

Making Mirrors: Premotor Cortex Stimulation Enhances Mirror and Counter-mirror Motor Facilitation

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Abstract

■ Mirror neurons fire during both the performance of an action and the observation of the same action being performed by another. These neurons have been recorded in ventral premotor and inferior parietal cortex in the macaque, but human brain imaging studies suggest that areas responding to the observation and performance of actions are more widespread. We used paired-pulse TMS to test whether dorsal as well as ventral premotor cor-

tex is involved in producing mirror motor facilitation effects. Stimulation of premotor cortex enhanced mirror motor facilitation and also enhanced the effects of counter-mirror training. No differences were found between the two premotor areas. These results support an associative account of mirror neuron properties, whereby multiple regions that process both sensory and motor information have the potential to contribute to mirror effects. ■

INTRODUCTION

TMS of primary motor cortex (M1) produces motor-evoked potentials (MEPs) in peripheral muscles. Action observation alters motor system activity, enhancing MEPs from the muscle, which would be involved in performing the observed movement (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). This matching, muscle-specific (“mirror”) motor facilitation may reflect the influence of mirror neurons that fire during both performance and observation of the same action (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992).

Mirror neurons have been recorded in macaque ventral premotor and inferior parietal cortex. However, functional brain imaging in humans suggests that areas responsive to both action observation and performance are more widespread than macaque mirror neuron areas. Among other areas, they include both ventral (PMv) and dorsal (PMd) premotor cortex (Gazzola, Rizzolatti, Wicker, & Keysers, 2007; Vogt et al., 2007; Gazzola, Aziz-Zadeh, & Keysers, 2006). It is therefore unclear whether both PMv and PMd contribute to mirror motor facilitation effects.

The exact relationship between mirror motor facilitation induced by M1 TMS and mirror neuron activity in PMv remains uncertain (Hickok, 2009). Avenanti, Bolognini, Maravita, and Aglioti (2007) disrupted mirror motor facilitation by repetitive TMS of PMv, suggesting it sends a motor command to M1 during action observation. This hypothesis may be tested using a trial-by-trial causal procedure such as paired-pulse TMS (Buch, Mars, Boorman, & Rushworth, 2010; Davare, Montague, Olivier, Rothwell, & Lemon, 2009; Davare, Lemon, & Olivier, 2008; O’Shea,

Sebastian, Boorman, Johansen-Berg, & Rushworth, 2007; Koch et al., 2006). This procedure also allows investigation of the timing of the influence of PMv on M1 during mirror motor facilitation. Moreover, the same technique can be used to compare the influence exerted by PMv and PMd.

In paired-pulse TMS, a conditioning pulse is applied to the brain area under investigation. This area’s task-related influence on M1 is measured by changes induced in MEPs evoked by a subsequent M1 pulse. In Experiment 1, we measured the mirror motor facilitation effect on single-pulse (M1 only) trials and compared this with the effect on paired-pulse trials, where the conditioning pulse was applied over either PMv or PMd. Enhancement of mirror motor facilitation on paired-pulse trials indicates functional involvement of the tested site in this effect. Thus, if PMv alone is involved in mirror motor facilitation, then enhancement should occur only in the PMv condition. In contrast, if PMd is also involved in mirror motor facilitation, there should be no difference between the two sites in enhancement of the mirror effect.

A control experiment was performed to verify that the results of Experiment 1 were due to the effect of the conditioning pulse on premotor cortex and not due to a direct effect of the conditioning TMS pulse on M1 (Mars et al., 2009; Davare et al., 2008; O’Shea, Sebastian, et al., 2007). In this experiment, both the conditioning and the test pulses were applied to M1, either within the same hemisphere (conditioning and test pulses applied to the same site) or between hemispheres (conditioning pulse applied to the right M1; test pulse applied to the left M1).

It has been shown that mirror motor facilitation can be reversed by “counter-mirror” sensorimotor training in which participants perform one movement while observing

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a movement involving a different muscle. After such training, action observation enhances MEPs from the non-matching muscle (Catmur, Walsh, & Heyes, 2007). It is not known whether these counter-mirror effects involve the same brain areas that are involved in mirror effects. A demonstration that counter-mirror effects involve PMv would provide strong evidence that the mirror system is forged by associative learning (Heyes, 2001, 2010). Alternatively, because PMd is involved in learning stimulus-response mappings (Mars, Piekema, Coles, Hulstijn, & Toni, 2007; Wise & Murray, 2000), it is possible that only PMd is involved in counter-mirror effects. Experiment 2, therefore, tested whether counter-mirror training alters the same areas that are involved in mirror motor facilitation, by repeating Experiment 1 after counter-mirror training.

METHODS

Experiments 1 and 2

Participants

Twelve right-handed participants (6 men, age = 18–32 years) received payment for taking part in these experiments. Participants were screened to exclude a family history of psychiatric or neurological disease and gave written informed consent. The order of premotor site stimulation (PMv or PMd first in both Experiments 1 and 2) was counterbalanced across participants, all of whom took part in both experiments. The study was approved by the Mid and South Buckinghamshire Research Ethics Committee (reference number 08/H0607/65) and carried out in accordance with the Declaration of Helsinki.

Action Observation TMS Testing Sessions (Experiments 1 and 2)

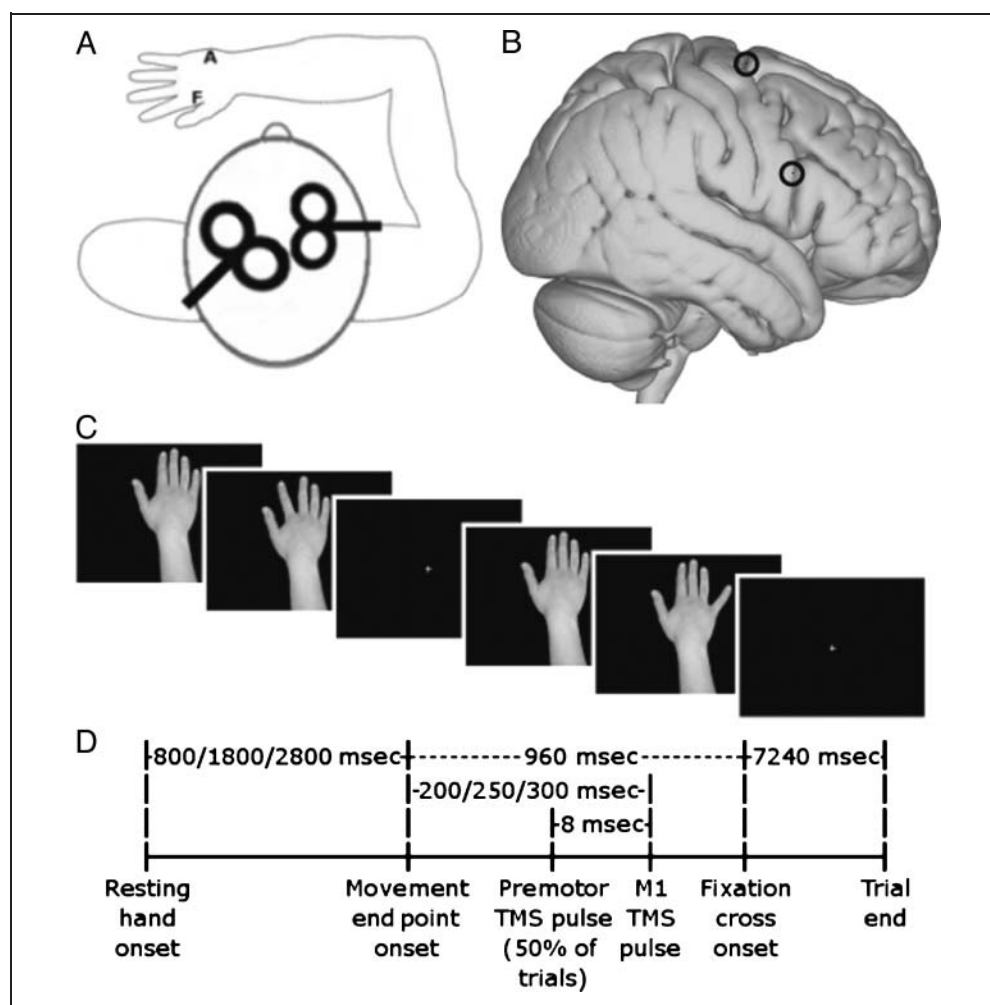
Each experiment consisted of two TMS sessions, with a minimum of a week between them. Only one premotor site (PMv or PMd) was stimulated in each session. Participants observed abduction movements of the index or little finger of a right hand while MEPs were recorded from the first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles of the participant's right hand (Figure 1). The observed movements were presented in an ego-centric perspective, that is, the perspective in which we most commonly observe our own movements, because this produces the strongest mirror motor facilitation during the observation of right-hand movements, irrespective of the observer's posture (Alaerts, Heremans, Swinnen, & Wenderoth, 2009). In addition, to ensure that there was no simple or orthogonal spatial compatibility between the observed movements and the muscle that would perform them, the participant's hand was oriented orthogonally to the observed hand and placed in the left hemispace (Press, Bird, Walsh, & Heyes, 2008; Cho & Proctor, 2004).

