# Bottom-Up and Top-Down Visuomotor Responses to Action Observation

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Action observation produces automatic "mirror" responses in the observers' motor system. However, in daily life, nonimitative actions are often required to be produced in response to others' acts, generating a conflict between automatic and voluntary responses. First, we used single-pulse transcranial magnetic stimulation (TMS) to assess the temporal dynamics of motor output in healthy volunteers preparing rule-based counter-imitative motor responses cued by different observed hand movements. Second, we applied the same paradigm after 1-Hz repetitive TMS (rTMS) of the left posterior parietal cortex (PPC) and of the left dorsolateral prefrontal cortex (dlPFC). The results showed an early (150 ms from onset of visual stimuli) stimulus-driven mirror response that was followed by a later (300 ms) rule-based nonmirror response. rTMS applied to the PPC modulated only the early mirror response. Conversely, rTMS to the dlPFC modulated specifically the late rule-based motor response. The data indicate that a fast bottom-up process mediated by the dorsal visual stream produces automatic imitative responses. Arbitrary rule-based visuomotor associations are on the contrary mediated by a slower system, relying on the prefrontal cortex. The 2 systems are mutually independent and compete for motor output in socially relevant situations only at a distal level.

Keywords: automatic, imitation, mirror, prefrontal, transcranial magnetic stimulation

## Introduction

The presence in an observer's visual field of a person acting upon an object triggers an automatic motor response similar to the observed one. This phenomenon, "motor mirroring," is thought to rely on the bottom-up activation of a cortical pathway along the dorsal visual stream (Cattaneo and Rizzolatti 2009). It can therefore be assimilated to other bottom-up visuomotor associations, occurring along parallel parieto-frontal systems such as the "visual grasp reflex," that, the saccade that positions over the fovea the projection of any salient visual feature appearing in the peripheral visual field (Theeuwes et al. 1998) or the "affordance" effect, that is, the grasp-related motor program that is triggered by the vision of the geometry of a graspable object (Gibson 1979; Tucker and Ellis 2004; Fischer and Dahl 2007; Buccino et al. 2009).

Everyday behavioral situations however demand that an individual responds to environmental stimuli with motor acts different from those preprogrammed in automatic visuomotor associations. Converging evidence in nonhuman primates indicates that this conflict is solved by activation of a neural route to action that is separate from, and parallel to, the bottom-up one. Physiological data clearly indicate that 2 different neural pathways encode bottom-up automatic visuomotor behavior (Rizzolatti et al. 1998) and top-down rule-based visuomotor associations (Tanji et al. 2007; Wise 2008). Anatomical studies in monkeys confirm the existence of a parieto-premotor pathway and a prefronto-premotor pathway that mediate bottom-up and top-down behavior, respectively, (Rizzolatti and Luppino 2001). In humans, psychological research has consistently shown the conflict between arbitrary and automatic visuomotor associations, leading to a dual route model of the control of sensorimotor behavior (reviewed in Kornblum et al. 1990; Hommel et al. 2002; McBride, Boy, et al. 2012). Such view was strongly corroborated by functional neuroimaging data (Passingham and Toni 2001). In humans, perhaps the strongest argument in favor of a dual route to action control comes from neuropsychological descriptions of prefrontal lesions resulting in failure of the executive system and full release of automatic interactions with the surrounding world, as for example in the "anarchic hand" syndrome (Marchetti and Della Sala 1998).

In the domain of motor mirroring, the interaction between automatic and voluntary behavior is poorly defined. One mainstream hypothesis on how the brain solves the conflict between automatic mirror tendencies and rule-based nonmirror behavior is that of fast reconfiguration of the visual tuning of mirror neurons (Catmur et al. 2007), thereby eliminating 1 of the 2 elements of conflict. We propose an alternative possibility, based on the dual-route model. Accordingly, a brief sensorimotor experience is not capable of reconfiguring in the short term the visuomotor associations that commonly produces imitative tendencies. On the contrary, the capacity of producing nonimitative behavior, at least in the short term, is supported by a neural route separate from that of automatic mirroring. A brief counter-imitative training would recruit this second route, leaving functionally unaltered the "mirror" route.

