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Motor facilitation of the human cortico-spinal system during observation of bio-mechanically impossible movements

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Neurophysiological and neuroimaging studies in the human and the monkey brain indicate that links between action observation and execution are much tighter than previously believed. Indeed, the mere observation of movements performed by other individuals brings about a clear increase in activity in specific fronto-parietal neural networks (mirror system). Here, we report a series of four single-pulse transcranial magnetic stimulation studies of the motor system, which show that observation of index and little finger movements brings about a facilitation of potentials recorded from muscles that would be involved in the actual execution of the observed action. Remarkably, however, a clear representational selectivity was found also during observation of bio-mechanically impossible index or little finger movements. Thus, in movement observation tasks, the human cortico-spinal system reacts similarly to the vision of bio-mechanically possible and impossible movements but it is able to detect which muscle would be involved in the actual execution of the observed movement. Importantly, this system may be more related to coding body part movements than precisely simulating their execution.

Keywords: Action observation; Bio-mechanically impossible finger movements; TMS; Mirror systems; Body image; Cortico-spinal motor excitability

Introduction

It has long been thought that cortical frontal areas are involved in motor planning and execution and posterior cortical regions in the processing of sensory inputs. Recent important neurophysiological studies in awake monkeys have shown that neurons in cortical frontal areas are not only recruited during action execution and planning but also during observation of actions performed by other individuals (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a). The discovery of these visuo-motor neurons, called "mirror neurons", has radically changed the notion of well-separate neural substrates for sensory and motor processing by suggesting that perception and action are much more tightly linked than previously believed. Based on evidence supporting the existence of mirror systems in humans (Buccino et al., 2001; Calvo-Merino et al., 2005; Cochin et al., 1999; Grafton et al., 1996; Grezes et al., 1999; Hari et al., 1998; Iacoboni et al., 1999; Rizzolatti et al., 1996b), it has been postulated that observation–execution matching systems play an important role in a number of processes ranging from observational learning to imitation and action understanding (Rizzolatti et al., 1996a, 2001).

Transcranial magnetic stimulation (TMS) studies provide evidence that action observation triggers specific facilitation of the muscles that would be involved in the actual execution of the observed movement (Fadiga et al., 1995; Strafella and Paus, 2000). This supports the notion that action execution is directly mapped onto the observer's motor system. In keeping with this view is the finding that motor facilitation induced by observation is higher for natural than unnatural hand orientations (Maeda et al., 2002) and for live acts rather than for video-clips (Jarvelainen et al., 2001). Also interesting is the result that while observation of actions performed by virtual hands activates only lateral occipital cortices with mainly sensory functions, observation of actions performed by a real hand activates a visuo-spatial network involved in action representation (Perani et al., 2001).

Relevant to the present research is that humans can "conceive" movements they cannot perform such as actions they have never learned, for example, very complex acrobatic exercises or impeccable tennis serves. It is even possible to try to imagine movements that can never be performed because of the constraints of human anatomy. Thus, knowing what to do does not always imply knowing how to do it. By using single-pulse TMS, we compared cortico-spinal neural activity contingent upon observation of bio-mechanically possible movements, commonly performed and seen in daily life, and observation of similar but bio-

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mechanically exaggerated movements that cannot be performed and seen in naturalistic contexts. We reasoned that if the mirror-matching system were strictly linked to execution, it would not be activated during observation of actions that cannot be performed. Thus, facilitation would be found only for bio-mechanically possible movements. By contrast, a comparable facilitation for bio-mechanically possible and impossible movements would indicate that human actions are coded in the cortico-spinal system regardless of whether or not they can be executed.

Materials and methods

Subjects

Eleven participants (8 women, two left handed, mean age ± SD = 30.0 ± 4.9, range 25–42) were tested in Experiment 1. Fourteen participants (one left handed, 8 women, mean age ± SD = 23.6 ± 3.1, range 19–28) took part in Experiment 2. Eleven participants (all right handed, 8 women, mean age ± SD = 26.4 ± 4.7, range 20–34) were tested in Experiment 3, and fifteen (one left handed, 11 women, mean age ± SD = 27.1 ± 6.1, range 20–46) in Experiment 4. Six right-handed participants (4 women, mean age ± SD = 24.6 ± 10.5, range 20–47) took part in Experiment 5. Manual preference was assessed by means of the standard Handedness Edinburgh Inventory (Oldfield, 1971). The local ethics committee approved the study and informed consent was obtained from all subjects prior to their participation. None of the participants had neurological, psychiatric, or other medical problems, nor had any contraindication to TMS (Wassermann, 1998). None of them complained of any discomfort or experienced any adverse effects during TMS.

Experimental apparatus

All experiments were programmed using E-prime 1.0 beta 5 software (Psychology Software Tools, Inc.) running on a PC and controlling sequence and duration of video-clips, and triggering of TMS and EMG recording.

