Modulation of primary motor cortex outputs from ventral premotor cortex during visually guided grasp in the macaque monkey

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Area F5, in the ventral premotor cortex of the macaque monkey, plays a critical role in determining the hand shape appropriate for grasp of a visible object. F5 neurones show increased firing for particular types of grasp, and inactivation of F5 produces deficits in visually guided grasp. But how is F5 activity transformed into the appropriate pattern of hand muscle activity for efficient grasp? Here we investigate the pathways that may be involved by testing the effect of single stimuli delivered through microwires chronically implanted in area F5 and in primary motor cortex (M1) of two macaque monkeys. The EMG responses from M1 test (T) stimulation were recorded from 4–11 contralateral hand, digit and arm muscles during reach-to-grasp of visually presented objects. Conditioning (C) stimulation of F5, at intensities subthreshold for motor effects, caused strong modulation (over twofold) of M1 test (T) responses. The pattern of facilitation was specific. First, facilitation of the T response was particularly evident at short C–T intervals of −1 to 1 ms. Second, this facilitation was only present in some muscles and during reach-to-grasp of a subset of objects; it did not appear to be simply related to the level of EMG activity in the muscles at the moment of cortical stimulation or indeed to the upcoming contribution of that muscle during grasp. At later C–T intervals (1–6 ms), F5 stimulation caused significant suppression of the test M1 response. The results are in keeping with the concept that during visually guided grasp, F5 modulates corticospinal outputs from M1 in a muscle- and grasp-specific manner.

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Research over the past decade has defined the basic circuits that are involved in the everyday performance of grasping visible objects (Jeannerod et al. 1995; Fagg & Arbib, 1998; Murata et al. 2000; Castiello, 2005; Umilta et al. 2007). Significant advances have been made possible by using non-invasive methods in humans backed up by anatomical and neurophysiological studies in macaque monkeys. The current view is that the anterior intraparietal region (AIP) is thought to encode a sensorimotor representation of an object’s visual properties; AIP sends major projections to area F5 of the ventral premotor cortex (Luppino et al. 1999; Borra et al. 2008). The discharge of F5 neurones during visual presentation of objects and their subsequent grasp (Murata et al. 1997; Raos et al. 2006) is compatible with encoding of general motor goals such as ‘hold’ or ‘grasp’ and also with the concept of a ‘motor prototype’, a description of how an action is to be made, for instance using whole hand prehension or precision grip (Rizzolatti & Luppino, 2001). Corticospinal projections from F5 and extensive reciprocal cortico-cortical projections to the primary motor area (M1) both provide possible routes through which F5 could influence hand shape (Muakkassa & Strick, 1979; Godschalk et al. 1984; He et al. 1993), but it is not yet established how F5 representations of grasping actions result in the specific patterns of muscular activation required for skilled grasp of different objects (Brochier et al. 2004).

The effect of F5 interactions with M1 have been investigated in sedated and anaesthetised macaque monkeys (Cerri et al. 2003; Shimazu et al. 2004). Single...
pulse stimuli delivered to M1 evoked direct (D) and indirect (I₁, I₂ and I₃) corticospinal volleys, which generated excitatory responses in hand motoneurons and muscles. In contrast, single stimuli delivered to F5 rarely produced detectable corticospinal outputs or overt motor responses. However, when these same F5 stimuli conditioned M1 stimuli there was a marked enhancement of the responses to M1 stimulation, and this appeared to result from a strong facilitation of the later corticospinal I₂ and I₃ waves evoked from M1. Facilitatory F5–M1 interactions occurred at short condition–test (C–T) intervals (0–1 ms) indicating a local interaction.

In the present study we used an identical approach to investigate F5–M1 interactions in awake, behaving monkeys performing a visuomotor grasping task. Stimuli were delivered through chronically implanted microwires located in the hand representations of area F5 and M1 in two monkeys. Stimuli were timed to occur as the monkey reached out to grasp a range of different objects. The first experiment demonstrated that the C–T facilitation observed in the sedated and anaesthetised monkey also occurs in the awake animal performing a task. At longer C–T intervals, we also found suppression of the M1 evoked response by F5 stimulation, which was not seen in the sedated monkey.

We postulated that if the pathways involved in the modulation of test responses from M1 were the same as those transmitting information required for grasp, the degree of F5 conditioning should vary with the type of grip used. In a second experiment, one of the monkeys was trained to grasp a set of four objects, two of which, a ring and a cube, could be grasped with either a hook or a side grip, allowing object and grasp to be dissociated. F5 conditioning of the M1 test response occurred in a specific manner, with facilitation of particular muscles for a subset of object-grasps.

A preliminary report of some of these findings has been published (Prabhu et al. 2005).