One hallmark of a dual route to action is the occurrence of response competition between automatic tendencies and rule-based responses. In particular, such competition can become evident as a biphasic pattern of motor output, with an initial tendency toward the automatic response (between 100 and 300 ms from visual stimuli, varying according to the sensory modality) and a later implementation of the arbitrary rule. This biphasic pattern becomes overt in the form of the so-called "partial errors" (Coles et al. 1995) that are produced in conflictual stimulus-response conditions. Alternatively, it can be demonstrated as a covert motor program, by means of any high-temporal resolution physiological measures of motor output such as electromyography (EMG), electroencephalography, dynamometry or motor evoked potentials (MEPs) to transcranial magnetic stimulation (TMS). The orderly alternation of early automatic and late voluntary responses seems to be pervasive to all domains of visuomotor behavior. It has been observed during visual search tasks (van Zoest and Donk 2006), during object-directed hand movements (Goslin et al. 2012) and during spatially oriented hand movements (Michelet et al. 2010). Depending on the visuomotor tasks, the processes responsible for the early and the late responses have received

the most disparate nomenclatures. It has been referred to as "stimulus-driven," "bottom-up," "automatic," "exogenous," or "the what system." Conversely, the process responsible for the late response has been defined as "goal-driven," "top-down," "voluntary," "executive," or "the how system" (Theeuwes et al. 1998; Passingham and Toni 2001; Siebold et al. 2011; McBride, Boy, et al. 2012; McBride, Sumner, et al. 2012; Barchiesi and Cattaneo 2013).

One recent work has demonstrated a similar biphasic pattern in the domain of motor responses to others' actions (Barchiesi and Cattaneo 2013). The authors asked participants to observe a hand performing an action and to implement a counter-imitative rule, that is, to perform an action opposite to, and mutually incompatible with, the observed one. As an after-effect of learning such counter-imitative training, participants exhibited an early (250 ms from video onset) pattern that was essentially imitative of the observed movement and a late (>300 ms from stimulus onset) pattern that was compatible with the arbitrary counter-imitative rule. These data only showed the aftereffect of the stimulus-response conflict and were potentially biased by a spatial compatibility effect. We therefore decided in the present work to test the automatic-voluntary conflict in motor mirroring during the actual implementation of a conflicting visuomotor task with nonspatially defined stimuli. In a second experiment, we explored with repetitive TMS (rTMS) the role of the putative human anterior intraparietal area (phAIP) and of the dorsolateral prefrontal cortex (dlPFC) in generating automatic and rule-based responses. The reason for choosing the phAIP is that, in models of the action observation network, related to hand movements, this is the node that receives visual information from the occipito-temporal cortex and relays it to the premotor cortex (Cattaneo et al. 2010; Arfeller et al. 2013). For a review, see Cattaneo and Rizzolatti (2009). It is therefore an obligatory passage upstream of the motor cortex in the bottom-up circuit. The dlPFC was chosen as a target area because it is a core node of the prefrontal executive system that selects different possible motor responses according to arbitrarily defined visuomotor rules (Passingham and Toni 2001; Toni, Ramnani, et al. 2001; Bunge et al. 2002, 2003). We preferred not to target the premotor cortex in the present study because it is a potential point of convergence of the voluntary and the automatic systems (Boussaoud et al. 1995).

## Materials and Methods

### General Experimental Design
In the present work, we investigated with single-pulse TMS (spTMS), delivered to the motor cortex, the covert modulation of motor cortex excitability while participants performed a delayed cued counter-imitative task, that is, they watched a hand performing 1 of 2 possible movements, wait for a GO-signal and perform the one movement that had not been performed by the observed hand. The early part (0–300 ms) of the delay period between the cue and the GO-signal was probed with spTMS at different time intervals. In this way we assessed how the observers' motor system was covertly modulated by the observation of the cue movement and by the implementation of the counter-imitative rule.