Electromyographic recording and transcranial magnetic stimulation

Surface electromyographic recording (EMG) was performed using Ag–AgCl cup electrodes (1 cm diameter) placed in a belly-tendon montage over the motor point of the first dorsal interosseus (FDI) in Experiments 1–4, abductor digiti minimi (ADM) in Experiments 1–3, and extensor indicis proprius (EIP) in Experiment 4. The EMG signal was recorded and amplified by a Viking IV D electromyograph (Nicolet Biomedical, U.S.A.). The band pass filter was set between 30 Hz and 2.5 kHz. The sampling rate of the MEP signal was fixed at 10 kHz. On each trial, EMG recording began 50 ms before the TMS pulse. EMG signals from both muscles were displayed in two high-sensitivity channels that could detect deviations of baseline of 50 μV. This procedure allows to detect any unwanted muscle contraction prior TMS pulses. Moreover, EMG signals were sent to loudspeakers. Thus, by using the EMG, auditory feedback subjects were able to avoid background electromyographic activity during the experiment. In Experiment 5, EMG was simultaneously recorded from FDI, ADM, and EIP during the execution of the three types of movements observed in Experiments 1–4. EMG signal was amplified at a gain of 1000× by a Digitimer D360 amplifier (Digitimer, Hertfordshire, England), band-pass filtered (20 Hz–2.5 kHz), and digitized by a CED Power 1401 controlled by means of the Spike2 software (Cambridge Electronic Design, Cambridge, England). The sampling rate was set at 333 Hz.

Focal TMS was performed with a figure-eight-shaped stimulation coil (outer diameter of each wing 9 cm), connected to a Magstim 200 Rapid® (Magstim, Whitland, Dyfed, UK). The coil was placed tangentially to the scalp, with the handle pointing backward and laterally at 45° away from the midline, approximately perpendicular to the line of the central sulcus. This orientation induced a posterior–anterior current in the brain. It was chosen based on the finding that the lowest motor threshold is obtained when the induced electric current in the brain is flowing approximately perpendicular to the central sulcus (Brasil-Neto et al., 1992; Mills et al., 1992).

In all experiments, the coil was placed on the left motor cortex in correspondence with the optimal scalp position (OSP), defined as the position from which MEPs with maximal amplitude were recorded. The OSP was detected by moving the intersection of the coil in 1-cm steps around the motor hand area of the left motor cortex and by delivering TMS pulses with constant intensity. During the recording session, the magnetic stimulus was delivered in correspondence with the OSP, at 130% of the resting motor threshold (rMT). The rMT was defined as the lowest stimulus intensity able to evoke MEPs with an amplitude of at least 50 μV in at least five out of ten trials. Since MEPs were simultaneously recorded from two muscles in all experiments, OSP and rMT were determined by using the higher threshold muscle, namely, ADM in Experiments 1–3 and EIP in Experiment 4. This procedure allowed to have clear and stable MEPs from the targeted muscles. Both MEP latency and amplitude were collected and stored on a computer for off-line analysis. Since no significant difference in latency in the different observation conditions was observed, this parameter will not be further considered. In order to control for the influence of spinal cord and peripheral nerve and muscle activity during movement observation, we recorded both F- and M-wave amplitudes. F-wave is considered an index of spinal cord excitability and M-wave an index of nerve and muscle excitability (Eisen, 1987; Kimura, 1993; Mercuri et al., 1996). F- and M-waves were evoked by supra-maximal electric stimulation of the ulnar nerve at the wrist (square wave pulses, duration 0.1 ms) and were recorded from FDI and ADM muscles (Experiment 2).

Experimental stimuli and procedure

In all observation conditions, different video-clips were presented on a 14-in. screen located at a distance of 80 cm from the subjects. Participants observed sequences of abduction/adduction movements of the right index finger (Experiment 1), the right little finger (Experiment 2), and the extension/flexion movement of the right index finger (Experiments 3 and 4). Based on the angular displacements of the fingers, movements were defined as bio-mechanically possible or impossible. Table 1 reports the range of angular displacement of the moving fingers for each of the four observational experiments. Video-clips with bio-mechanically impossible movements were constructed by changing the position of the target finger (index in Experiments 1, 3, 4 and the little finger in Experiment 2). Presentation of series of single frames (with the target finger in different angular displacement positions)

Table 1
Angular displacement (in degrees) of the movements seen in the different observation conditions

	Possible	Impossible
Index finger abduction/adduction (Exp. 1)	0–45	60–120
Little finger abduction/adduction (Exp. 2)	0–35	45–90
Index finger extension/flexion (Exp. 3–4)	0–45	110–160

Range of angular displacements (in degrees) of the three types of movements observed in the four TMS experiments. The bio-mechanically possible movements can be easily performed and likely to be often seen in daily life. By contrast, the bio-mechanically impossible movements cannot be performed and seen in naturalistic contexts.

each lasting 33.2 ms, at a frequency of 30.1 Hz, allowed to obtain the animation effect. Care was taken that in bio-mechanically impossible movement videos, all the angular displacements were clearly beyond the natural limits of the metacarpo-phalangeal joint (see Table 1).

