMG recordings (Fig. 2B) from monkey CS13. The mean latency in this case ranged from 7.3 to 9.5 ms (grand average: 8.6 \( \pm \) 0.5 ms, \( n = 5 \) averages from 5 sessions).

In the other monkey (CS14), which was larger (body weight: 4.4 versus 3.1 kg for CS13), the mean latency of EMG responses to M1 stimulation in different sessions ranged from 9.7 to 14.0 ms (grand average: 12.5 \( \pm \) 1.1 ms, \( n = 32 \) separate averages from 4 sessions). Examples of averaged responses to single M1 stimuli in CS14 are shown in Fig. 3A; note again the variation in onset latency in different sessions. In this monkey, we were able to compare the thenar EMG responses evoked from M1 with those obtained by direct stimulation of the medullary pyramidal tract (PT). Single PT shocks (250 \( \mu \text{A} \)) evoked short-latency EMG responses with little variation in latency (cf. Olivier et al. 2001); the mean onset latency was 8.3 ms (4 sessions); this latency is indicated by the vertical dashed line in Fig. 3A.

Because the M1-evoked responses had substantially longer latencies than those evoked by direct corticospinal volley excited from the PT, it is unlikely that the responses from M1 were due to a direct or D wave in the corticospinal tract. A D wave from M1 would be expected to discharge the target motoneurons at about the same latency as from the PT with a small correction needed for the additional conduction time of \( \sim 1.0 \) ms from cortex to pyramid (Maier et al. 1997; Olivier et al. 2001). This delay is included in Fig. 3A by placing the PT stimulus delivery 1.0 ms later than the M1 stimulus (long arrow and arrowhead, respectively). Thus the predicted latency for a D wave mediated response from M1 would be \( \sim 9.3 \) ms (8.3 + 1.0, dashed vertical arrow in Fig. 3A), substantially shorter than the observed mean value, 12.5 ms. Thus we can conclude that the M1 evoked responses probably resulted from later descending I waves (Lemon et al. 1987; Maier et al. 2002; Porter and Lemon 1993; Shimazu et al. 2002). The typical interval between D and successive I waves is 1.0–1.6 ms (Edgley et al. 1997; Lemon 2002). Thus the corrected latency difference between the M1 and PT evoked responses (\( \sim 3.2 \) ms) suggests that thenar motoneurons were discharging after the arrival of the I\(_2\) wave.

**Paucity of thenar EMG responses to single- or double-shock stimulation of F5**

Single F5 shocks of \( \leq 100 \mu \text{A} \) (CS13) and 200 \( \mu \text{A} \) (CS14) never produced any clear EMG effects (Fig. 3B, gray line). This was also true for double shocks separated by 3 ms (Figs. 2A and 3D), unless high intensities were used. For example, in Fig. 3, double F5 shocks at 150 \( \mu \text{A} \) produced no response (Fig. 3D), whereas at 180 and 200 \( \mu \text{A} \), small responses were observed (Fig. 3, E and F). Responses from F5 were significantly later than from M1 (paired \( t \)-test \( P = 0.007 \), \( n = 12 \) pairs of averages from 2 sessions in CS14); measured from the second F5 shock, the latency of responses to F5 was on average 1.5 ms \( \pm 1.0 \) ms longer than those from M1.

**FIG. 3.** Timing of M1 responses and characteristics of their facilitation by F5 stimulation. A: EMG response to test M1 stimulation. Monkey CS14, light sedation. 5 different averages (each 50 sweeps) of responses in thenar muscle to single M1 shocks (at arrowhead, 180 \( \mu \text{A} \)) have been superimposed. The shortest response latency was \( \sim 12.5 \) ms. The vertical dashed line (PT response) indicates onset latency of response in the same muscle to single shocks delivered directly to the pyramidal tract (PT, 250 \( \mu \text{A} \)). The PT stimulus onset has been placed 1.0 ms after the M1 shock to allow for cortex to pyramid conduction time. B and C: Monkey CS14, light sedation. B: a single F5 conditioning shock (intensity: 80 \( \mu \text{A} \)) produced no thenar muscle EMG response when given alone. A single M1 shock (intensity: 180 \( \mu \text{A} \)) produced a clear response which was facilitated by the conditioning shock (F5+M1), with a reduction in onset latency. C: a double F5 shock of the same intensity, given alone, produced a small EMG response, and produced more powerful facilitation of the M1 response, with further shortening in latency. 50 sweeps for each condition. D–F: raising the intensity of the F5 stimulus (double shocks, 3-ms separation) from 150 to 180 \( \mu \text{A} \) and then to 200 \( \mu \text{A} \) produced small EMG responses when given alone (see E and F). These stimuli also produced greater facilitation of the M1 test response, with successive reductions in conditioned EMG response latency (F5 + M1). 50 sweeps for each condition.
Characteristics of facilitation from F5