Two different experiments were performed. In Experiment 1, we explored with spTMS the excitability of the participants' motor cortex at several time intervals after movement onset in order to define with high-temporal resolution the time-course of stimulus-locked covert motor activity. In Experiment 2, we investigated the brain circuitry that

is associated with the covert motor modulation demonstrated in Experiment 1. Experiment 2 was divided into 2 subexperiments with 2 different groups of participants that were tested over 2 different brain regions. In one subgroup, offline 1-Hz rTMS was applied to the dlPFC and, in the other subgroup, 1-Hz rTMS was applied to the putative human homolog of the phAIP of the macaque (Rizzolatti et al. 1998). After rTMS, the modulation of the motor cortex in the counter-imitative task was assessed similarly to Experiment 1.

### Experiment 1

#### Participants and Experimental Design
Twenty-one healthy participants (5 males, mean age 22 years) took part in the experiment, which was approved by the Ethical Committee of the University of Trento and was conducted in compliance with the revised Helsinki declaration (World Medical Association General Assembly 2008). All participants gave written informed consent to the experiment and were screened for contraindication to TMS (Rossi et al. 2009). We tested all participants during a session of counter-imitative training as defined in (Catmur et al. 2007) and in (Barchiesi and Cattaneo 2013). They were required to move their little finger when they saw an index finger moving and vice versa. In every trial, participants saw the biological stimulus and then waited for spTMS over the motor cortex which served as GO-signal to perform the response. SpTMS was delivered at different interstimulus intervals (ISIs), that is, at 0, 100, 150, 200, 250, and 300 ms from the onset of the stimulus. MEPs to spTMS were recorded and were the source of the main dependent variable after data processing. The experimental setup and trial structure are shown in Figure 1.

#### Trial Structure
Stimuli were presented using E-Prime 2.0 software on a 22 inches monitor with a refresh rate of 60 Hz. Trials were 5-s long and started with a white fixation cross on a black background for 1000 ms. Participants were then shown a still frame depicting the dorsum of a hand oriented horizontally but with fingers pointing either leftward or rightward for 1000 ms which was followed by another still frame of the same hand that was touching an object with the index finger or with the little finger for 1500 ms. The quick succession of the 2 images produced an apparent motion of the finger. Trials with leftward or rightward orientation of the fingers were randomly intermixed in the same block. The whole set of visual stimuli is shown in Figure 2. In the following frame, the participants were given a feedback related to the performance of their manual response. If they responded within 1500 ms, they were then presented with a green number correspondent to their reaction time in case of a correct response, or with the word "Wrong" in case of an incorrect response. If no response was given within 1500 ms, they were presented with the phrase "No Response." The feedback frame lasted for 500 ms and was followed by a black background until the total trial duration (5 s) was reached. It should be noted that the orientation of the observed hands was orthogonal to that of the subject (which was pointing anteriorly) in order to avoid any spatial mapping of the motor response on the cue movement.