Bio-mechanically possible and impossible movements were presented in separate blocks. A woman's hand was used in Experiment 1 and a man's hand in Experiments 2–4. Each video-clip was presented for 3 s. TMS stimuli were delivered at randomized intervals between 1500 and 2500 ms after the onset of the videos. In Experiment 1, there was a block in which subjects kept their eyes closed; in Experiment 2, a block in which participants observed an expanding–contracting circle. Frequency of expansion–contraction was comparable to that of the different finger movements (about 1 Hz). Observational blocks of a still hand were used in Experiments 1, 3, and 4. The order of the different video-clips was counterbalanced across subjects. Subjects were seated on a comfortable armchair in a dimly illuminated room with their right hand resting on a pillow. They were instructed to keep their hands still and to completely relax their muscles. Subjects were also instructed to pay attention to the stimuli presented on the screen and were informed that at the end of the experimental session they would have to answer questions about the videos. In all experiments, after each block, questions were asked about the handedness and gender of the hand stimuli, the order of presentation of the four blocks, and the spatial location of the hand on the monitor. No overt response was required at any time during stimuli presentation and data collection. In each block, 15 MEPs were collected simultaneously from two muscles. While TMS delivered for 15 min with a 0.9-Hz frequency may induce a clear decrease of MEP amplitude, TMS delivered for 1 h at 0.1 Hz frequency did not induce any change of excitability (Chen et al., 1997). Thus, to avoid any influence of TMS on MEPs modulation contingent upon movement observation, we used an inter-trial interval of about 10 s.

Importantly, after TMS session, participants were asked to score along a five-point Likert scale whether the presented image bothered them. A score of 0 indicated no aversion and a score of 4 indicated maximal effects.

In Experiment 5, subjects performed the three types of possible movements observed in Experiments 1–4, in three different blocks. Fifteen cycles of three movements were performed in separate blocks, one for each type of movement (index abduction/adduction, little finger abduction/adduction, or index extension/flexion). The order of the three blocks was counterbalanced across subjects. The angular displacement during execution was kept identical to that of the observation conditions (Table 1, left column). This procedure allowed to maximize analogies between execution (Exp. 5) and observation (Exp. 1–4) experiments in which subjects saw three movement cycles.

Statistics

In all experiments, peak-to-peak MEP amplitudes from each muscle (FDI, ADM, or EIP) were analyzed by a series of repeated measures analyses of variance (ANOVAs) with observation conditions as main factor. The main factor had four levels in Experiment 1 and three in Experiments 2, 3, and 4. M-wave (mV) and F-value amplitudes (expressed as percentage of the M-wave) recorded in Experiment 2 were submitted to repeated measures ANOVAs with muscle (FDI and ADM) and condition (expanding circle, possible and impossible movements) as main factors. Post hoc multiple comparisons were performed by using the Duncan test. Subjective aversion scores induced by observation of bio-mechanically possible and impossible movements were compared by means of paired t tests.

In Experiment 5, raw maximal EMG amplitudes (MEA) in each trial were computed. For each muscle, a grand average of MEA during execution of the three types of movements was computed and was used as baseline. For each muscle, raw MEA were normalized by using the following formula: (raw MEA − grand average MEA) * 100. This procedure allowed to carry out a 3 (muscle: FDI, ADM, or EIP) × 3 (type of movement: index abduction/adduction, little finger abduction/adduction, index extension/flexion) ANOVA with repeated measures. Post hoc multiple comparisons were performed by using the Duncan test.

Results

Experiment 1

We first sought to determine whether the possible modulation of cortico-spinal excitability contingent upon the mere observation of actions was present not only for possible but also for impossible movements. In the first experiment, motor evoked potentials (MEPs) to TMS of the cortical site representing first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles were recorded while subjects observed three different types of video-clips. Images of the dorsal surface of a still right hand were presented in one observation block. In the other two, subjects observed sequences of abduction/adduction movements of the right index finger with angular displacements within (possible) or outside (impossible) the physiological range of the metacarpo-phalangeal joint. It is important to note that FDI would be crucially involved if abduction/adduction movements of the index finger were actually performed. By contrast, the ADM muscle does not play any role in the observed index finger movement. A condition where participants kept their eyes closed was also performed. Analysis of MEP amplitudes recorded from FDI showed the significance of the main factor observation condition [$F_{(3,30)} = 8.77$, $P = 0.0002$]. Post hoc comparisons showed that MEP amplitude in the no observation (1.52 ± SEM 0.41 mV) and observation of still hand (1.41 ± SEM 0.29 mV) conditions differed significantly from MEP amplitude in the observation of possible (2.04 ± SEM 0.45 mV) and impossible movements (2.30 ± SEM 0.41 mV) (P values ≤ 0.01; Fig. 1A, left part) which in turn did not differ from one another. By contrast, analysis of MEP amplitudes recorded from ADM were comparable in all observation conditions [$F_{(3,30)} = 1.31$, $P = 0.29$] (Fig. 1A, right part).