SINGLE VERSUS DOUBLE SHOCKS. Figure 3, B and C, illustrates the facilitation produced by single and double F5 shocks, respectively; the condition-test interval was 6 ms. In both cases, the conditioned response was larger and earlier, but the effects were more pronounced with the double F5 shocks (intershock interval of 3 ms).

INTENSITY OF STIMULATION. Stronger F5 stimulation also greatly increased the facilitation of responses evoked from M1. In Fig. 3, D–F, the intensity of paired F5 shocks delivered 4.5 ms before the M1 shock was varied; the M1 shock intensity was kept constant at 175 μA. Figure 3D shows that the conditioned response (F5 + M1) was clearly facilitated by F5 stimulation (intensity 150 μA). Stronger F5 stimulation at 180 μA (Fig. 3E) and 200 μA (Fig. 3F) produced dramatic increases in the amplitude of the conditioned response. Delivered alone, these F5 stimuli evoked only small EMG responses.

LATENCY OF RESPONSES. It is noticeable that compared with the test responses, the onset latency of the conditioned responses was clearly shortened, and this occurred in an intensity-related manner. Thus the latency shortening (condition-test) was ~0.5 ms at 150 μA (Fig. 3D), 1.5 ms at 180 μA (Fig. 3E), and 3.0 ms at 200 μA (Fig. 3F). These average values conceal considerable sweep-by-sweep variation in latency, as noted in the preceding text. Detailed examination of the latency of individual sweeps revealed a tendency for latencies to cluster at particular values, separated by ~1.0 ms. For example, in Fig. 2C, the superimposed sweeps show that the larger responses occurred at preferred latencies just over 1 ms apart. Figure 4 shows the results of analysis for 104 conditioned responses recorded in one session; in this case there are hints of clustering at latencies of 10–12 ms. It is possible that this periodicity reflects the discharge of thenar motoneurons in association with successive I waves generated from M1 whose amplitude is modulated by conditioning shocks to F5 (see DISCUSSION).

LOCATION OF EFFECTIVE ELECTRODES. Figure 5 demonstrates the specificity of the effects from F5. The most pronounced facilitation (Fig. 5A) was observed from one pair of F5 electrodes: electrode 10 as cathode (−) and 9 as anode (+; see inset in Fig. 5). A second pair (electrodes 8−6+) was also effective, as shown in Fig. 5C, but no facilitation was obtained from three other combinations using electrodes 9, 7, and 6 as...
cathode (Figs. 5, B, D, and E, respectively). Both the effective cathodes (electrodes 8, 10) lay in the deep laminae (V or VI); all other electrodes were in the superficial layers (II or III; see Fig. 1D).

**Time course of F5-evoked facilitation**

Figure 6 shows examples from CS13 and CS14 of facilitation of M1 responses by conditioning F5 shocks delivered at different condition-test (C-T) intervals. In the left hand series (Fig. 6, A–D), a single F5 shock was used; a double shock (separation 3 ms) was used in the series on the right (Fig. 6, E–H). Clear facilitation was observed with short C-T intervals (1.6 ms in Fig. 6A, 3.0 ms in Fig. 6E) and at longer intervals ranging from 6 to 10 ms.