#### Task and Data Logging
Participants were seated in a dimly lit room with the head positioned on a chinrest at 0.6 m from the presentation screen. Their right arm was positioned orthogonally to the body, and their right hand was positioned on a custom-made response box. The participants were instructed to wait for the TMS pulse as their GO-signal and then to move sideways the finger opposite to the one that they saw in the video, as fast as they could until it reached the side plates of a custom-made response box (schematized in Fig. 1). This was constituted by 2 metal plates placed close to the index and to the little finger; these plates were connected to the ground pins of the computer's parallel port. Two adhesive rings were tightened on participants' index and little fingers; on the side of each ring a piece of metal was connected to the input pins of the computer's parallel port. When one of the metal pieces, on the rings, touched the nearest plate, a response was detected and the corresponding response time (RT) was logged.
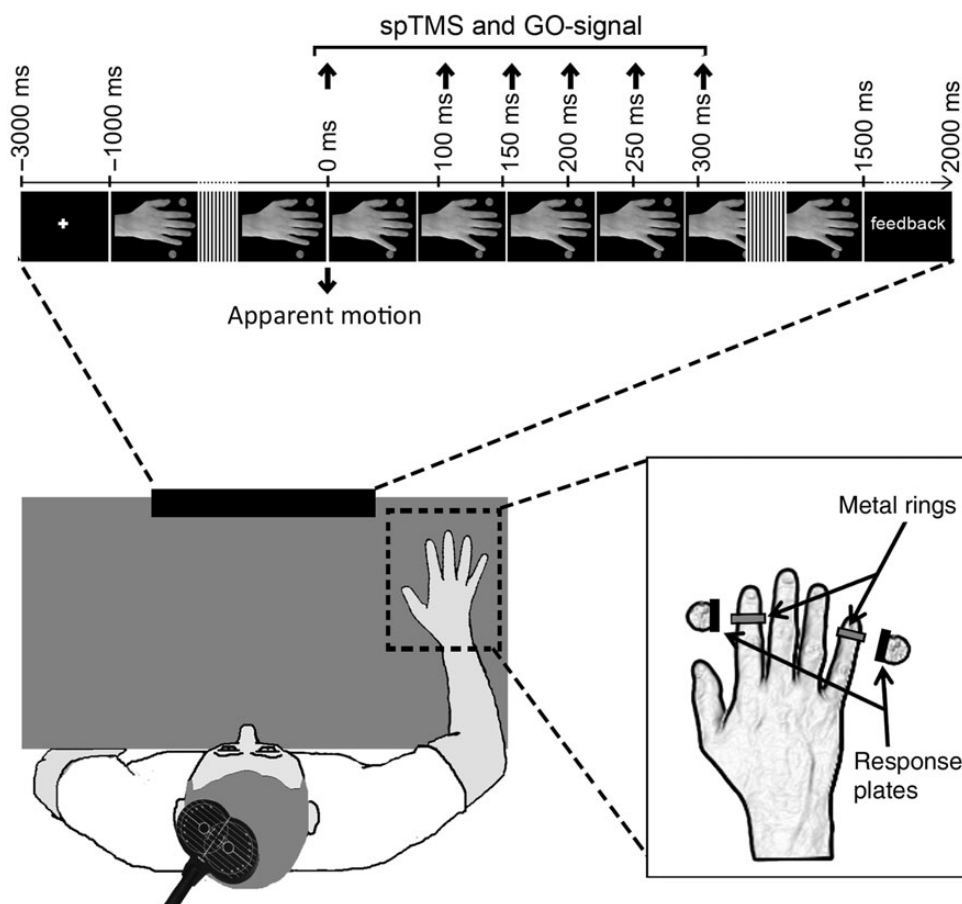
**Figure 1.** Experimental setup. The upper panel indicates the trial structure. The time of change between the first and the second frame of the stimulus hand is defined as $t = 0$ ms and referred to as "stimulus onset." Participants were stimulated over the primary motor cortex with spTMS at 6 different time intervals from stimulus onset. TMS was the GO-signal that prompted participants to produce the rule-based response (i.e. moving the finger other than the one moving in the video stimuli). The time of contact between the finger and the response plates (schematized in the lower right panel) was logged as "response time."

### Single-Pulse TMS

SpTMS was delivered with a biphasic Magstim Rapid (Magstim, Dyfed, UK) stimulator connected to a custom-made figure-of-eight coil with outer winding diameter of 50 mm. The coil was positioned with the handle pointing backward at 45° from the midline over the optimum scalp location where MEPs, with the maximal amplitude, could be obtained from the first dorsal interosseus (1DI) and abductor digiti minimi (ADM) muscles with the minimum stimulus intensity. Then the resting motor threshold (RMTh) for the 1DI muscle was determined. RMTh is defined as the minimum intensity at which TMS produces MEP amplitude of at least 50 μV in 5 of 10 trials (Rossini et al. 1994). The stimulation intensity was then set at 120% of the RMTh. In the present experiment the MEPs evoked by the same TMS pulse were recorded simultaneously from the 2 separate muscles. This experimental approach requires the motor threshold for both muscles to be as near as possible. However, it is common experience that this is not achievable in all participants. We tackled this problem by using 1 target muscle (the 1DI) and then excluding participants in which the stimulation at 120% of the RMTh evoked responses, in the 2 muscles, with amplitudes that differed by more than 1 mV. Three participants were excluded from the analysis for this reason. The final population analyzed was therefore of 18 participants. SpTMS (which served as GO-signal) was applied at 6 different ISIs, corresponding to 0, 100, 150, 200, 250, and 300 ms. A total of 576 trials were presented in each experiment, corresponding to 96 repetitions per each of the 6 ISIs.