**Methods**

**Ethical approval**

Two adult purpose-bred monkeys (Macaca fascicularis, CS15, male, weight 8.5 kg; M. mulatta, M39, female, weight 5.0 kg) were used in this study. All procedures were approved by the UCL Institute of Neurology Ethical Review Process in accordance with the UK Animals (Scientific Procedures) Act 1986. All procedures were carried out at the UCL Institute of Neurology.

**Behavioural task**

The trial sequence for the visuomotor task used is summarised in Fig. 1A (Brochier et al. 2004; Umulta et al. 2007). The monkey sat in a dimly lit room. At the start of each trial the monkey used both hands to gently press two waist-level ‘homepads’. After 200 ms an object mounted on a shuttle device in front of the monkey was illuminated, and a light from a red LED was reflected onto the object through a half-mirror. After a variable period (1 ± 0.8 s), the monkey was cued, by either the LED switching to green (M39) or by a tone (CS15), to release the homepad underneath the trained hand and then to reach, grasp and pull the object into a target displacement zone. The delay between homepad release and object displacement was typically 300–400 ms. The shuttle was held by a weak spring and object displacement was monitored by Hall-effect sensors; correct positioning was indicated by a tone. The object had to be held for 1 s in the displacement zone (4 to 14 mm from the rest position, requiring a force of between 0.9 and 2.4 N, respectively). There was an inter-trial interval of 1–2.5 s, without object illumination. Monkeys performed the task with the right (CS15) or left (M39) hand for food rewards. Both monkeys were trained to use specific hand postures when grasping: CS15 grasped a cone, while M39 grasped a disc, plate, cube or ring (Fig. 1B). The cube and the ring could be grasped either by a side grip (between the thumb and the side of the index finger) or with a hook grip (in which the index finger was inserted into a groove at the back of the cube, or into the ring). Thus M39 performed six different object-grasps. M39 was head-restrained throughout the sessions; CS15 was head free, restrained only by a loose-fitting neck collar. The monkeys performed 150–600 trials per session.

**Mapping procedures and implantation of cortical microwires**

In summary the whole procedure involved the following steps. First, an MRI scan was used to guide the implantation of a recording chamber that gave access to the hand areas of both M1 and the ventral premotor cortex (area F5). Second, detailed mapping of these hand areas was carried out using repetitive intracortical microstimulation (rICMS) and single unit recording. Next, chronic arrays of stimulating microwires were implanted in F5 and M1. Finally, EMG electrodes were implanted in selected digit, hand and arm muscles. All surgeries for implantation of the cortical chamber, cortical arrays and EMG electrodes were performed under deep general anaesthesia, induced with 10 mg kg⁻¹ ketamine I.M. and, after intubation, were maintained with 2–2.5% isoflurane in 50 : 50 O₂ : N₂O. Full aseptic procedures were observed, and antibiotics and analgesics given postoperatively.

The mapping of the hand representations of F5 and M1 in M39 (right hemisphere) was done in the awake state, during performance of the task described above, using a multiple electrode approach for simultaneous
recording of grasp-related neurons in both cortical areas (see Umilta et al. 2007). In CS15 a single tungsten microelectrode driven by a hydraulic microdrive and located in the chamber with an X–Y positioner was used (see Cerri et al. 2003) and mapping of the left hemisphere was carried out under light sedation with ketamine and medetomidine HCl (Domitor; Ramsgate, Kent, UK). The doses were 3.6 mg kg\(^{-1}\) ketamine and 0.044 mg kg\(^{-1}\) Domitor, both given i.m.).

The rICMS used for mapping consisted of twenty 0.2 ms biphasic constant current pulses at 333 Hz, delivered at 0.5 Hz through a metal microelectrode with impedances of ca 1 M\(\Omega\). Sites in M1 and F5 which produced digit movements with rICMS currents of 5–40 \(\mu\)A were mapped. When mapping was complete, arrays of four to five low impedance (\(\sim 20 \text{ k}\Omega\)) elgiloy microwire electrodes (2–5 mm long, horizontal interelectrode distance 1–1.3 mm) were implanted in M1 and F5 (Cerri et al. 2003; Shimazu et al. 2004). A slit was cut in the dura, centred on the low-threshold rICMS points, and the array inserted into the underlying cortex. The precise orientation of the implant was again guided by structural MRI (Baker et al. 1999). The F5 array was implanted in the inferior bank of the arcuate sulcus, just lateral to the spur, and the M1 array in the rostral bank of the central sulcus (Fig. 2A, C). Each array was connected to a separate miniature D-connector mounted on the monkey’s cranium.