On half of trials, right premotor cortex was stimulated 8 msec before left M1. This time interval was based on pre-

vious studies of premotor–M1 interactions (Buch et al., 2010; Davare et al., 2008; O'Shea, Sebastian, et al., 2007). Contralateral rather than ipsilateral premotor cortex was chosen because, due to the proximity of PMd and M1, it is only possible to stimulate contralateral PMd when an M1 coil is present. For PMv–M1 investigations, it is possible to stimulate either contralateral or ipsilateral PMv. When the contralateral PMv conditioning pulse is applied at 110% of motor threshold, as in the current study, the effects seen after contralateral PMv conditioning pulses (right PMv–left M1; Buch et al., 2010) are similar to those seen after ipsilateral PMv conditioning pulses (left PMv–left M1; Davare et al., 2008). Although it is possible to obtain different effects by stimulating unilaterally either left or right PMd (Johansen-Berg et al., 2002; Schluter, Rushworth, Passingham, & Mills, 1998), paired-pulse TMS experiments with contralateral PMd–M1 coil positions are unaffected by dominance and lateralization (O'Shea, Sebastian, et al., 2007, found no differences between left PMd–right M1 and right PMd–left M1 conditions), and so they may provide an index of any functional interactions that occur, regardless of their hemispheric origin.

M1 stimulation was applied using an 80-mm figure-of-eight coil to the left hemisphere optimal scalp position for the FDI and ADM muscles. This position was defined as the location at which the lowest intensity stimulation was required to produce MEPs in both muscles. During the experiment, M1 TMS was applied at this location at the minimum intensity required to produce MEPs of approximately 1 mV in both muscles. Premotor stimulation was applied using a 70-mm figure-of-eight coil. The scalp locations of the premotor sites were determined using Brainsight frameless stereotaxy (Rogue Research, Montreal, Canada). The Montreal Neurological Institute coordinates were averaged from previous studies investigating action observation or grasping (PMv: 57, 12, 23) (Davare et al., 2008; Avenanti et al., 2007; Urgesi, Candidi, Ionta, & Aglioti, 2007; Aziz-Zadeh, Koski, Zaidel, Mazziotta, & Iacoboni, 2006; Costantini et al., 2005; Buccino et al., 2001) and action selection (PMd: 25, –7, 68) (O'Shea, Johansen-Berg, Trief, Gobel, & Rushworth, 2007; O'Shea, Sebastian, et al., 2007; Amiez, Kostopoulos, Champod, & Petrides, 2006; Davare, Andres, Cosnard, Thonnard, & Olivier, 2006; Chouinard, Van Der Werf, Leonard, & Paus, 2003). For both PMv and PMd, premotor stimulation was applied at an intensity of 110% of resting motor threshold (rMT) for the right hemisphere. rMT was defined as the lowest intensity stimulation required to produce MEPs of at least 50 μ V in the left FDI on at least three of five trials when applied over the optimal scalp position (Rossini et al., 1994). TMS was delivered by two monophasic Magstim 200 stimulators (Magstim, Dyfed, UK). MEPs were recorded using Ag–AgCl electrodes in a belly-tendon montage. The EMG signal was acquired using a CED 1902 amplifier, a CED 1401 analog-to-digital converter, and the Signal software (Cambridge Electronic Design, Cambridge, UK). The signal was sampled at 5000 Hz and band-pass

Figure 1. (A) Diagram of experimental setup. TMS was applied to the left M1 and right premotor cortex (the PMd testing session is shown) while MEPs were recorded from right FDI (F) and ADM (A). (B) Location of TMS sites in right premotor cortex. PMd: 25, -7, 68; PMv: 57, 12, 23 (Montreal Neurological Institute coordinates). (C) Sequence of events depicted for two trials. (D) Timeline of events during each trial. The M1 TMS pulse was delivered at one of three time intervals after the finger movement. On paired-pulse trials, the premotor TMS pulse was delivered 8 msec before the M1 pulse.



filtered between 10 and 1000 Hz with a 50-Hz mains frequency notch filter.

Each trial started with presentation of an image of a right hand at rest. After a variable duration (800, 1800, or 2800 msec), the image was replaced with that of the hand at the end point of an index or little finger abduction movement, creating the appearance of a finger movement. The movement image remained on the screen for 960 msec, after which it was replaced by a fixation cross for 7240 msec. At a variable interval after the appearance of the movement image (200, 250, or 300 msec), the TMS pulse to M1 was applied. On half of trials, this was preceded by 8 msec by the premotor TMS pulse. Stimulus presentation and pulse timings were controlled by Presentation software (Neurobehavioral Systems, Albany, CA).

Each experimental session consisted of five blocks. Participants rested for a mean duration of 2.6 min ($SEM = 0.4$ min) between blocks. Each block contained 36 trials, comprising a fully factorial combination of the above variables (resting image duration, movement identity, time interval to TMS pulse, and TMS pulse type) presented in a randomized order. To maintain participants' attention to the stimuli, an attentional task was used. On a randomly selected 11% of trials, a faint flesh-colored circle was presented

on the moving finger. Participants were instructed to attend to this circle. A question screen was presented four times per block, whereby participants responded using their left hand as to whether a circle had been present on the preceding trial. To make this task demanding, the color of the circle was very similar to that of the hand. It was set to the mean color of the hand stimulus, calculated by finding the mean intensity of the red, green, and blue components of every colored pixel in the hand image. This resulted in a challenging task.