### Electromyographic Recordings and Processing of MEPs

The EMG signal from the subjects' right hand was collected by means of 2 pairs of surface Ag/AgCl electrodes positioned on the skin overlying the belly and tendon of the 1DI and of the ADM muscles. The EMG signal from the 2 muscles was collected by 2 analog amplifier channels (CED 1902 unit: Cambridge Electronic Design, UK), amplified 1000 times and digitized (4 KHz sampling rate) by means of a CED power 1401 analog-to-digital converter, controlled by the Signal software (CED 1902 unit: Cambridge Electronic Design, UK). Recordings were digitally band-pass filtered between 20 and 2 KHz with a notch filter at 50 Hz. The data extracted from each of the 2 recorded EMG channels were 1) the peak-peak amplitude of MEPs, which was used to produce the main experimental variable, and 2) the maximum and minimum values of spontaneous activity in the 120-ms preceding the MEP, which was used to check for voluntary muscular activity. Trials with maximum–minimum activity exceeding 50 μV on any of the 2 EMG channels were discarded, in order to avoid analyzing trials with anticipation of responses with respect to the GO-signal. After trimming the data, the MEPs were normalized within each muscle by simply dividing the amplitudes of single MEPs by the grand average of MEPs from that same muscle from the whole experiment.

At this point, within each trial, the 2 MEPs recorded from the 2 muscles were classified as "congruent MEP" and "incongruent MEP." The congruent MEP was the one recorded from the muscle corresponding to the prime mover of the observed act (i.e., the 1DI muscle when observing index finger abduction and the ADM muscle when observing little finger abduction). Vice versa, the incongruent MEP was the one recorded from the muscle not involved in the production of the observed act. The pool of congruent MEPs was therefore composed by half of MEPs form the ID1 muscle and half of MEPs form the ADM muscle and the same was true for the pool of incongruent MEPs. Congruent and incongruent MEPs were averaged separately within each ISI. The variations of their relative amplitudes indicated either an
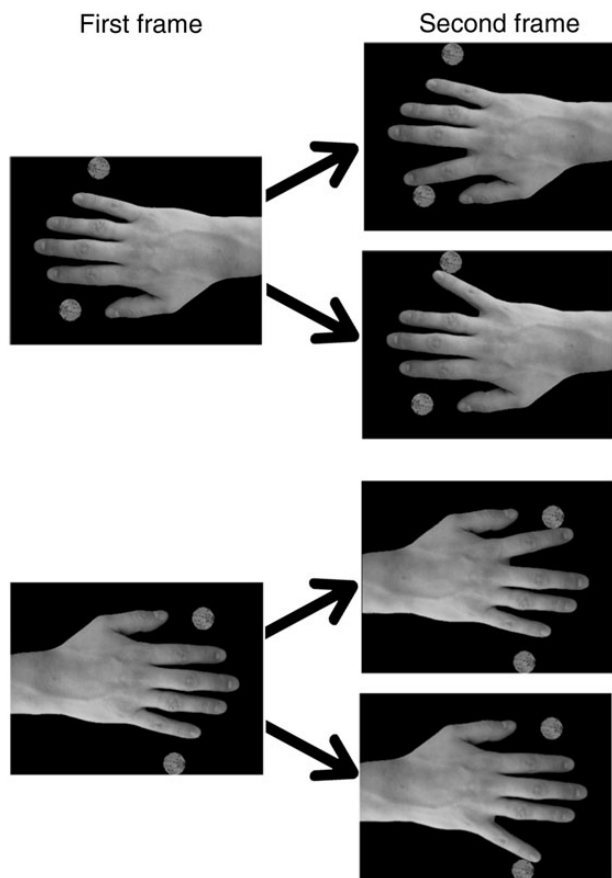
**Figure 2.** Visual stimuli. The succession of the 2 frames generating apparent motion is shown. All hands were presented horizontally (orthogonal to the participant's right hand) and were randomly oriented leftward or rightward. The second frame represented either an index finger movement or a little finger movement.

imitative response if the congruent MEPs were larger than the incongruent MEPs, or a counter-imitative response whether congruent MEPs were smaller than the incongruent MEPs.