### Implantation of EMG electrodes

Subcutaneous EMG electrodes were chronically implanted over a number of muscles and connected to a miniature D-connector on the monkey’s back (Miller et al. 1993; Brochier et al. 2004). In M39 EMGs were recorded from the following 11 muscles: first dorsal interosseus (1DI), thenar (Th), abductor digiti minimi (AbDM), abductor pollicis longus (AbPL), flexor digitorum superficialis (FDS) and profundus (FDP), palmaris longus (PL), extensor digitorum communis (EDC), extensor digitorum digits 4 and 5 (ED4,5), brachioradialis (BrR) and the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The behavioural task (A), hand postures used to grasp the objects (B) and the timing of cortical stimulation (C)

A, to start the trial the monkey pressed both hands on the homepads. After 200 ms an object was illuminated and light from a red LED was reflected onto the object, which the monkey fixated. The monkey was cued to grasp after a variable time from the start of object illumination (0.8–1.8 s) by the LED changing from red (R) to green (G). An additional auditory cue was given to CS15. Both monkeys were then required to reach out, grasp the object and pull it into a displacement zone for 1 s to receive a food reward. A further auditory cue was given when the hold period was completed. B, both monkeys were trained to use specific hand postures to grasp the objects. M39 was trained to use the left hand to grasp a disc, plate, cube and ring. The cube and the ring could be grasped by either a side grasp or a hook grasp; the latter involved insertion of the index finger only. CS15 used its right hand to grasp a cone. C, averaged, rectified EMG activity recorded from palmaris longus (M39, 67 trials) and thenar muscles (CS15, 215 trials) illustrating the time of cortical stimulation (arrows) during reach to grasp (50 ms after homepad release for M39 and 100 ms for CS15). Averages are referenced to homepad release. Epochs for the analysis of ongoing EMG during cortical stimulation (1–3) and hand shaping (7–9) in M39 are shown by the grey bars.
Table 1. Summary of experimental conditions for studies in the two monkeys (M39 and CS15)

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<th>CS15 (head-free)</th>
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<td>Task</td>
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<td>Stimulation delay after</td>
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<td>Muscles tested</td>
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<td>AbPL, EDC, FDS</td>
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<td>Extrinsic AbPL, EDC, FDP, EDC, PL,</td>
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anterior deltoid (AD). In CS15, thenar, AbPL, EDC and FDS were implanted (see Table 1 for a summary). The location of the EMG electrodes was verified in a terminal experiment by muscle twitches evoked from electrical stimulation (~0.5 V) through the back connector and by post-mortem dissection.

**Experimental protocol: recording and stimulation**

To examine the interaction between F5 and M1 stimulation in the awake monkey, single monophasic stimuli, 0.2 ms in duration, were delivered to pairs of microwires in M1 and in F5 from a Neurolog NL800 stimulus isolator (Digitimer Ltd, Welwyn Garden City, UK). Test (T) stimuli delivered to M1, conditioning (C) stimuli to F5, or combined conditioning–test stimuli (C–T) were given in an interleaved fashion to counteract any slow changes in excitability during the course of the recording session, with one stimulus (T, C or C–T) being tested in a given trial. A number of different C–T intervals were tested with F5 leading M1 by intervals of 1 up to 6 ms. It is known that the major interaction in terms of corticospinal output from M1 is on the late I waves (I2 and I3), which do not arise until some 3–5 ms after the M1 shock (Shimazu et al. 2004). Therefore we also tried F5 conditioning to test the earliest possible interaction, with both shocks together (C–T = 0 ms) and with the F5 shock delivered 1 ms after the M1 shock (C–T interval of −1 ms).

Stimuli were delivered while the monkey grasped a single object (M39: the disc and CS15: the cone; see Fig. 1B). In a separate study in M39, the monkey was tested with all six object-grasps in four experimental sessions over a period of 7 days. In some sessions, three objects were presented in blocks of 150 trials (50 per object) so that the same number of C, T and C–T stimuli were delivered for grasp of each object. In other sessions, to counteract any order effects, objects were presented in pseudorandom order (27–77 trials per object). No difference in the C–T response was observed for sessions with block vs. pseudorandom presentations.

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EMG signals were amplified ×2000, highpass filtered (30 Hz) (Neurolog EMG amplifier, NL824, Digitimer) and sampled at 5 kHz using an A–D interface (PCI-6071E, National Instruments, Austin, TX, USA). Behavioural events (homepad release and object displacement) were recorded along with EMG activity.

**Histology**

At the end of the procedure, both monkeys underwent a terminal experiment under general anaesthesia. Small electrolytic lesions were placed at the cortical stimulation sites by passing DC current (20 μA for 20 s, tip positive). The animal was given an overdose of barbiturate and perfused through the heart with formal saline. The entry points of microwire arrays visible in the fixed cortical tissue were photographed. All stimulation sites were confirmed histologically (Suzuki & Azuma, 1976). Frozen sections (50 μm) were cut in the parasaggital plane, mounted, and Nissl stained. Each section was inspected carefully for electrode tracks, and sample sections were digitized.