Data Analysis

For each muscle for every trial, the 100-msec period before the M1 pulse was checked for background EMG activity; if this was found, the data from both muscles for this trial were rejected. Peak-to-peak MEP size was measured for each muscle for every trial. For each muscle, if MEP size was less than 0.15 mV on three or more consecutive trials, the data for these trials were removed because this indicated that M1 coil placement was not accurate. MEP sizes were normalized by dividing by the mean MEP size for single-pulse trials for each muscle for each block of trials. This controlled for any variation in coil position between blocks and intersession or interindividual variability in

MEP size. For the single-pulse analysis, mean normalized MEP size was calculated for each condition. For the paired-pulse analyses, to simplify data presentation, MEP mirror ratios were calculated for each condition. For each muscle, MEP size during observation of the movement which that muscle would perform was divided by MEP size during observation of the other movement. An MEP mirror ratio greater than 1 indicates mirror motor facilitation.

Counter-mirror Training (Experiment 2)

Experiment 2 took place at an average of 4 weeks after Experiment 1. Twenty-four hours before each TMS session in Experiment 2, participants were given counter-mirror training for approximately 1 hour. Each training session was therefore half as long as that given by Catmur et al. (2007); this was to make the overall length of the experiment tolerable for participants. Each trial began with presentation of the hand at rest. After a variable time interval (800, 1600, or 2400 msec), the image was replaced with the end point of an index or little finger abduction movement, which remained on the screen for 960 msec, after which it was replaced with a blank screen for 3500 msec. Participants were instructed to perform a little finger abduction movement as soon as they saw an index finger abduction movement and to make an index finger abduction movement when they observed a little finger abduction movement. The participant's hand was positioned in an orthogonal plane to the observed hand, as for the TMS sessions. Twelve blocks of trials were presented, with 36 trials per block. Each trial type (3 time intervals \times 2 observed finger movements) was repeated six times per block in a randomized order. Speed and accuracy were rewarded by additional payment (£0.50 extra per block of trials in which two or fewer errors were made, and mean response time was less than 400 msec).

EMG was recorded from the right FDI and ADM with Ag–AgCl electrodes. The EMG signal was acquired using a CED 1902 amplifier, sampled at 3000 Hz and band-pass filtered between 20 and 3000 Hz. Response times were calculated off-line in the following manner: a window of 20 msec width was passed over the EMG data in 1-msec steps, starting from the onset of the movement stimulus, and the standard deviation of the data within this window was measured. When this value reached $2.75 \times$ the standard deviation of the period 100 msec before the onset of the movement stimulus for three 20-msec windows in succession, this was taken to be the start of the response, and the elapsed time since the onset of the movement stimulus was measured. Response times were verified on a plot of the data for every trial, and errors were recorded. Mean response times were calculated for each block of trials.

Control Experiment

Participants

Twelve right-handed participants (6 men, age = 19–31 years) received payment for taking part in this experiment. Seven

of these participants (original participant group) took part in Experiments 1 and 2, a minimum of 9 months previously; the remaining five participants were new. The order of M1 site stimulation (within-hemisphere or between-hemisphere first) was counterbalanced across participants. Participants were screened and the study was approved in the same manner as for Experiments 1 and 2.

Action Observation TMS Testing Sessions

The experiment consisted of two TMS sessions (within- and between-hemisphere conditions), administered on the same day with a minimum of 22 min (mean \pm SEM = 29 ± 1 min) between conditions. Each session was identical to those administered in Experiment 1, with the exception that for the within-hemisphere condition, only one 80-mm figure-of-eight TMS coil, over left hemisphere M1, was used; thus, both conditioning and test pulses were applied to the left M1. For the between-hemisphere condition, as for Experiment 1, conditioning pulses were applied using a 70-mm figure-of-eight TMS coil. These pulses were applied to the right M1. On half of trials, as before, only one TMS pulse was administered, whereas on the other half of trials, a conditioning pulse was applied to M1 (either left or right hemisphere) 8 msec before the test pulse. As before, test pulse intensity was set at the minimum intensity required to produce MEPs of approximately 1 mV in both muscles of the right hand, whereas conditioning pulse intensity was set at an intensity of 110% of right hemisphere rMT. For the within-hemisphere condition, TMS pulses were delivered through a BiStim module (Magstim). As before, each condition consisted of five blocks. Participants rested for a mean duration of 1.7 min (SEM = 0.4 min) between blocks.

RESULTS

Experiment 1

Attentional Task

The mean \pm SEM percentage of correct responses for the attentional task was $79.4\% \pm 4.5\%$. Scores from this task were entered into a one-way ANOVA with factor of TMS site (PMv, PMd). There was no significant effect of TMS site.

MEP Data

To verify the initial presence of a mirror motor facilitation effect, a preliminary analysis was performed on the normalized MEP sizes, from single-pulse trials only, in both PMv and PMd conditions. Repeated measures ANOVA was performed with factors of recorded muscle (FDI and ADM), observed movement (index finger and little finger), and time interval between movement onset and M1 pulse (200, 250, and 300 msec). There was a significant interaction between recorded muscle and observed movement, $F(1, 11) = 7.842, p = .017$, indicating mirror motor facilitation

(MEPs from each muscle were higher during the observation of the movement in which it would be involved than during observation of the other movement; see Figure 2A). No other effects or interactions reached significance.

To simplify data presentation, MEP mirror ratios were used in all subsequent analyses. For each muscle, MEP size during observation of the movement which that muscle would perform was divided by MEP size during observation of the other movement (e.g., for the FDI muscle, which performs index finger abduction, MEP size during observation of index finger movements was divided by MEP size during observation of little finger movements). An MEP mirror ratio greater than 1 indicates mirror motor facilitation. Figure 2B illustrates the same data as shown in Figure 2A but in simpler format, showing mirror motor facilitation in both muscles.

MEP mirror ratios for the single- and paired-pulse trials were compared across the two premotor sites. Repeated measures ANOVA was performed with factors of site (PMv and PMd), recorded muscle (FDI and ADM), TMS pulse type (single and paired), and time interval (200, 250, and 300 msec). There was a significant interaction between pulse type and time interval, $F(2, 22) = 3.711$, $p = .041$. Figure 3 demonstrates that MEP mirror ratios were greater than 1 for both single and paired TMS pulse types at all time intervals, all $t(11) > 2.3$, all $p < .05$, indicating mirror motor facilitation. However, stimulation of premotor cortex at 300 msec after the onset of the observed movement enhanced mirror motor facilitation beyond that seen during stimulation of M1 alone. Simple

effects analysis confirmed that paired-pulse stimulation enhanced mirror motor facilitation only at an interval of 300 msec after the onset of the observed movement, $F(1, 11) = 5.933$, $p = .033$. There were no significant effects or interactions involving the factor of site [main effect of site: $F(1, 11) = 0.006$, $p = .942$; interaction between site, pulse type, and time interval: $F(2, 22) = 1.173$, $p = .328$; simple effect of site on paired-pulse trials at 300 msec: $F(1, 11) = 1.747$, $p = .213$], indicating no difference between the effects of stimulation of the two premotor sites on the mirror ratios.

Although the use of mirror ratios simplifies data presentation, they do not indicate whether the enhancement of mirror motor facilitation is due to an increase in MEP size in the congruent muscle-movement condition (FDI MEPs during observation of index finger movements and ADM MEPs during observation of little finger movements) or a decrease in the incongruent condition (FDI MEPs to little finger movements and ADM MEPs to index finger movements). Mean MEP size for the congruent and incongruent muscle-movement conditions is shown in Figure 4. This suggests that premotor stimulation enhances congruent MEPs, $t(11) = 3.209$, $p = .008$.