### Data Analysis

Any trial with no response, error responses or with EMG activity detected in the 2 target muscles prior to the TMS pulse was discarded from further analysis. All the data were grouped according to several experimental variables, namely they were characterized by ISI, that is, the interval between the cue and TMS (6 levels: 0, 100, 150, 200, 250, 300 ms), MOVEMENT, that is, the movement that was shown in the cue pictures, (2 levels: index or little finger movements) and ORIENTATION, that is, the side to which the fingers were pointing in the cue stimuli (2 levels: right or left). Finally, in the MEPs' analysis, a further factor was added, CONGRUENCE, indicating whether the MEP was congruent or incongruent with the observed movement (2 levels: congruent or incongruent).

A first analysis was carried out on RTs, defined as the time of contact of the finger with the touch-sensitive target, which were averaged within each experimental condition and entered an ANOVA with ISI, MOVEMENT, and ORIENTATION as within-subjects factors. Raw MEPs from single trials were first normalized dividing the amplitude of the potentials by the mean value of potentials within that experimental condition. This procedure was carried out to equalize the amplitudes of the MEPs from the 2 muscles (1DI and ADM). Normalized MEPs were analyzed by means of an ANOVA with ISI, MOVEMENT, ORIENTATION, and CONGRUENCE as within-subjects factors. In order to account for possible violations of the sphericity assumptions, all within-subjects effects of the ANOVAs were corrected by means of

the Geisser–Greenhouse lower-bound adjustment (Geisser and Greenhouse 1958; Keselman and Rogan 1980; Berkovits et al. 2000) as calculated by the STATISTICA 6.0 (StatSoft, Inc.) software package. All post hoc comparisons were carried out with Bonferroni-corrected *t*-tests where appropriate.

### Experiment 2

#### Participants and Experimental Design

Thirty healthy participants (9 males, mean age 22 years, 1 left handed) took part in the experiment and were divided in 2 equal groups of 15 volunteers who received 1-Hz rTMS over the left dlPFC, (prefrontal group, 3 males, mean age 23 years) or over the left phAIP (parietal group, 6 males, mean age 20 years). None of the participants took part in more than 1 experiment. All participants gave written informed consent to the experiment and were screened for contraindication to TMS (Rossi et al. 2009).

The general experimental design was to test participants with spTMS on the motor cortex in trials structured identically to those of Experiment 1, but after offline modulation with 1-Hz rTMS of cortical regions distant from the motor cortex. The experiment's assumption was that any selective effect on motor cortex excitability, by neuromodulation of a remote cortical site, indicates that the stimulated area is involved in the visuomotor circuit underlying the experimental task. The task, the trial structure, the spTMS parameters, as well as the EMG recordings were identical to those of Experiment 1. Only the number of ISIs was reduced to 2 time-points, 150 and 300 ms from the onset of the cue movement, which were the ISIs of interest according to the results of Experiment 1. Also the number of repetitions per condition was reduced to 32 per time interval, corresponding to 64 trials per each of the 3 blocks. This choice was dictated by the need to shorten the experimental blocks within the time-window of the aftereffects of the 10-min train of rTMS. The aftereffects of 1-Hz rTMS are expected to be about as long as the duration of the train of stimulation itself (Chen et al. 1997, 2003).

In both the parietal and the prefrontal groups, each participant underwent 3 consecutive experimental blocks. The first one was performed in baseline conditions, without rTMS ("no-rTMS" block). In the remaining 2 blocks, offline 1-Hz rTMS was first applied to the cortical target (left dlPFC or the left phAIP) and was followed by the experimental task with spTMS. In 1 of these 2 blocks ("low-intensity rTMS" block), rTMS was delivered at low intensity and in the other one ("high-intensity rTMS" block) it was delivered at high intensity. The order of the "no-rTMS" block was fixed (first of the series), while the order of the "low-intensity rTMS" and the "high-intensity rTMS" blocks was balanced between participants.