**Analysis**

Separate averages of rectified EMG activity were compiled for each condition (C, T, C–T) with all averages referenced to the timing of the conditioning (C) stimulus. Averages comprised data from 25–121 trials per condition. For each muscle, the peak amplitude of the EMG response recorded in each trial was then measured at the latency...
predicted from the average evoked response. To control for the trial-by-trial variation in response amplitude and background EMG activity during the task, the peak amplitude of the response to each stimulus was normalised by dividing it by the mean amplitude of the background EMG activity present in the 18 ms pre-stimulus period for that trial. The modulation of the M1 test (T) response by the conditioning (C) F5 stimulus was measured for each C–T interval, by calculating the ratio of the normalised responses i.e. [conditioned (C–T) response/background]/[test stimulus (T) response/background]. In the second study in M39, the C–T response was normalised to the mean T response for that session and object-grasp.

A Kruskal–Wallis test was performed for each muscle on the ratio of the normalised C–T/T response from data pooled across session, with the factor of C–T interval or object-grasp. If significant for C–T interval or object-grasp, Wilcoxon’s signed-rank tests were then performed separately for each C–T interval or object-grasp, comparing the T and C–T response in each session or data pooled across sessions.

EMG activity from one session (180 trials) with no cortical stimulation was used to assess muscle activity during the time of cortical stimulation and hand shaping for the six object-grasps (M39); a distinct and reproducible pattern of activity was present for grasp of each object (Brochier et al. 2004). EMG activity was rectified and averaged, and the mean EMG level during the visual presentation period was subtracted. The average EMG amplitude at the time window of interest was then normalised by the peak activity for that muscle across

Figure 2. The cortical location of microwire arrays in the two monkeys
A and C, surface diagrams. Entry points of the microwires are indicated by numbers 1–10. The cathode (−ve, black circle) and anode (+ve, grey circle) most commonly used for stimulation in M1 and F5 are indicated. The arcuate sulcus (AS) and spur (Sp), central sulcus (CS) and principal sulcus (PS) are shown. B and D, parasagittal histological sections. Sections are taken at the level indicated by the horizontal arrows in A and C. Sections in the upper row show location of cathodal electrodes (section a for F5 and c for M1 in each case). Sections in the lower row show location of anodes (section b for F5 and d for M1). Dots in sections indicate presence of large lamina V pyramidal cells.
grasps of different objects and all time points (i.e. the start of object illumination to the end of the hold period). The maximum value for any muscle was assigned a value of 1 and the minimum, a value of 0. This allowed the relative level of EMG activity for each object-grasp to be compared across muscles. As the movement time (the time from homepad release to object displacement) was different for each object (330 to 420 ms), a normalised time scale as used by Brochier et al. (2004) assisted comparison of EMG activity across grasps. The time from homepad release to just before object displacement was divided into nine epochs (Fig. 1C, M39). The EMG activity was examined at the time of cortical stimulation (epochs 1–3) and during hand shaping (epochs 7–9), just before contact with the object and its displacement.

**Results**

**Facilitation by F5 stimulation of EMG responses evoked from M1**

Stimuli were delivered shortly after the monkey released the homepad (50 ms after HPR in M39 and 100 ms in CS15), when EMG activity related to reach and grasp was beginning to increase (Fig. 1C). During this period, a single F5 conditioning (C) stimulus significantly
enhanced the evoked response to an M1 test (T) stimulus. An example of this facilitation is shown in Fig. 3A, which shows superimposed EMG responses from the PL muscle in M39 to 30 successive interleaved C, T and C–T stimuli. F5 stimulation alone (200 μA) evoked no consistent responses (Fig. 3A, grey sweeps). Single test (T) shocks given to M1 alone (150 μA; black dashed sweeps) evoked responses with a latency of 8–10 ms but with variable amplitude and low probability (4/10 sweeps). However, when conditioned with an F5 stimulus (200 μA, C–T = 0 ms; continuous black sweeps), larger and more consistent responses were evoked (8/10 sweeps). The average evoked responses in PL from this session are shown in Fig. 3B (lower panel); they confirm that while F5 alone produced no EMG response, when it conditioned the M1 stimulus the amplitude of the response was significantly enhanced (P < 0.05, Wilcoxon’s signed-rank test).

In M39, 17 recording sessions were carried out over four weeks; 62 separate data sets were recorded. In 15 sessions facilitation was observed in at least one muscle (31/62 data sets). All of the 11 muscles tested in this monkey showed responses to M1 stimulation alone and nine showed clear facilitation of the M1 test response from F5 in at least two sessions. These effects were obtained with a variety of different electrode combinations and stimulation intensities.