Aversion scores significantly different from 0 were obtained only during observation of impossible abduction/adduction movements of the index finger [mean 0.81 ± SEM 0.18, $t(10) = 4.5$, $P = 0.001$]. Thus, results clearly show a selective motor facilitation of the muscle that would actually be involved in executing the observed movement. Remarkably, facilitation was comparable for possible and impossible movements. It is relevant that neurons in the monkey primary motor cortex responsive to moving stimuli in the absence of any overt movement have recently been described (Merchant et al., 2004a,b). The facilitation reported in Experiment 1 occurred in the two dynamic conditions. Thus, it may be that facilitation is related to activation of neural substrates mapping moving objects instead of actions. Although the somatotopy of the effect speaks against this possibility, a second experiment (Experiment 2) was carried out in which a non-corporeal moving stimulus was also used. Moreover, to further qualify the specificity of the facilitation effect, video-clips of abduction/adduction movements of the little finger were used to rule out that the modulation of FDI found in Experiment 1 could be ascribed to its larger representation in the motor cortex.

Experiment 2

MEPs were recorded from both FDI and ADM muscles during observation of blocks of possible and impossible movements of the right little finger. Importantly, this movement relies almost entirely upon activation of ADM while FDI has no role. Crucially, subjects were also asked to observe a non-corporeal moving stimulus, namely, an expanding–contracting circle. Analysis of MEP amplitudes recorded from ADM (Fig. 1B, right part) showed the significance of the main effect observation condition [$F_{(2,26)} = 7.18$; $P = 0.003$]. Post hoc comparisons showed significantly lower MEP amplitude during observation of the expanding circle (0.82 ± SEM 0.19 mV) with respect to observation of possible (1.07 ± SEM 0.26 mV; $P < 0.05$) and impossible movement conditions (1.29 ± 0.30 mV; $P = 0.001$). MEP amplitudes recorded from FDI