Control Experiment

Attentional Task

The mean \pm SEM percentage of correct responses for the attentional task was $89.0\% \pm 2.6\%$.

Figure 2. (A) Mean \pm SEM of normalized MEP sizes for single-pulse trials in Experiment 1, collapsed across TMS sites and time intervals. (B) Data from panel A expressed as MEP mirror ratios. For each muscle, MEP size during observation of the movement which that muscle would perform was divided by MEP size during observation of the other movement, for example, index/little for the FDI muscle, which performs index finger abduction movements. A value greater than 1 indicates mirror motor facilitation.

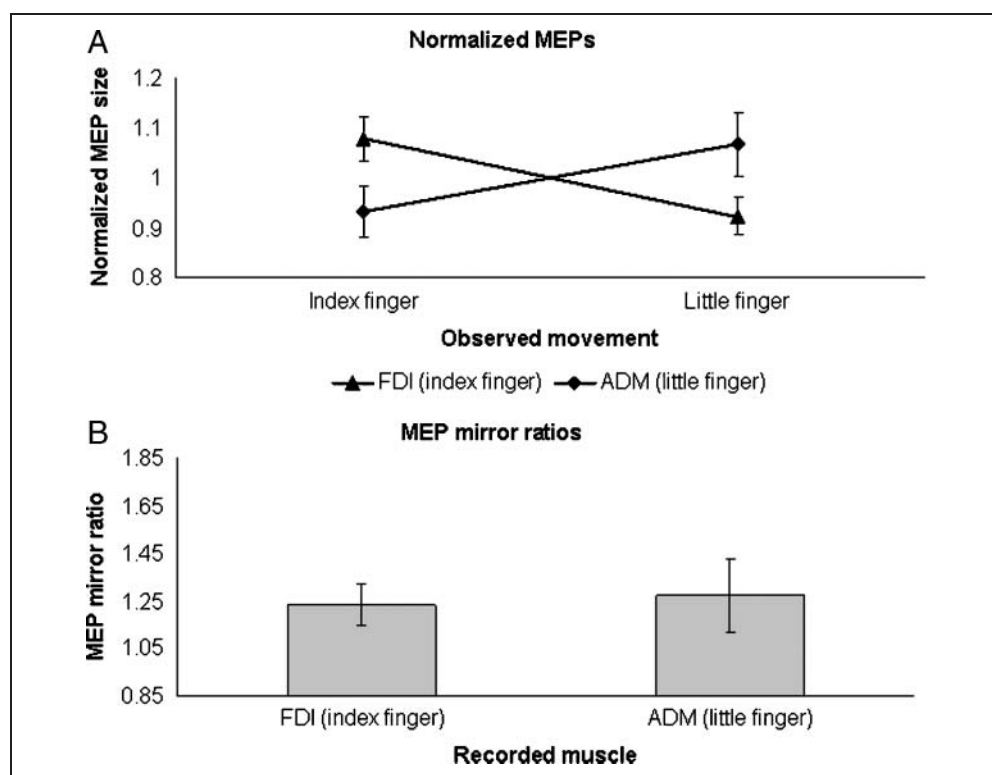
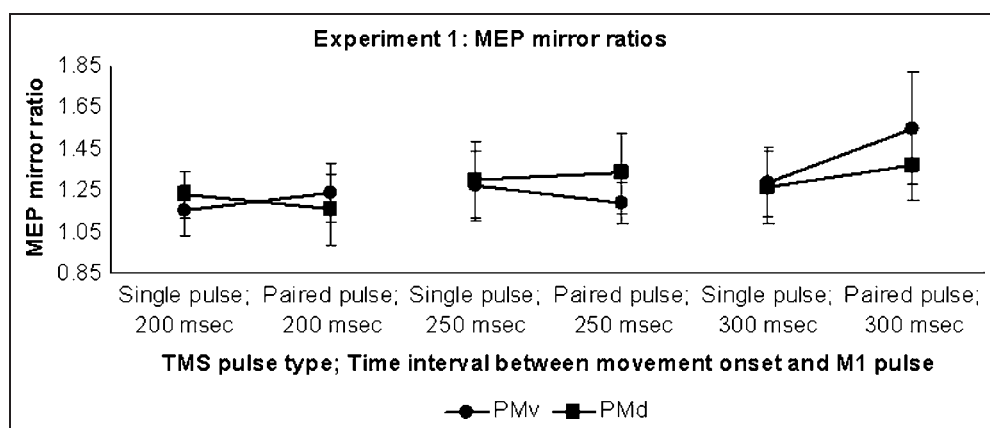


Figure 3. Mean \pm SEM of MEP mirror ratios for Experiment 1. Stimulation of premotor cortex (the paired-pulse condition) at 300 msec after the onset of the observed movement enhanced mirror motor facilitation. There were no differences between the two premotor sites.



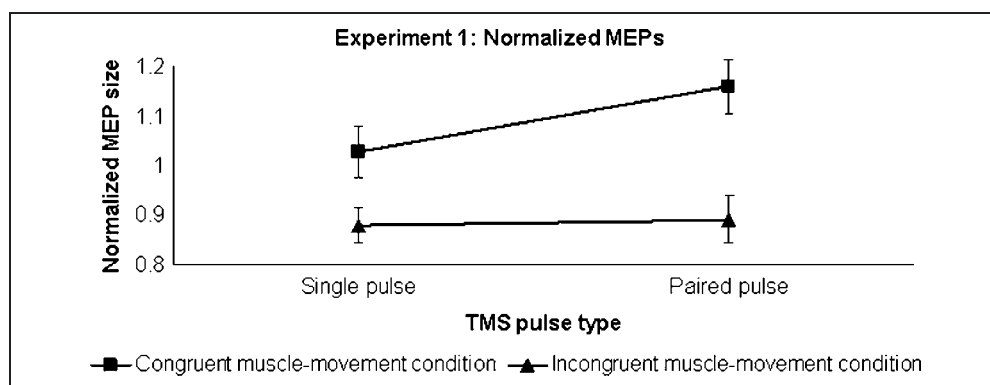
MEP Data

MEP mirror ratios were entered into repeated measures ANOVA with between-subjects factor of participant group (original and new) and within-subjects factors of site (within hemisphere and between hemisphere), recorded muscle (FDI and ADM), TMS pulse type (single and paired), and time interval (200, 250, and 300 msec). Figure 5 displays the effect of paired-pulse TMS over M1 at each of the three time intervals. Unlike in Experiment 1, there was no interaction between time interval and pulse type, $F(2, 20) = 0.129$, $p = .879$. There was also no main effect of pulse type, $F(1, 10) = 0.231$, $p = .641$. This indicates that a conditioning pulse to M1 does not influence mirror motor facilitation in the same way as a conditioning pulse to premotor cortex.

In addition, a trend toward a main effect of recorded muscle was observed (the FDI showed greater mirror ratios than the ADM), $F(1, 10) = 4.5$, $p = .060$. This trend is likely a result of the relative difficulty of successfully stimulating the cortical representation of the ADM muscle because of its smaller size.

This control experiment confirmed that the result of Experiment 1 was not due to the direct effect of the conditioning pulse on M1 but was specific to the premotor sites tested.

Figure 4. Mean \pm SEM of normalized MEP sizes for the 300-msec time interval in Experiment 1, collapsed across TMS sites. MEPs are expressed in terms of congruency between recorded muscle and observed movement (congruent = mean of FDI MEPs during observation of index finger movements and ADM MEPs during observation of little finger movements; incongruent = mean of FDI MEPs during observation of little finger movements and ADM MEPs during observation of index finger movements).