The muscles that yielded the most frequent effects were PL and AbPL. In PL, the average facilitation (± S.E.M.) of the M1 evoked response by F5 conditioning at C–T = 0 ms was 2.31 ± 0.17 (n = 105 sweeps per condition, recorded in three sessions over an eight day period using the optimal electrode combination, see below). Thus, the M1 response was increased 2.31-fold by F5 conditioning. For AbPL, the average facilitation at C–T = 0 ms was 1.96 ± 0.17 (n = 105 sweeps per condition, same three sessions and electrode combination) (all P < 0.05).

Significant facilitation by F5 conditioning was also observed in the second monkey (CS15). An example is shown in Fig. 3B (upper panel) of evoked responses from the AbPL muscle. A single shock to F5 (110 μA) given alone evoked no response, but when it conditioned the M1 response (C–T = 0 ms) the test response to a single M1 shock (180 μA) was significantly augmented (P < 0.01, Wilcoxon’s signed-rank test). In this monkey, all four muscles showed responses to test M1 stimulation. Facilitation from F5 was observed in at least one muscle in 8/10 sessions (total of 30 data sets). Of the four muscles tested in this monkey, three showed significant facilitation in at least two sessions. The muscles that yielded the most frequent effects were thenar and AbPL. At C–T = 0 ms the average facilitation (± S.E.M.) of the M1 evoked response in thenar EMG was 2.07 ± 0.17 (n = 95 sweeps per condition from two sessions) (P < 0.001), and in AbPL it was 3.25 ± 0.39 (n = 121 sweeps per condition from two sessions over two days) (P < 0.001).

Suppression of M1 test responses from F5

We found that at longer C–T intervals it was possible to observe significant suppression by F5 stimulation of EMG test responses evoked from M1. Figure 3C shows examples from the thenar muscles in CS15 and from AbPL in M39. In both cases, the response evoked by combined F5 and M1 stimulation was significantly smaller than the test M1 response (P < 0.05). Suppression from F5 was less common than facilitation in both monkeys; it was seen in 5/17 sessions (7/11 tested muscles) in M39 and 4/10 sessions (2/4 muscles) in CS15. In M39, AbPL suppression was significant (P < 0.05) at C–T intervals of 1.2 ms (F5–M1/M1 ratio (± S.E.M.): 0.9 ± 0.09), 1.4 ms (0.75 ± 0.08) and 2.4 ms (0.74 ± 0.07). In this monkey, the AbPL muscle could be facilitated from the same F5 and M1 sites using shorter C–T intervals (not illustrated). In CS15 suppression was significant in AbPL and thenar at C–T = 6 ms (0.62 ± 0.08 and 0.75 ± 0.12, respectively; both P < 0.05). In this monkey, AbPL was facilitated at shorter intervals from the same site (see Fig. 3B, upper panel).

The influence of C–T interval on F5–M1 interaction

A total of 17 different C–T intervals were tested in M39 (range from −0.8 to 3.5 ms) and five C–T intervals (from −1 to 6 ms) were tested in CS15. Responses in all sampled muscles were examined for conditioning effects. An initial Kruskal–Wallis test showed a significant effect of C–T interval for both M39 and CS15 (both P < 0.001). The number of times that averaged muscle responses showed evidence of facilitation and suppression by F5 of M1 responses at different C–T intervals is plotted in Fig. 4.

![Figure 4. Incidence of F5-induced facilitation and suppression](jp.physoc.org)
Each occurrence indicates the presence of these effects in a full data set for that muscle (with interleaved C, T and C–T stimuli). Facilitation (Fig. 4, black columns) was seen in both monkeys at short C–T intervals of −1 ms (M1 before F5), or at 0 and 1 ms, with the largest effects generally at C–T = 0 ms. Facilitation was also present in some muscles at longer C–T intervals of 3 or 3.5 ms, but not seen at longer intervals of up to 6 ms. In contrast, suppression was never observed at short intervals (−1, 0 or 1 ms), but was present at 1.2, 1.4, 2.4 and 6 ms (Fig. 4, grey columns).

**Location of electrodes in F5 and M1 and the EMG responses evoked from them**

Electrode positions were verified by post-mortem histology. In both monkeys, the tips of the M1 electrodes were confirmed to lie in the anterior bank of the central sulcus, while the F5 electrodes were in the inferior bank of the arcuate sulcus, just lateral to the arcuate spur (Fig. 2A and B for M39 and Fig. 2C and D for CS15). The microwire arrays were aimed at sites in M1 and F5 which previous mapping experiments had revealed as yielding digit movements with HCMS (see Methods). In M39 single shocks delivered to 4/5 M1 and 2/5 F5 electrodes yielded short-latency EMG responses in hand/digit muscles, while in CS15 this was true for 2/4 M1 and 2/5 F5 electrodes. The other electrodes were ineffective or evoked responses in other body parts (e.g. face, elbow or shoulder).