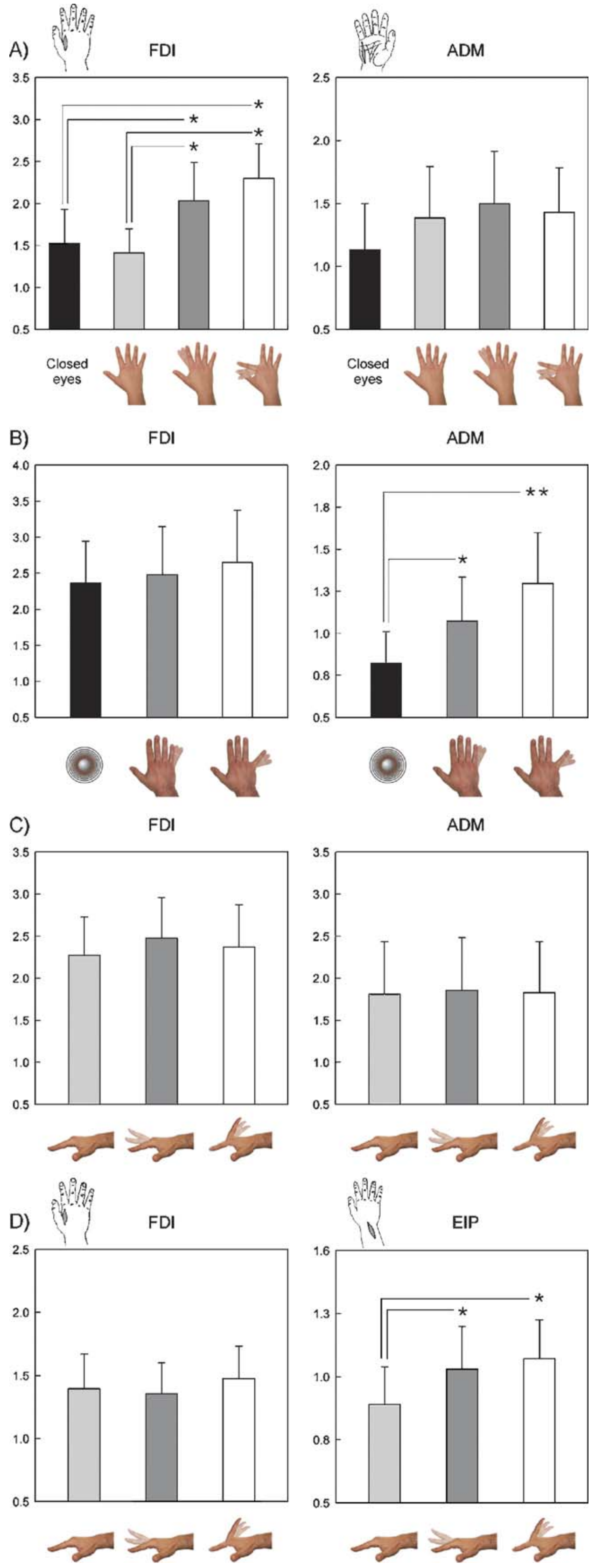

Fig. 1. (A) MEP amplitudes recorded from FDI (left part) and ADM (right part) muscle in the three observation conditions and in the eyes-closed condition. In this experiment, abduction/adduction movements of the index finger were observed. Modulation was found only for the FDI, the muscle actually involved in executing the observed movement. (B) MEP amplitudes recorded from FDI (left part) and ADM (right part) muscle in three observation conditions. In this experiment, abduction–adduction movements of the little finger were observed. Modulation was found only for the ADM, the muscle actually involved in executing the observed movement. The first column refers to observation of a contracting–expanding circle. (C) MEP amplitudes recorded from FDI (left part) and ADM (right part) muscle in the different observation conditions. In this experiment, possible and impossible extension/flexion movements of the index finger were observed. Interestingly, no modulation of MEP amplitude was found. (D) MEP amplitudes recorded from FDI (left part) and EIP (right part) muscle in the different observation conditions. In this experiment, possible and impossible extension/flexion movements of the index finger were observed. Modulation was found only for the EIP, the muscle that, as shown in Experiment 5, is maximally involved in actual execution of the observed movement. The different MEP amplitudes recorded from FDI during observation of index finger extension/flexion in Experiments 3 and 4 are likely to be due to inter-individual variability. Legend: Mean (±SEM) values in mV. $*P \leq 0.05$; $**P \leq 0.001$.

were comparable in all observation conditions [$F_{(2,26)} = 1.02$, $P = 0.37$] (Fig. 1B, left part). Impossible abduction/adduction movements of the little finger were scored as more aversive than observation of the same possible movement [1.64 ± SEM 0.16 vs. 0; $t(13) = 9.07$, $P \leq 0.0001$]. Results of Experiment 2 confirm the modulation of MEP amplitude found in Experiment 1 and indicate that the effects cannot be attributed to low-level cues provided by a moving stimulus but are specific for body actions.

In order to explore the neural levels at which the observed facilitation may have occurred, indices of spinal cord (F-wave) and nerve and muscle (M-wave) excitability were also collected (see Materials and methods). No changes in F- or M-wave amplitudes were found (Table 2) thus suggesting that the neural modulation during action observation likely originates upstream with regard to the spinal cord.

Experiment 3

We further assessed the specificity of the MEP facilitation found in Experiments 1 and 2 by recording MEPs from FDI and ADM during observation of both possible and impossible extension/flexion movements of the index finger. Three observation conditions (still hand, possible movement, impossible movement) were carried out. It is important to note here that FDI had little or no involvement and ADM no involvement at all in the actual execution of the observed movement. Thus, if facilitation were based on the functional involvement of a given muscle in the observed action, little or no MEP facilitation should have been found. Fig. 1C shows that the three observation conditions did not differ either for FDI [$F_{(2,20)} = 0.53$, $P = 0.60$] or for ADM [$F_{(2,20)} = 0.16$, $P = 0.85$].

Bio-mechanically impossible extension/flexion movements of the index finger were scored as more aversive than observation of the same possible movement [mean 2.9 ± SEM 0.16 vs. 0; $t(10) = 17.88$, $P \leq 0.0001$]. It is relevant that, despite the high aversion effect induced by observation of impossible movements, no MEP facilitation was found in this experiment. Thus, aversion per se does not imply any specific MEP facilitation.

Experiment 4

To further assess the degree of specificity of the facilitation effect, we carried out an experiment with the same observational conditions as Experiment 3. The main difference was that MEPs were recorded not only from FDI but also from extensor indicis proprius (EIP), a forearm muscle which is likely to be massively involved in finger extension movements.

Analysis of MEP amplitudes recorded from EIP showed the significance of the observation condition [$F_{(2,28)} = 4.52$, $P = 0.019$] (Fig. 1D, right part). Post hoc comparisons showed the following: observation of still hand (0.88 ± SEM 0.14 mV) significantly differed from observation of possible (1.03 ± SEM 0.16 mV) and impossible (1.07 ± SEM 0.15 mV) movements (P values were 0.03 and 0.01, respectively), which in turn did not differ from one another. By contrast, analyses of MEP amplitudes recorded from FDI (Fig. 1D, left part) were comparable in all observation conditions [$F_{(2,28)} = 0.66$; $P = 0.52$] thus confirming the pattern of results obtained in Experiment 3.

Bio-mechanically impossible extension/flexion movements of the index finger were scored as more aversive than observation of the same possible movements [mean 2.84 ± SEM 0.14 vs. 0; $t(14) = 19.34$, $P \leq 0.0001$].

Experiment 5

Results from Experiments 1–4 would suggest that facilitation effects are specific for the muscles that would be maximally involved in actual execution of the observed movements. To assess that this was actually the case, we carried out an experiment in which EMG activity of FDI, ADM, and EIP muscles during execution of the three types of possible movements observed in Experiments 1–4 was recorded.