Experiment 2

The action observation TMS experiment was repeated 24 hours after counter-mirror training (one training session per premotor site; see Methods).

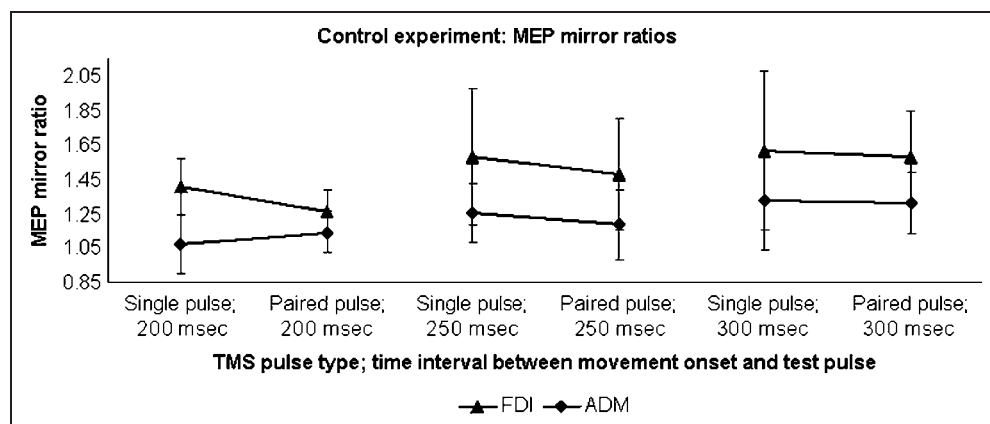
Counter-mirror Training: Response Time Data

Response times from the two counter-mirror training sessions were entered into ANOVA with factor of training block (1–24). There was a significant effect of block, $F(23, 253) = 1.865$, $p = .011$: Response time decreased over the course of training, indicating learning of the counter-mirror association (see Figure 6).

Attentional Task

The mean \pm SEM percentage of correct responses for the attentional task was $81.9\% \pm 4.0\%$. Scores from the attentional task were entered into ANOVA with factors of training (pretraining and posttraining) and TMS site (PMv and PMd). There were no significant effects or interactions.

Figure 5. Mean \pm SEM of MEP mirror ratios for the control experiment, where both conditioning and test TMS pulses were applied over M1.



MEP Data

MEP mirror ratios for the 300-msec time interval (at which a significant effect of premotor stimulation was observed in Experiment 1) were subjected to repeated measures ANOVA with factors of training (pretraining and posttraining), TMS site (PMv and PMd), recorded muscle (FDI and ADM), and TMS pulse type (single and paired). An interaction between training and pulse type was observed, $F(1, 11) = 11.272, p = .006$: The enhancement of mirror motor facilitation seen in Experiment 1 was reversed, such that the effect of counter-mirror training (which abolished the mirror effect) was enhanced by paired-pulse stimulation (Figure 7). There was no difference between the effects of stimulation of the two premotor sites on this interaction, $F(1, 11) = 1.048, p = .328$. Simple effects analysis of the posttraining mirror ratios demonstrated a significant effect of TMS pulse type, $F(1, 11) = 4.947, p = .048$: After counter-mirror training, paired-pulse stimulation produced a significant reduction in mirror motor facilitation. Again, there was no difference between stimulation of the two premotor sites, $F(1, 11) = 0.000, p = .987$.

An additional interaction between TMS site and recorded muscle was observed, $F(1, 11) = 10.704, p = .007$: Mirror ratios were greater for the ADM muscle during the sessions including stimulation of PMv than of PMd. Because this interaction was not affected by TMS pulse type or training, it is of limited theoretical significance. It most likely resulted from suboptimal M1 coil position for the

ADM muscle during PMd stimulation because of the close location of the M1 and PMd coils and the smaller cortical representation of the ADM muscle.

Prepulse EMG Analysis

Analyses were performed for all experiments to ensure that the reported TMS results could not be due to differences in muscle contraction between the conditions (e.g., it is possible that participants might have actually selected the observed action, increasing the contraction of the muscle involved in the observed action and thus producing a spurious mirror effect). The root mean square of the background EMG in the 100 msec before the TMS pulse was calculated for all trials included in the MEP analyses. These values were entered into the same ANOVAs as those used in the MEP analyses. None of the results that were significant in the MEP analyses reached significance in the EMG root mean square data. The MEP results are therefore not due to systematic differences in muscle contraction between conditions.

DISCUSSION

The current study is among the first to use paired-pulse TMS to investigate the interaction between brain regions during action observation (see also Koch et al., 2010; Lago et al., 2010). It suggests that premotor–M1 connections

Figure 6. Mean response times during counter-mirror training (Experiment 2). Each training session consisted of twelve blocks of trials.

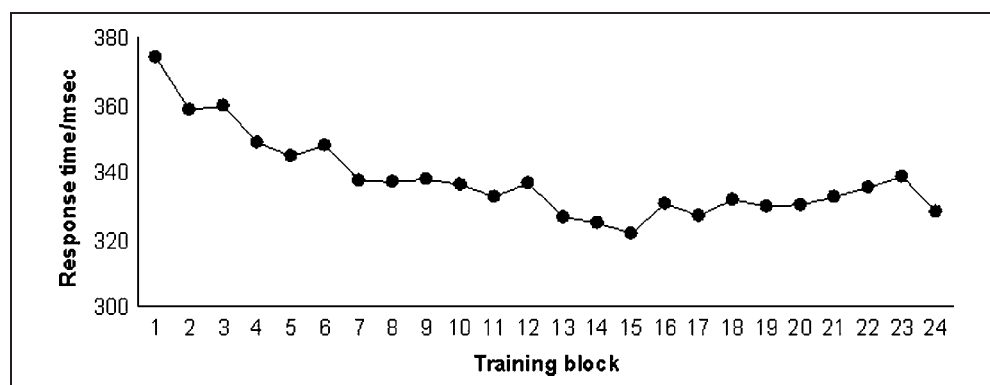
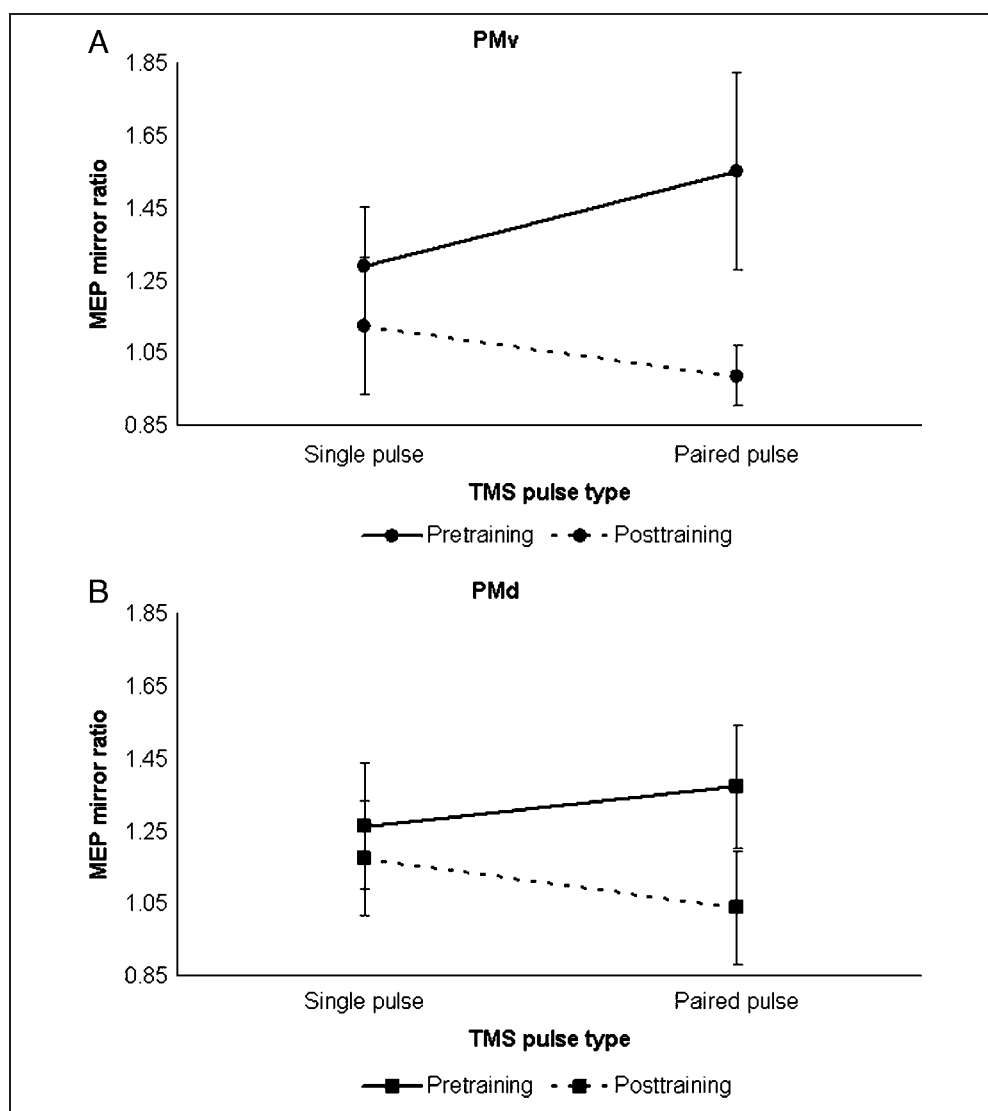