In both M39 and CS15 the test responses from M1 were all evoked from a cathode in the deep lamina (V or VI). In M39 M1 responses were evoked from electrode 4 as cathode and 3 as anode (Fig. 2A). The tip of electrode 4 was in lamina VI (Fig. 2B, section c) and that of electrode 3 at the lamina III–V border (Fig. 2B, section d). These M1 test responses were facilitated by two different F5–M1 combinations, using F5 cathodes 6 and 7 (Fig. 2A). The most consistent effects resulted from F5 stimulation using electrode 7, with electrode 8 as the anode. These F5 electrodes were located in lamina V (Fig. 2B, section a) and lamina II (Fig. 2B, section b), respectively. This M1–F5 pairing produced facilitation of 4/10 tested muscles (EDC, AbPL, PL and BrR). Other combinations produced different effects. For example, at longer C–T intervals, test responses in AbPL and PL from M1 were suppressed by F5 stimulation using electrode 9 as cathode (tip in lamina V) and 8 as anode. Other test responses evoked from M1 (electrode 2 as cathode, lamina VI) were also facilitated from F5 electrodes 7 (cathode) and 8 (anode).

In case CS15, only one electrode pairing evoked a conditioning effect. In M1, electrode 4 as the cathode was deep in lamina V (Fig. 2D, section c) while electrode 2 as the anode was at the V–VI border (Fig. 2D section d). EMG responses evoked from this M1 electrode pair were strongly facilitated by F5 stimulation using electrode 8 as cathode and 6 as anode (Fig. 2C). These electrodes were both located in lamina III (Fig. 2D, sections a and b). This M1–F5 pairing produced significant modulation of the M1 test response in three muscles (facilitation or suppression, depending on C–T intervals, see above). In summary, both facilitation and suppression of M1 test responses could be obtained from F5 (M39 three electrode combinations; CS15 one combination) but these were not related in any obvious manner to the layer in which the F5 electrode was located.

**Grasp specific F5 conditioning effects**

The second part of the study examined whether the F5 conditioning effect of stimuli delivered 50 ms after the onset of the reach-to-grasp movement (HPR event in Fig. 1C) was related to the shape of the upcoming grasp. This was carried out in M39, which was trained to grasp four objects, two of which, the cube and the ring could be grasped by either a hook grip or a side grip (see figurines of grasps in Fig. 5A). The cube side grip was performed in each of the four sessions, the disc in three sessions, the plate twice, and the cube hook grip, and both grips of the ring were performed once. M1 stimulation intensity was set at 150 μA and F5 stimulation at 175 μA; the latter did not evoke any clear EMG responses when given alone. With this stimulus configuration, and using a C–T interval of 3.5 ms, M1 responses were facilitated in 6/10 of muscles tested; here we concentrate on two with the most consistent effects, PL and BrR.

Examples of the evoked responses from F5, M1 and F5–M1 stimulation during the reach to grasp of the six object-grasps are shown in Fig. 5A. A Kruskal–Wallis test showed a significant effect of object-grasp for BrR and PL muscles on the C–T ratio (both, P < 0.001). Both muscles showed significant facilitation (all P < 0.0001) during reach to grasp of the disc (F5–M1/M1 ratio (± S.E.M.): BrR 1.92 ± 0.11 and PL 1.68 ± 0.08 n = 162, three sessions). F5 conditioning also facilitated the test response for side grips of both the cube (BrR: 1.62 ± 0.09 n = 209 P < 0.001; PL: 1.36 ± 0.07 n = 209 P < 0.0001, four sessions) and the ring (BrR 2.87 ± 0.30 n = 37, P < 0.0001; PL 1.85 ± 0.14 n = 37 P < 0.05, one session), but there was no significant facilitation of either BrR or PL for the hook grip of either the cube or the ring. Finally there was no facilitation of either muscle for grasp of the plate.

The differential effect of F5 conditioning for the six object-grasps could be related to the level of ongoing EMG activity at the time of cortical stimulation, with the EMG responses evoked by corticospinal volleys modulated by the level of excitability of the target motoneurones (Lemon et al. 1987; Kischka et al. 1993; Bennett & Lemon, 1994; Devanne et al. 1997). However, the polar plots in Fig. 5B show that when the cortical stimuli were delivered early during the reach (epochs 1–3, see Fig. 1C), the level...
of EMG activity in both BrR and PL was similar for all six object-grasps (Fig. 5B, grey lines and diamonds), so this is unlikely to explain the variation in F5–M1 facilitation during reach-to-grasp of specific objects. This variation is shown in Fig. 5B by plotting the difference between the amplitude of the conditioned (F5–M1) and the test (M1) EMG responses (black lines and triangles).