Normalized maximal EMG amplitudes for each muscle and each type of movement are reported in Fig. 2. Moreover, raw EMG amplitude of a representative trial in a representative subject is shown in Fig. 3.

The 3 (muscle: FDI, ADM, or EIP) × 3 (type of movement: index abduction/adduction, little finger abduction/adduction, index extension/flexion) ANOVA on normalized Maximal EMG amplitudes showed the significance of the interaction [$F_{(4,20)} = 25.94$; $P < 0.00001$]. Post hoc comparisons showed the following: the FDI muscle was significantly more active during index finger abduction/adduction than during little finger abduction/adduction ($P = 0.0006$) and index finger extension/flexion (0.004). The ADM muscle was significantly more active during little finger abduction/adduction than during index finger abduction/adduction ($P = 0.001$) and index finger extension/flexion ($P = 0.001$). The EIP muscle was significantly more active during index finger extension/flexion than index finger abduction/adduction ($P = 0.01$) and little finger abduction/adduction ($P = 0.03$) (see Fig. 2). In a similar vein, the rather specific relation between type of movement and the activated muscle is confirmed by the following: during index ab/adduction, FDI was more active than ADM ($P = 0.001$) and EIP ($P = 0.01$); during little finger ab/adduction, ADM was more active than FDI ($P = 0.001$) and EIP ($P = 0.007$); during index extension/flexion, EIP was significantly more active than FDI ($P = 0.02$) and ADM ($P = 0.001$).

Discussion

The main result of the present study is that action observation selectively facilitates the cortico-spinal system when

Table 2

M- and F-wave mean amplitudes (±SEM) during the three conditions and for each muscle

	M-wave			F-wave		
	EC	PM	IM	EC	PM	IM
FDI	17.86 ± 1.2	17.84 ± 1.2	17.76 ± 1.2	0.62 ± 0.3	0.53 ± 0.2	0.58 ± 0.2
ADM	15.53 ± 0.6	15.63 ± 0.6	15.57 ± 0.6	1.21 ± 0.9	1.20 ± 0.7	1.22 ± 0.8

Mean values (±SEM) for M- (mV) and F-waves (in % of M-waves). Legend: EC = observation of expanding circle; PM = observation of bio-mechanically possible movement; IM = observation of bio-mechanically impossible movement.

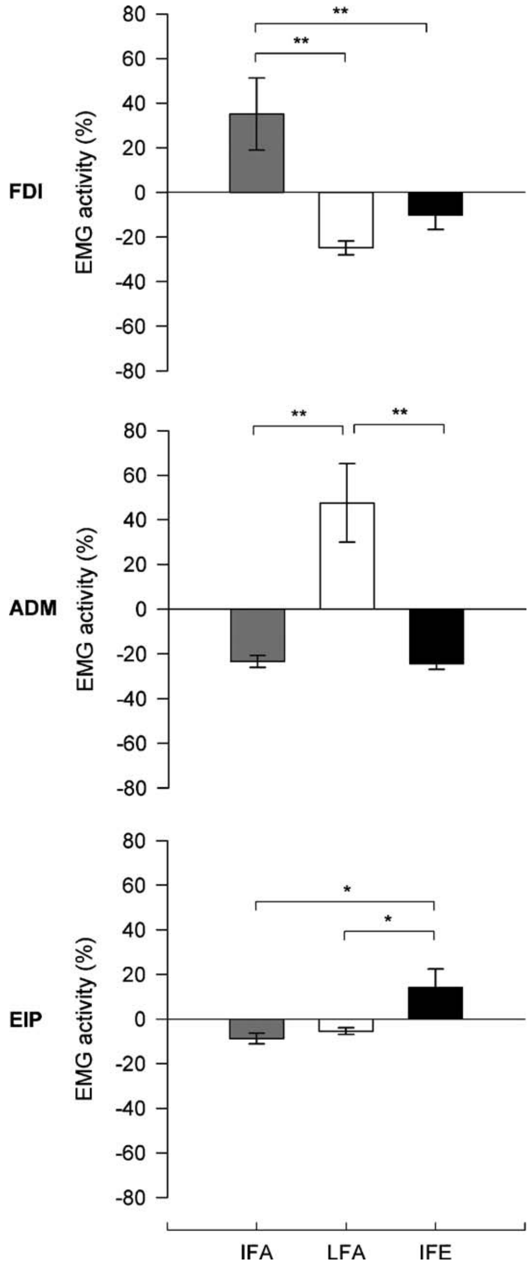

Fig. 2. Normalized EMG amplitude values (means in % of deviation from the baseline ± SEM) for each muscle (rows) and each type of performed movement (columns). Positive deviations from the baseline indicate higher EMG activity. The higher EMG amplitude of a given muscle indicates its preferential involvement in a given type of movement. Legend: $*P \leq 0.05$; $**P \leq 0.001$. IFA = index finger abduction/adduction, LFA = little finger abduction/adduction, IFE = index finger extension/flexion.

viewing possible movements or movements that being well beyond the normal range of joint mobility, are bio-mechanically impossible.