Figure 7 shows the full time course of facilitation produced by double (A) or single (B) shocks in monkey CS14. In each series, at each C-T interval, we delivered 50 shocks per condition (F5 alone, M1 alone, and F5 + M1). The duty cycle was 500 ms between each condition, and different C-T intervals were tested in a block design. F5 stimulation alone produced no EMG responses. For each sweep, we measured the peak voltage of the conditioned response (F5 + M1) at the latency predicted by the average response (i.e., at 10–15 ms, see Fig. 6, A–H). This value was then normalized by dividing it by the peak voltage of the response evoked by the test shock (M1 alone) that had just preceded it (i.e., delivered 500 ms earlier). The • in Fig. 7 are the means ± SE of 40 such ratio measurements: the five largest and five smallest responses were excluded. This procedure minimized the effect of slow changes in the level of excitability during the course of experiment, largely caused by the need to administer small additional doses of ketamine from time to time. These changes are indicated by •, which plot the mean amplitude (±SE) of the responses to the test (M1 alone) shocks. Overall there was no significant correlation between the amplitudes of the test and conditioned responses, suggesting that these slow changes did not contribute to the ratio measurements.

**Double shocks.** For the data shown in Fig. 7A, the intensity of the test stimulus was a single M1 shock (180 μA), and it was conditioned by two shocks to F5 (each 80 μA). Separation between the F5 shocks was 3 ms, and C-T interval was measured from the second shock. A one-way ANOVA confirmed that there was a significant relationship between C-T interval and the amount of facilitation (df = 7, F = 8.6, P < 0.001). A Wilcoxon signed-ranks test revealed that the first significant effect was at 3 ms (P < 0.001, ratio = 4.5). Although a large effect was also present at C-T of 1.0 ms (ratio: 3.1), this did not reach significance because of the large variance in response amplitude. Overall conditioning effects were large, with the ratio of conditioned response to test response (Fig. 7A, •) rising to >12 at C-T of 10 ms. No remaining conditioning effect was observed at C-T of 30 ms. Similar results were obtained in the other monkey (CS13), again with a consistent early facilitatory effect at C-T of 3.0 ms.

**Single shock.** In another session (Fig. 7B), we tested the effects of a single F5 shock (also 80 μA). The overall effect was weaker than with double shocks (compare vertical axes in Fig. 7, A and B), but again the ANOVA showed that a C-T interval-dependent facilitation was present (df = 7, F = 2.7, P < 0.01). No significant effect was present for simultaneous delivery of F5 and M1 (C-T = 0). The first significant point was at 1.0 ms (P < 0.05). From 6 ≤ 15 ms responses were all significantly larger and returned to baseline at 30 ms.

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**FIG. 6.** Examples of the time course of the facilitation from F5. Examples of responses to condition (F5), test (M1) and condition + test (F5 + M1) stimuli obtained from CS14 (A–D) and CS13 (E–H), using different condition-test (C-T) intervals. In CS14, facilitation was observed with C-T intervals as short as 1.6 ms (A), and clear facilitation was also present with intervals of 3.6, 6.0, and 10.0 ms (B–D). In CS13, where double F5 shocks were used, there were clear effects at 3.0 ms after the second shock (E), with somewhat weaker effects at longer intervals (F–H). Percentage values give the peak of the conditioned/test response [(F5 + M1)/M1 × 100%]. In A–D, M1 test intensity was 1 × 180 μA and F5 condition intensity 1 × 80 μA. In E and F, M1 test intensity was 1 × 70 μA and F5 condition intensity was 2 × 70 μA; 50 sweeps for each condition.

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**FIG. 7.** Time course of the facilitation from F5 using single and double shocks. Graphs show the time course of facilitation from F5 using either a double (A, 3-ms interstimulus interval) or single (B) 80-μA shock. The test M1 shock was 1 × 180 μA in both cases. The mean ± SE of the peak amplitude of the response to the test (M1 alone) shock is plotted (○; right-hand scale); each point is the mean of 40 observations collected at different times in the recording session, and these data illustrate the variation in the test response probably due to administration of small additional doses of ketamine throughout the session. Condition alone (F5), test alone (M1) and condition + test shocks (F5+M1) were delivered in sequence, and for each successive sequence the peak amplitude of the conditioned response (F5 + M1) was normalized by the amplitude of the test M1 response (■, left-hand scale). The mean ratio ± SE is plotted for each C-T interval tested. The baseline ratio was set at 1.0 (●). For statistical comparison, a Wilcoxon signed-ranks test was carried out on 40 pairs of conditioned vs. test responses at each C-T interval; significant differences are indicated *P < 0.05, **P < 0.001. For technical reasons, the data in B for C-T = 0 ms were collected during a separate recording session and scaled appropriately. Note the early onset of facilitation after conditioning with either single (B) or double (A) F5 shocks, and the much larger effects of the latter (note the difference in the left-hand scales).