Figure 5. Grasp specificity of F5 conditioning of M1 stimulation and EMG activity during reach-to-grasp. A, averaged evoked responses from F5 conditioning (C) (grey traces), M1 test (T) stimuli (dashed traces), and combined F5–M1 stimulation at C–T = 3.5 ms (black traces) recorded from brachioradialis (BrR) and palmaris longus (PL) for the six object-grasps in M39 (shown at left). Stimuli delivered 50 ms after homepad release. Wilcoxon’s signed-rank test: \(* P < 0.05, ** P < 0.001, *** P < 0.0001\). All figures are with 10% outliers removed. Averages are of 23–67 trials per condition. B, polar plots showing the relative amount of facilitation obtained for the six object-grasps tested. This is plotted as the amplitude of the conditioned response (F5–M1) minus that of the test response alone (M1), normalised to the maximum value (= 1). The plots also show the normalised ongoing muscle activity during the period when cortical stimuli were delivered (grey line and diamonds) and during hand shaping prior to grasp (dashed lines and squares) of the six objects (30 trials per object-grasp), again normalised to the maximum value (= 1). The relevant periods of EMG activity are indicated by grey bars in Fig. 1C. These EMG activity data were collected from a session in which no stimulation was given.
A second possibility is that the F5 conditioning reflects the upcoming pattern of EMG activity for hand shaping. F5 neurones are known to show grasp-specific discharge both before and early in the reach-to-grasp movement (Murata et al. 1997; Raos et al. 2006; Umilta et al. 2007). The average level of EMG activity during hand shaping (epochs 7–9, see Fig. 1C) is shown by the dashed lines and squares in Fig. 5B. For BrR, the largest F5–M1 facilitation was obtained for side grip of the ring, followed by grasp of the disc, and side grips of the cube; although all of these grasps were associated with relatively high levels of activity in BrR and are possibly related to the semi-supinated posture of the forearm for these grasps, there was again no obvious relationship between the degree of facilitation and the level of EMG activity during hand shaping.

Again for PL, although there was generally strong facilitation for grasps associated with a high level of EMG activity in this muscle (e.g. during grasp of the disc and side grasp of the cube) the largest F5–M1 facilitation was for trials involving side grip of the ring which was not associated with particularly high level of EMG activity. For the plate, a large but relatively unfacilitated response was present for a specific subset of grasps.

The specific nature of F5–M1 interactions

The modulation of M1 responses by F5 stimulation is unlikely to be due solely to the general shift in excitability that occurs during the reach-to-grasp movement. First, the modulatory action of F5 stimulation was seen in most but not all of the muscles tested (9/10 in M39 and 3/4 in CS15). Second, modulation was far more common in some muscles than others. These included PL, a wrist flexor that also tenses the palmar aponeurosis, an important action for whole hand grasp. Modulation of responses was also common in extrinsic (AbPL) and intrinsic (thenar) muscles acting on the thumb and in the 1DI muscle acting on the index finger. All of these muscles show grasp-specific changes in activity during reach-to-grasp (Brochier et al. 2004). Moreover, the modulation did not seem to be related in any simple manner to the level of ongoing EMG activity (Fig. 5A and B). The overall impression is that sites in F5 are able to modulate M1 output in a muscle-specific fashion.

The origin of F5 effects and the possible sites of F5–M1 interaction

EMG responses to M1 stimulation all arose from electrodes located in the deeper cortical laminae (V, VI) in the anterior bank of the central sulcus. The facilitation and suppression of these responses from F5 involved a number of F5 sites, all located in the inferior bank of the arcuate sulcus (cf. Cerri et al. 2003; Shimazu et al. 2004), in the same region where we have recorded neurons with grasp-specific activity (single neuron data from M39 was included in the study of Umilta et al. 2007). Effects were evoked from F5 electrodes located in a number of different cortical laminae (including lamina II and V). The physical stimulus spread at the intensities used here (110–200 µA) would be in the order of 1 mm (Ranck, 1981; Lemon, 1984) so it is unlikely that there was spread of current from M1 to F5 or vice versa. In our experience the current intensity of effective single shocks is always higher for chronically implanted microwires than when using sharp electrodes in acute penetrations (typically 5–20 µA). This is probably due to oedema and gliosis that follows the implant of the chronic electrode, and to a decrease in electrode tip impedance.

We have discussed elsewhere the likely sites of interaction between F5 and M1 (Shimazu et al. 2004; Schmidlin et al. 2008). While a subcortical site has not been excluded, the short latency of the C–T interactions and their abolition by the reversible inactivation of the M1 hand area both indicate a local site, most likely within M1 itself. Interactions between F5 and M1 probably result from the extensive reciprocal cortico-cortical connections between the M1 and F5 hand areas (Muakkassa & Strick, 1979; Godschalk et al. 1984; Dum & Strick, 2005; Dancause et al. 2006). A recent inactivation study showed that the integrity of the M1 hand area was critical for motor
effects in the hand to be generated by F5 stimulation with repetitive ICMS (Schmidlin et al. 2008).