Experiments 1 and 2 showed a selective motor facilitation of the muscle that would be involved in actual execution of the observed movement. Importantly, the facilitation was comparable for possible and impossible movements. Experiments 3 and 4 further qualified the facilitation effect. The type of observational somatotopy appears to be even finer than previously held. Indeed, the observational facilitation was absent in Experiment 3 in which there was no direct role of the recorded muscles in the actual execution of the observed movements. In the same vein, in Experiment 4, the facilitation occurred only for EIP muscle, which although probably less represented in the cortico-spinal system than FDI and ADM, is crucially involved in the actual performance of the observed action. Importantly, the highly specific somatotopy of the facilitation effect is not based on the functional relevance of a given muscle in the cortico-spinal system but on its dynamic role in coding the observed action. TMS evidence supports the notion that hand muscles are more heavily represented in the cortico-spinal system than forearm muscles (Krings et al., 1998). Yet, facilitation of FDI, which is a functionally important and well-represented muscle, was not always found during the observation of moving index finger. Rather, facilitation of both hand (FDI or ADM) and forearm muscles (EIP) was found for observed movements that would activate the corresponding muscle if actually performed.

This result significantly extends previous work on the specificity of observational mapping by suggesting that observation-related motor facilitation does not derive from coding muscles per se but from coding the role played by each muscle in a given action. Although it has been speculatively suggested that facilitation of MEPs during motor observation reflects neural processing carried out in higher-order motor areas that are part of the mirror circuit (Fadiga et al., 1995), single-pulse TMS studies provide information on the functional status of the neural pathway comprised between the primary motor cortex and the spinal cord. Unlike paired-pulse TMS (e.g., Strafella and Paus, 2000), single-pulse TMS does not specifically locate the observational facilitation to the primary motor cortex. It is thus likely that the motor facilitation contingent upon action observation found in the present and in previous TMS studies (Fadiga et al., 1995; Gangitano et al., 2001) is related to an increase of activity in the cortico-spinal system. Analysis of the H-reflex seems to indicate that neural activity in the spinal cord may be either inhibited as well as excited depending upon the phases of the observed movement (Baldissera et al., 2001). The absence of modulation of F- and M-waves (Experiment 2) would suggest that the facilitation of MEP amplitude during observation of bio-mechanically possible and impossible movements found in the Experiments 1, 2, and 4 likely originates upstream with regard to the spinal cord.

A number of recent studies support the notion that action understanding is based on a direct “mirror” matching of the seen action in the observer’s motor system rather than on the pure visual analysis of the elements that form the observed action (Rizzolatti et al., 2001). Indeed, behavioral studies indicate that action observation directly influences execution of one’s own actions (Brass et al., 2001; Craighero et al., 2002).

There is clear evidence that the primary motor cortex in humans and monkeys is involved in much more complex functions than previously believed. The primary motor cortex, for example, may play a crucial role in somatic perception of limb movement (Naito et al., 2002) or in the coding of complex postures (Graziano et al., 2002). Relevant to the present discussion is that the illusion of limb movement contingent upon a frequency-specific vibration of muscle tendons can induce the feeling that limbs are entering a table or that impossible angular displacements are reached. Interestingly, this type of illusion seems to originate in the primary motor cortex (Naito et al., 1999).

The increase of MEP amplitude contingent upon observation of specific actions has been interpreted as neurophysiological proof of