Figure 7. Mean \pm SEM of MEP mirror ratios for Experiments 1 (pretraining) and 2 (posttraining) for the time interval 300 msec after movement onset. Stimulation of premotor cortex enhanced the effect of counter-mirror training by increasing the extent to which training reduced mirror motor facilitation. Both premotor sites are shown: (A) PMv: Training \times TMS Pulse Type interaction, $F(1, 11) = 8.025$, $p = .016$. (B) PMd: Training \times TMS Pulse Type interaction, $F(1, 11) = 5.934$, $p = .033$.



modulate the M1 corticospinal response to observed actions at around 300 msec after movement onset. The current study also provides evidence supporting the involvement of premotor–M1 projections in counter-mirror as well as mirror motor facilitation. Stimulation of premotor cortex at this time interval resulted in enhancement of mirror motor facilitation and also in enhancement of the effects of counter-mirror training. No differences were seen between the two premotor sites in either case.

Mirror motor facilitation produced by M1 TMS provides evidence of muscle-specific matching of observed and performed actions. Experiment 1 showed that these mirror effects are enhanced by premotor cortex stimulation. This result supports the use of mirror motor facilitation effects as an index of premotor mirror neuron activity. The results of Experiment 1 also suggest that mirror properties are present across lateral premotor cortex and therefore may not be restricted to those areas of the brain from which mirror neurons have been recorded in the macaque.

The suggestion that PMd has mirror properties might appear to support claims of a discontinuity between the

characteristics of mirror neurons recorded in the macaque and mirror effects measured in the human brain (Hickok, 2009; Dinstein, Thomas, Behrmann, & Heeger, 2008; Turella, Pierno, Tubaldi, & Castiello, 2008). However, although mirror neurons have only been recorded in macaque PMv and inferior parietal cortex, neurons with mirror-like properties (showing similar responses during both the observation and the performance of a reaching task) have been reported in macaque PMd (Cisek & Kalaska, 2004). The results of Experiment 1 therefore support the description of these PMd neurons as mirror neurons. Similarly, although the present experiments used gestural movement stimuli and the macaque mirror neurons respond preferentially to the observation of actions on objects (Turella et al., 2008), there is some evidence demonstrating that mirror neurons can respond to non-object-related gestures (Kraskov, Dancause, Quallo, Shepherd, & Lemon, 2009; Ferrari, Gallese, Rizzolatti, & Fogassi, 2003). Questions remain, however, as to whether in either species, mirror neurons mediate action understanding (Hickok, 2009; Brass, Schmitt, Spengler, & Gergely, 2007).

Experiment 2 showed that stimulation of both premotor sites enhanced the effects of counter-mirror training. This result supports the suggestion that counter-mirror training alters the same brain areas that were involved in the original mirror motor facilitation and thereby provides further support for the theory that mirror neurons acquire their matching properties through associative learning (the Associative Sequence Learning [ASL] theory; Heyes, 2010; Hickok, 2009; Heyes & Ray, 2000).

The ASL theory suggests that any motor areas with appropriate neuroanatomical connections to sensory areas have the potential to show mirror effects, given sufficient mirror experience (Heyes, 2010). Sources of mirror experience, in which observation and performance of the same action occur in a contingent manner, include observing one's own actions, being imitated (especially as an infant), and engaging in synchronous actions with others (e.g., dance). ASL therefore provides an explanation for the presence of multiple brain areas responding to both observation and performance of actions in brain imaging studies.

The stimuli used in the current experiment made use of apparent motion by presenting the end points of finger movements. This allows precise investigation of the timing of the involvement of premotor cortex in mirror and counter-mirror motor facilitation. Other paired-pulse investigations of premotor cortex, using identical coil arrangements to those used here, have shown that its effects on M1 tend to occur at early time intervals. For example, PMd is involved in action selection at 75–100 msec after the onset of a cue to move or at 50 msec in a simple response task (O'Shea, Sebastian, et al., 2007; Koch et al., 2006). Recent results from PMv suggest that it too first influences M1 at about the same time, 100 msec after the appearance of an object to be grasped (Buch et al., 2010). PMv continues to influence M1 even after movement onset as the grasping movement unfolds and then again in a distinctive manner whenever an adjustment to the movement needs to be made (Buch et al., 2010). The macaque data give limited information about the latency of mirror neuron responses because the recordings do not generally show when the observed movement commenced. Certain studies suggest, however, that mirror neurons begin firing approximately 250 msec after the onset of the observed hand movement (Kraskov et al., 2009; Umiltà et al., 2001; di Pellegrino et al., 1992), which is consistent with the present data, in which effects of premotor stimulation were seen at 300 msec after movement onset.

It therefore appears that mirror motor facilitation effects are present much later in premotor cortex than are other effects of action selection and grasping. It is possible that observed movements are more complex than the kind of cues typically used in action selection tasks and therefore require longer to process. The requirement to make a speeded response during action selection and grasping experiments may also increase attention to the stimuli and hence reduce processing time.