**DISCUSSION**

We show here that single shock stimulation of macaque ventral premotor cortex, or area F5, while itself evoking any significant EMG responses in the wrist and digit muscles, could produce a large and long-lasting facilitation of responses evoked from M1. The results presented here could be of considerable importance in that they demonstrate an excitatory interaction between two key parts of the motor network that mediate visuomotor cortical control of skilled hand movements.

**Nature of the responses to M1 stimulation.**

Before considering the facilitatory effects of F5, we need to understand the nature of the test responses evoked from M1. The tips of effective electrodes were in the deep cortical layers in the anterior bank of the central sulcus, located within the wrist/digit area of M1 (as defined by previous mapping with rICMS). We used single bipolar stimuli with intensities of 70–200 μA to activate a significant proportion of the cortical output to the thenar muscles. The physical spread of the currents used should be <1 mm and confined to the stimulated area (Lemon 1984; Ranck 1975; Tokuno and Nambu 2000). Single-pulse stimulation gives least temporal and spatial facilitation and provides the best means of documenting relatively direct connectivity, similar to that demonstrated by single neuron cross-correlation techniques (see Cheney and Fetz 1985; Lemon et al. 1987; Park et al. 2001).

Bipolar stimulation of M1 gives rise to a complex succession of small D and I waves in the corticospinal tract (Maier et al. 2002). The rather focal nature of the stimulation is supported by the fact that the amplitude of these waves amount to only a few percent of the fast volley evoked by supramaximal stimulation of the tract at the level of the pyramidal tract (PT) (Maier et al. 2002; Shimazu et al. 2002). It is likely that the EMG responses we observed resulted from the discharge of motoneurons after temporal summation and facilitation of corticospinal inputs associated with successive D and I waves (Boniface et al. 1991; Day et al. 1989; Maier et al. 2002). In contrast to the EMG responses evoked by direct stimulation of the PT, those evoked from M1 showed rather late and variable onset latencies (Figs. 2–4), consistent with motoneurons reaching threshold after arrival of later I waves (I3 or I4).

**Location of electrodes giving rise to facilitation from F5**

The sites within F5 that were effective in facilitating outputs from M1 all lay in the deep layers of the inferior bank of the arcuate sulcus, several millimeters lateral to the spur (Fig. 1). This is in the distinctive subdivision of area F5 (F5ab of Rizzolattti et al. 1998; PMvr of Garbet et al. 1999) from which wrist and finger movements can be obtained by low-threshold rICMS (Godschalk et al. 1995) and which receives visual-related inputs from the parietal area AIP (Rizzolatti et al. 1998; Luppino et al. 1999; Tanne et al. 2002). In this subdivision, recordings have been made from so-called “canonical neurons,” which have both “visual” and “motor” properties: they respond to the visual presentation of particular objects as well as discharging when the monkey grasps the object (Rizzolatti and Luppino 2001). Thus the facilitatory effects we have observed could be of importance for F5-M1 interactions during visuomotor transformations related to hand function. The results hint that such interactions are organized in a rather focused fashion: only certain combinations of electrodes within the F5 microwire array were effective (Fig. 5), and this may reflect the known somatotopical relationships between F5 and M1 (Gentilucci et al. 1988; Kurata and Tanji 1986; Rizzolatti et al. 1988).