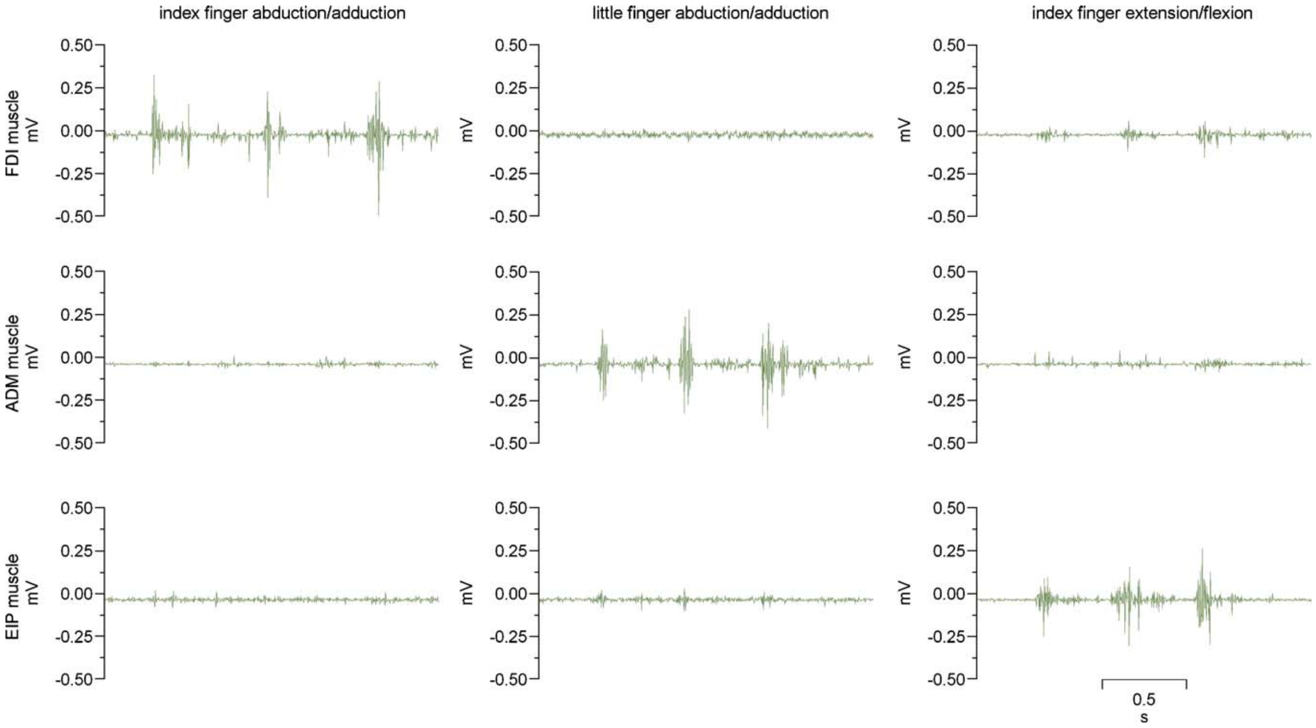

Fig. 3. EMG activity recorded from three muscles (FDI, ADM, EIP) during execution of the three types of possible movements observed in Experiments 1–4 (data from a typical trial of a representative subject). The higher EMG amplitude of a given muscle indicates its preferential involvement in a given type of movement. Note that to match observation and execution conditions, triplets of movements were performed on each trial. Time is plotted on abscissa and EMG amplitude (in mV) on the ordinate axis.

the existence of a mirror system in humans that matches the observed action onto the observer's motor cortex (Fadiga et al., 1995; Rizzolatti and Craighero, 2004). A novel and somewhat unexpected result of our study is that observational mapping in the human cortico-spinal system is similar for bio-mechanically possible and impossible movements. This effect is puzzling because it de-emphasizes the role of action observation in action execution and learning. Indeed, the impossible movements presented in our study can never be learned and performed. Yet, their observation brings about a clear cortico-spinal facilitation. This result seems to contradict the PET study that investigated perception of movement paths in an apparent motion task with human figures as presented objects (Stevens et al., 2000). Two pictures of static lateral views of a human figure, which differed only because the right upper limb was placed in front of or behind the right lower limb, were presented. Short (350 ms) or long (750 ms) stimulus onset asynchronies (SOAs) were used. While at long SOAs subjects reported that the arm moved around the lower limb (possible path), short SOAs presentations induced the perception that the upper limb moved through the lower limb (biologically impossible path). Activation of visual areas concerned with analysis of moving stimuli was found during perception of both possible and impossible paths of movement; by contrast, primary motor areas were activated only during perception of possible paths thus suggesting that these regions process actions which conform to the capabilities of the observer (Stevens et al., 2000). One way of accounting for this discrepancy is that, even though angular displacements were clearly beyond the limits of the metacarpo-phalangeal joint, subjects may have coded them as an exaggeration of possible movements. It is important to emphasize, however, that no previous TMS study assessed the facilitation effect of observing possible and impossible movements and thus, no direct comparison with our study can be drawn. An alternative, somewhat counterintuitive, explanation is that even impossible movements are coded in the frontal mirror system as human actions that can be learned through practice. At any event, even the most parsimonious interpretation of our results implies that excitation of the human cortico-spinal system contingent upon action observation is related more to coding of the specificity of moving body parts than to a detailed reading of the kinematics of the observed movement in terms of its biological plausibility. Therefore, computations about whether an action can actually be performed or learned are probably carried out in neural structures different from the motor cortical pathway upstream with regard to the spinal cord, which is explored in our research. This view is supported by a magnetoencephalographic study reporting that neural activity in extrastriate occipital areas was higher during observation of distorted than natural postures (Avikainen et al., 2003). Also relevant is a study in which oscillatory activity in the gamma band was measured during observation of images of point-light displays depicting recognizable, upright walkers and non-recognizable, inverted walkers. Only recognizable stimuli evoked activity in parietal and temporal regions thus indicating that analysis of plausibility is carried out in non-motor regions (Pavlova et al., 2004). Therefore, we hypothesize that relevant information on the bio-mechanical plausibility of the observed action derives from the sensory (e.g., parietal structures) more than from the motor nodes of the mirror circuits underlying action observation. Obviously, TMS is not adept to disclose the entire neural network underlying the effects of observing actions. However, our suggestion is supported by an fMRI study showing a significantly higher parietal activation (in BA areas 7 and 40) during observation of bio-mechanically impossible than possible movements (Costantini et al., 2005).

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