Given this later presence of mirror motor facilitation effects in premotor cortex, it might be expected that, even on single-pulse trials, mirror facilitation would increase as the time interval between the movement and the TMS pulse increases. This was not observed in the present experiment. It is possible that noise within the system prevents the detection of a premotor effect at earlier time intervals; that is, we only see the impact of the premotor cortical stimulation at 300 msec because this is when it is strong enough to overcome the noise in the system. In other words, the modulating influence of premotor cortex may be present at an earlier time interval but detected only when it is strongest at 300 msec. Such an explanation suggests that the effect of premotor stimulation might have increased across the three time intervals in the present Experiment 1, and indeed follow-up analysis indicated that the factor of TMS pulse type showed a trend toward a linear effect across the three time intervals, $F(1, 11) = 4.358$, $p = .061$.

Despite the fact that enhancement of mirror motor facilitation was seen on trials where premotor cortex was stimulated, it is possible that the enhancement is not due to premotor–M1 connections but to those between another area and M1. If this were the case, premotor stimulation could, by virtue of its projections to M1, reveal the effects of this other area rather than the functional effects of premotor–M1 connections. The control experiment excludes this possibility by testing whether any stimulation of M1 with the same pulse timing and intensity parameters is sufficient to enhance mirror motor facilitation. Application of the conditioning TMS pulse to M1 did not have the same effect on mirror motor facilitation as a conditioning pulse to either PMv or PMd. This indicates that the enhancement of mirror motor facilitation seen in Experiment 1 (and the enhancement of the counter-mirror training effect in Experiment 2) is specific to stimulation of premotor cortex.

Although the current experiments support the involvement of premotor cortex in mirror motor facilitation, the exact route of this involvement has yet to be determined. For example, rather than following a premotor–M1–motoneuron pathway, it is possible that the effects of action observation on corticospinal excitability are the result of direct connections from premotor cortex to spinal motoneurons. In this case, the MEPs measured after the test (M1) pulse in the current experiments would measure motoneuron excitability without a concurrent change in M1 excitability. A recent study demonstrating mirror properties in pyramidal tract neurons in macaque premotor area F5 supports this possibility (Kraskov et al., 2009). However, M1 neurons have also been shown to respond to action observation (Dushanova & Donoghue, 2010), suggesting that M1 does receive action-specific input during action observation. Our current results provide evidence supporting for the first time the involvement of premotor cortex in this process. Note that if M1 does indeed have mirror-like properties, it is possible that with different intensity and/or pulse timing parameters, paired-pulse

stimulation of M1 would enhance mirror motor facilitation; however, our control experiment was not designed to test this possibility.

The present study stimulated premotor cortex contralateral to the stimulated M1 because of the practical difficulties in stimulating PMd ipsilateral to M1 (it is not possible to fit both the M1 and the PMd coils onto the head at the same time). It is therefore possible that stimulation of ipsilateral premotor cortex might have produced a different pattern of results. However, where both ipsilateral and contralateral paired-pulse stimulation of premotor cortex is possible (i.e., for PMv), previous work has found similar effects for both ipsilateral and contralateral stimulation (Buch et al., 2010; Davare et al., 2009). In addition, when the effects of contralateral PMd–M1 stimulation have been compared for left and right hemispheres, no differences were found (O’Shea, Sebastian, et al., 2007).

A related concern is that the observed hand was ipsilateral to the stimulated premotor cortex (i.e., the movements were performed by a right hand, and right premotor cortex was stimulated). It is possible that a greater mirror response would have been found if premotor cortex contralateral to the observed hand had been stimulated. However, in the one study that has specifically measured mirror neuron responses to the observation of actions performed by ipsilateral versus contralateral hands, no such effect was found. In fact, a greater number of mirror neurons responded to movements performed by the ipsilateral rather than the contralateral hand (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). This result supports the use, in the present experiment, of movements performed by the hand ipsilateral to stimulated premotor cortex. Finally, neuroimaging studies that have measured brain responses to both action observation and execution have tended to find bilateral responses, suggesting that the mirror system is not strongly lateralized (Catmur et al., 2008; Gazzola et al., 2007; Montgomery, Isenberg, & Haxby, 2007; Vogt et al., 2007; Aziz-Zadeh et al., 2006; Cunnington, Windischberger, Robinson, & Moser, 2006; Buccino et al., 2004; Koski, Iacoboni, Dubeau, Woods, & Mazziotta, 2003; Koski et al., 2002).

The present study strongly suggests that the widely studied mirror motor facilitation effect during action observation does indeed index the activation of specific mirror representations, at similar time points, in premotor cortex. In addition, it demonstrates similar modulation of mirror motor facilitation by stimulation of PMv and PMd. Finally, the finding that the same areas are involved in counter-mirror effects supports the suggestion that these effects—and mirror properties in general—arise from associative learning.

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REFERENCES

- Alaerts, K., Heremans, E., Swinnen, S. P., & Wenderoth, N. (2009). How are observed actions mapped to the observer’s motor system? Influence of posture and perspective. *Neuropsychologia*, *47*, 415–422.
- Amiez, C., Kostopoulos, P., Champod, A. S., & Petrides, M. (2006). Local morphology predicts functional organization of the dorsal premotor region in the human brain. *Journal of Neuroscience*, *26*, 2724–2731.
- Avenanti, A., Bolognini, N., Maravita, A., & Aglioti, S. M. (2007). Somatic and motor components of action simulation. *Current Biology*, *17*, 2129–2135.
- Aziz-Zadeh, L., Koski, L., Zaidel, E., Mazziotta, J., & Iacoboni, M. (2006). Lateralization of the human mirror neuron system. *Journal of Neuroscience*, *26*, 2964–2970.
- Brass, M., Schmitt, R. M., Spengler, S., & Gergely, G. (2007). Investigating action understanding: Inferential processes versus action simulation. *Current Biology*, *17*, 2117–2121.
- Buccino, G., Binkofski, F., Fink, G. R., Fadiga, L., Fogassi, L., Gallese, V., et al. (2001). Action observation activates premotor and parietal areas in a somatotopic manner: An fMRI study. *European Journal of Neuroscience*, *13*, 400–404.
- Buccino, G., Vogt, S., Ritzl, A., Fink, G. R., Zilles, K., Freund, H. J., et al. (2004). Neural circuits underlying imitation learning of hand actions: An event-related fMRI study. *Neuron*, *42*, 323–334.
- Buch, E. R., Mars, R. B., Boorman, E. D., & Rushworth, M. F. (2010). A network centered on ventral premotor cortex exerts both facilitatory and inhibitory control over primary motor cortex during action reprogramming. *Journal of Neuroscience*, *30*, 1395–1401.
- Catmur, C., Gillmeister, H., Bird, G., Liepelt, R., Brass, M., & Heyes, C. (2008). Through the looking glass: Counter-mirror activation following incompatible sensorimotor learning. *European Journal of Neuroscience*, *28*, 1208–1215.
- Catmur, C., Walsh, V., & Heyes, C. (2007). Sensorimotor learning configures the human mirror system. *Current Biology*, *17*, 1527–1531.
- Cho, Y. S., & Proctor, R. W. (2004). Influences of multiple spatial stimulus and response codes on orthogonal stimulus-response compatibility. *Perception & Psychophysics*, *66*, 1003–1017.
- Chouinard, P. A., Van Der Werf, Y. D., Leonard, G., & Paus, T. (2003). Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. *Journal of Neurophysiology*, *90*, 1071–1083.
- Cisek, P., & Kalaska, J. F. (2004). Neural correlates of mental rehearsal in dorsal premotor cortex. *Nature*, *431*, 993–996.
- Costantini, M., Galati, G., Ferretti, A., Caulo, M., Tartaro, A., Romani, G. L., et al. (2005). Neural systems underlying observation of humanly impossible movements: An fMRI study. *Cerebral Cortex*, *15*, 1761–1767.
- Cunnington, R., Windischberger, C., Robinson, S., & Moser, E. (2006). The selection of intended actions and the observation of others’ actions: A time-resolved fMRI study. *Neuroimage*, *29*, 1294–1302.
- Davare, M., Andres, M., Cosnard, G., Thonnard, J. L., & Olivier, E. (2006). Dissociating the role of ventral and dorsal premotor