We have shown that the late I-waves evoked in corticospinal neurons by single M1 stimuli are particularly enhanced by conditioning stimuli delivered to F5, and that these late I-waves then elicit enhanced responses in motoneurones supplying hand muscles (Cerri et al. 2003; Shimazu et al. 2004). In a terminal experiment on one of the monkeys in this study (CS15) we were able to confirm that the F5 sites that facilitated EMG responses also strongly facilitated I₂ and I₃ waves (H. Shimazu et al. unpublished observations).

Because the late I-waves do not leave the cortex until some time after the M1 shock has been delivered (probably around 4 ms for the I₂ wave), inputs from F5, which can arrive in M1 as early as 1 ms after F5 shock (Godschalk et al. 1984; Ghosh & Porter, 1988; Tokuno & Nambu, 2000), have ample time to influence the circuits generating the I₁-waves, even at short C–T intervals (−1 to 1 ms, see Fig. 4). Facilitation was also seen at later intervals (Fig. 4; 3 to 3.5 ms) and this might represent effects on the next (I₃) wave.

### Comparison of evoked responses in the sedated vs. awake behaving monkey

The results here extend previous work in the sedated monkey (Cerri et al. 2003; Shimazu et al. 2004) to the awake behaving monkey. In both the sedated and awake animal F5 facilitation of M1 test responses is large and reproducible, and occurs at discrete C–T intervals, which may reflect the inherent periodicity of the I-waves. The degree of facilitation evoked by single F5 shocks is similar in the sedated animal (up to 4 fold increase) and awake animal (up to 3 fold). It is noticeable that while single F5 shocks, given alone, never evoked any clear EMG responses in the sedated monkey (Cerri et al. 2003), we found that strong single shocks did so in the awake monkey executing an active movement. This presumably reflects the much more excitable state of the F5–M1 network in the awake state (see Koch et al. 2006).

### Suppression of M1 responses by F5 stimulation

Another key difference is that in the awake monkey we found consistent evidence for suppression from F5 of M1 test responses. In our previous studies in sedated and anaesthetised macaques, all of the F5–M1 interactions were facilitatory in nature, and this was true over a range of C–T intervals. In the present study, we found that by varying the C–T interval we could observe either facilitation or suppression in several muscles, and these different effects could be evoked from the same combination of F5 electrodes. Facilitation occurred mostly at short intervals while suppression was seen at longer C–T intervals (> 1 ms; Fig. 3C, Fig. 4). Thus in the awake behaving monkey, it would appear that the selective modulation of M1 outputs by F5 involves both inhibitory and excitatory processes; the inhibitory effects may be selectively blocked by sedation or anaesthesia. In the awake monkey, Tokuno & Nambu (2000) reported that stimulation of the premotor cortex exerted early excitation of some PTNs followed by longer lasting inhibition, which is in keeping with our findings on EMG responses. Interestingly, most TMS studies of premotor–motor cortex interactions in humans have highlighted suppression effects (Civardi et al. 2001; Gerschlager et al. 2001; Munchau et al. 2002), and a number of intrinsic circuits are known to give rise to inhibition of M1 corticospinal neurons (Ilic et al. 2002; Kujirai et al. 1993); these circuits may well be influenced by F5 inputs. However, during certain types of active grasp, facilitation may dominate (Davare et al. 2008). The capacity to balance suppression and facilitation of M1 outputs to hand muscles would allow F5 precise control over the shape of the hand during grasp.

### F5–M1 interactions can be grasp-specific

The facilitation of M1 outputs from F5 varied for grasp of different objects (Fig. 5). The specific pattern of facilitation did not appear to reflect the ongoing level of EMG activity at the time of cortical stimulation, which, only 50 ms after the onset of the reach-to-grasp movement, was rather similar for all six object-grasps (Fig. 5B; see Brochier et al. 2004).

Since F5 neurones often show activation prior to movement that reflects the subsequent grasp (Murata et al. 1997; Raos et al. 2006; Umilta et al. 2007) and since F5 may encode ‘motor primitives’ that make up a grasp prototype (Rizzolatti & Luppino, 2001), the interaction between F5 and M1 may predict the upcoming EMG activity at the time of hand-shaping. Evidence for such an idea has been deduced from TMS studies in humans preparing to grasp specific objects (Castiello, 2005; Prabhhu et al. 2007). In the present study, two of the muscles studied showed facilitation for some types of grasp and not for others (Fig. 5A). However, there was no fixed relationship between the degree of activation of the muscle for a particular grasp and the presence of significant facilitation from F5 (Fig. 5B). Thus this experiment demonstrates the grasp-specific nature of F5–M1 interactions in the awake monkey. Further investigation at other time points during the reach-to-grasp behaviour will be needed to fully elucidate the evolving interaction between these two key components of the grasping circuit.
References


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