**Characteristic features of F5 facilitation**

The facilitatory effects observed were of large amplitude with the test response being increased several fold by F5...
stimulation; double shocks produced stronger effects than single. It is noteworthy that single F5 shocks never produced any EMG responses when tested alone (Figs. 2, 3, and 6). This is consistent with the general lack of short-latency excitatory postsynaptic potentials in hand motoneurons after F5 stimulation (Shimazu et al. 2002). Although it has been shown that there is a concentration of corticospinal neurons in the same ventral premotor region that we have stimulated (Dum and Strick 1991) and our most effective F5 electrodes had tips located in the deeper cortical layers (V/VI), corticospinal projections from this subdivision of F5 do not reach the lower cervical cord in the macaque (Galea and Darian-Smith 1994; He et al. 1993). The absence of such projections makes it unlikely that the F5-M1 interaction we have observed occurs at the level of the hand motoneurons themselves, but this conclusion requires confirmation in the form of direct recordings from motoneurons.

Indirect transmission via propriospinal neurons located within the upper cervical segments (to which F5 corticospinal neurons do project, He et al. 1994) remains a possibility, although a number of our earlier studies have suggested that such transmission is rather weak in the macaque monkey (Maier et al. 1998; Nakajima et al. 2000; Olivier et al. 2001) and does not target intrinsic hand motoneurons (Pierrot-Deseilligny 1996).

Another possible site of interaction is within M1 itself. There are numerous cortico-cortical projections from F5 to M1 (Ghosh et al. 1987; Godschalk et al. 1984; Jeannerod et al. 1995; Lu et al. 1994; Matelli et al. 1986; Muakassa and Strick 1979). Pyramidal tract neurons in M1 can be excited from ventral premotor cortex with latencies as short as 1–3 ms (Ghosh and Porter 1988; Tokuno and Nambu 2000). Such short-latency excitatory effects are consistent with the latency of the first significant evidence for facilitation from F5 (1–3 ms; see Figs. 6 and 7). Again, more direct evidence will be needed to identify whether the interaction we have observed occurs at cortical or subcortical sites or at both.

**Excitation and inhibition**

It is striking that all of the effects we observed were facilitatory: there was no sign of inhibition in any of the interaction experiments, at any C-T interval (Fig. 7). Yet Tokuno and Nambu (2000) found that most M1 PTNs were inhibited by ventral premotor cortex stimulation: of 33 PTNs, only 11 showed signs of early excitation, but in all of these, there followed a long period of inhibition, lasting ~100 ms; the remaining PTNs showed only inhibitory responses. While these findings argue against M1 as the site of interaction, it must be pointed out that the spinal targets of these PTNs were not identified and so may have been different to those giving rise to the EMG responses that we have studied. Again, further work is needed.

**Facilitation and I waves**

We observed several “jumps” in the latency of the conditioned response when the intensity of the conditioning F5 shock was increased (Fig. 3, D–F) with a tendency for the conditioned responses to cluster at particular latencies (Figs. 2C and 4). These effects had a periodicity of 1.0–1.5 ms. As discussed in the preceding text, this may reflect the discharge of thalamic motoneurons in response to successive I waves generated in M1 at intervals of 1–1.6 ms (Edgley et al. 1990, 1997; Kernen and Wu 1967; Maier et al. 2002; Patton and Amassian 1954; Ziemann and Rothwell 2000). Tokuno and Nambu (2000) also found some evidence for periodicity in the responses of M1 PTNs and other neurons in response to stimulation of nonprimary motor areas.

Thus we speculate that our results could be explained by conditioning shocks to F5 modulating the amplitude of different corticospinal I waves. Waxing and waning of motor evoked potentials in response to paired-pulse TMS of human motor cortex has also been attributed to I-wave periodicity (Ziemann et al. 1998). I waves probably derive from synaptic activation of corticospinal neurons through chains of cortical interneurons (Amassian et al. 1987; Ziemann and Rothwell 2000), and these interneurons could be sites for convergence of synaptic inputs originating both within M1 and beyond M1, that is, from other motor cortical areas with strong projections to it, such as F5 (Ghosh et al. 1987; Godschalk et al. 1984; Tokuno and Nambu 2000). We raise the possibility that such pathways are also activated by rICMS and speculate that this is one of the mechanisms mediating motor responses evoked from F5.

**Conclusions**

We demonstrate a robust facilitatory action of F5 stimulation on motor outputs to the hand generated from M1. Such a pathway may be of importance for F5-M1 interactions during visuomotor transformations related to hand function. The sites of this interaction and the underlying mechanisms involved will require further investigation (Shimazu et al. 2002).

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