- cortex in precision grasping. *Journal of Neuroscience*, *26*, 2260–2268.
- Davare, M., Lemon, R., & Olivier, E. (2008). Selective modulation of interactions between ventral premotor cortex and primary motor cortex during precision grasping in humans. *Journal of Physiology*, *586*, 2735–2742.
- Davare, M., Montague, K., Olivier, E., Rothwell, J. C., & Lemon, R. N. (2009). Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. *Cortex*, *45*, 1050–1057.
- di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: A neurophysiological study. *Experimental Brain Research*, *91*, 176–180.
- Dinstein, I., Thomas, C., Behrmann, M., & Heeger, D. J. (2008). A mirror up to nature. *Current Biology*, *18*, R13–R18.
- Dushanova, J., & Donoghue, J. (2010). Neurons in primary motor cortex engaged during action observation. *European Journal of Neuroscience*, *31*, 386–398.
- Fadiga, L., Fogassi, L., Pavesi, G., & Rizzolatti, G. (1995). Motor facilitation during action observation: A magnetic stimulation study. *Journal of Neurophysiology*, *73*, 2608–2611.
- Ferrari, P. F., Gallese, V., Rizzolatti, G., & Fogassi, L. (2003). Mirror neurons responding to the observation of ingestive and communicative mouth actions in the monkey ventral premotor cortex. *European Journal of Neuroscience*, *17*, 1703–1714.
- Gallese, V., Fadiga, L., Fogassi, L., & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain*, *119*, 593–609.
- Gazzola, V., Aziz-Zadeh, L., & Keysers, C. (2006). Empathy and the somatotopic auditory mirror system in humans. *Current Biology*, *16*, 1824–1829.
- Gazzola, V., Rizzolatti, G., Wicker, B., & Keysers, C. (2007). The anthropomorphic brain: The mirror neuron system responds to human and robotic actions. *Neuroimage*, *35*, 1674–1684.
- Heyes, C. (2001). Causes and consequences of imitation. *Trends in Cognitive Sciences*, *5*, 253–261.
- Heyes, C. (2010). Where do mirror neurons come from? *Neuroscience and Biobehavioral Reviews*, *34*, 575–583.
- Heyes, C. M., & Ray, E. D. (2000). What is the significance of imitation in animals? *Advances in the Study of Behavior*, *29*, 215–245.
- Hickok, G. (2009). Eight problems for the mirror neuron theory of action understanding in monkeys and humans. *Journal of Cognitive Neuroscience*, *21*, 1229–1243.
- Johansen-Berg, H., Rushworth, M. F., Bogdanovic, M. D., Kischka, U., Wimalaratna, S., & Matthews, P. M. (2002). The role of ipsilateral premotor cortex in hand movement after stroke. *Proceedings of the National Academy of Sciences, U.S.A.*, *99*, 14518–14523.
- Koch, G., Franca, M., Del Olmo, M. F., Cheeran, B., Milton, R., Alvarez, S. M., et al. (2006). Time course of functional connectivity between dorsal premotor and contralateral motor cortex during movement selection. *Journal of Neuroscience*, *26*, 7452–7459.
- Koch, G., Versace, V., Bonni, S., Lupo, F., Gerfo, E. L., Oliveri, M., et al. (2010). Resonance of cortico-cortical connections of the motor system with the observation of goal directed grasping movements. *Neuropsychologia*, *48*, 3513–3520.
- Koski, L., Iacoboni, M., Dubeau, M. C., Woods, R. P., & Mazziotta, J. C. (2003). Modulation of cortical activity during different imitative behaviors. *Journal of Neurophysiology*, *89*, 460–471.
- Koski, L., Wohlschlagel, A., Bekkering, H., Woods, R. P., Dubeau, M. C., Mazziotta, J. C., et al. (2002). Modulation of motor and premotor activity during imitation of target-directed actions. *Cerebral Cortex*, *12*, 847–855.
- Kraskov, A., Dancause, N., Quallio, M. M., Shepherd, S., & Lemon, R. N. (2009). Corticospinal neurons in macaque ventral premotor cortex with mirror properties: A potential mechanism for action suppression? *Neuron*, *64*, 922–930.
- Lago, A., Koch, G., Cheeran, B., Marquez, G., Sanchez, J. A., Ezquerro, M., et al. (2010). Ventral premotor to primary motor cortical interactions during noxious and naturalistic action observation. *Neuropsychologia*, *48*, 1802–1806.
- Mars, R. B., Klein, M. C., Neubert, F. X., Olivier, E., Buch, E. R., Boorman, E. D., et al. (2009). Short-latency influence of medial frontal cortex on primary motor cortex during action selection under conflict. *Journal of Neuroscience*, *29*, 6926–6931.
- Mars, R. B., Piekema, C., Coles, M. G. H., Hulstijn, W., & Toni, I. (2007). On the programming and reprogramming of actions. *Cerebral Cortex*, *17*, 2972–2979.
- Montgomery, K. J., Isenberg, N., & Haxby, J. V. (2007). Communicative hand gestures and object-directed hand movements activated the mirror neuron system. *Social Cognitive and Affective Neuroscience*, *2*, 114–122.
- O’Shea, J., Johansen-Berg, H., Trief, D., Gobel, S., & Rushworth, M. F. (2007). Functionally specific reorganization in human premotor cortex. *Neuron*, *54*, 479–490.
- O’Shea, J., Sebastian, C., Boorman, E. D., Johansen-Berg, H., & Rushworth, M. F. (2007). Functional specificity of human premotor-motor cortical interactions during action selection. *European Journal of Neuroscience*, *26*, 2085–2095.
- Press, C., Bird, G., Walsh, E., & Heyes, C. (2008). Automatic imitation of intransitive actions. *Brain and Cognition*, *67*, 44–50.
- Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: Basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*, 79–92.
- Schluter, N. D., Rushworth, M. F., Passingham, R. E., & Mills, K. R. (1998). Temporary interference in human lateral premotor cortex suggests dominance for the selection of movements. A study using transcranial magnetic stimulation. *Brain*, *121*, 785–799.
- Turella, L., Pierno, A. C., Tubaldi, F., & Castiello, U. (2008). Mirror neurons in humans: Consisting or confounding evidence? *Brain and Language*, *108*, 10–21.
- Umiltà, M. A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C., et al. (2001). I know what you are doing. A neurophysiological study. *Neuron*, *31*, 155–165.
- Urgesi, C., Candidi, M., Ionta, S., & Aglioti, S. M. (2007). Representation of body identity and body actions in extrastriate body area and ventral premotor cortex. *Nature Neuroscience*, *10*, 30–31.
- Vogt, S., Buccino, G., Wohlschlagel, A. M., Canessa, N., Shah, N. J., Zilles, K., et al. (2007). Prefrontal involvement in imitation learning of hand actions: Effects of practice and expertise. *Neuroimage*, *37*, 1371–1383.
- Wise, S. P., & Murray, E. A. (2000). Arbitrary associations between antecedents and actions. *Trends in Neurosciences*, *23*, 271–276.