Both the no-TMS and the low-intensity rTMS blocks were assumed to be control conditions for the main experimental intervention, that is, the high-intensity stimulation. The low-intensity rTMS block represents robust baseline measure because it accounts for all TMS effects that are not related to cortical stimulation, such as noise, cutaneous sensations, pain, weight, and position of the TMS coil. According to data obtained on the motor cortex, (Fitzgerald et al. 2006), and on the temporal cortex (Arfeller et al. 2013), intensities lower than 80% of RMTh should be ineffective. However, there are no available data in literature on how low should TMS intensity be, in order not to produce efficient stimulation of the parietal or prefrontal cortices with 1-Hz frequency. Therefore, the ineffectiveness of low-intensity TMS can only be inferred post hoc, by comparing it to a block with no stimulation at all. As a result, in the present experiment, we needed to adopt 2 control conditions in order to highlight only the effects of TMS due to cortical stimulation.

#### Repetitive TMS, Target Definition, and Neuronavigation

Repetitive TMS was delivered offline prior to MEP collection with a biphasic Magstim Rapid (Magstim, Dyfed, UK) stimulator connected to a figure-of-eight coil with outer winding diameter of 70 mm. The intensity of "high-intensity rTMS" was set to 100% of the RMTh, and the intensity of the "low-intensity rTMS" was set to 40% of the RMTh. Stimuli were delivered at 1 Hz for 10 min. The Talairach coordinates of the left

dlPFC target were identified averaging the results of 5 previous imaging studies investigating the learning of arbitrary visuomotor associations (Toni and Passingham 1999; Schluter et al. 2001; Toni, Rushworth, et al. 2001; Bunge et al. 2002, 2003) and corresponded to [$x = -52$, $y = 32$, and $z = 20$] for dlPFC. For the left phAIP position, we used the coordinates indicated in 2 recent large meta-analyses of imaging studies on action observation (Caspers et al. 2010; Molenberghs et al. 2012), corresponding to [$x = -42$, $y = -46$, and $z = 57$]. The left dlPFC and left phAIP sites on the subject's scalp were automatically identified using the SoftTaxic Evolution Navigator system (E.M.S., Bologna, Italy) that can operate in the absence of radiological images on the basis of digitized fiducial points on the skull which are related to standard cerebral anatomy. Therefore, although individual magnetic resonance images were not available, Talairach coordinates of cortical sites, underlying coil locations, were automatically estimated for the participants by the navigator system, on the basis of an MRI-constructed stereotaxic template.

*Data Analysis*

The participant exclusion criteria were the same described in Experiment 1 and 2 participants were excluded from the analysis; thus, the final population resulted in 28 participants divided in 2 groups of 14 participants each. Any trial with no response, error responses or with EMG activity detected in the 2 target muscles prior to the TMS pulse was discarded from further analysis. The data were grouped according to the between-subjects factor TARGET (2 levels: prefrontal and parietal) and the within-subjects factors BLOCK (3 levels: no-rTMS, low-intensity rTMS, and high-intensity rTMS), ISI (2 levels: 150 and 300 ms), MOVEMENT (2 levels: index or little finger movements), ORIENTATION (2 levels: right or left) and, limitedly to MEP analysis, CONGRUENCE (2 levels: congruent or incongruent). The RTs were analyzed by means of a TARGET\*BLOCK\*ISI\*MOVEMENT\*ORIENTATION ANOVA. As in Experiment 1, raw MEPs from single trials were first normalized by dividing the amplitude of the single potentials by the grand average of the MEPs from that same muscle. This procedure was done separately for the 3 blocks.

The normalized MEPs were then analyzed by means of a TARGET\*BLOCK\*ISI\*MOVEMENT\*ORIENTATION\*CONGRUENCE ANOVA. In order to account for possible violations of the sphericity assumptions, all within-subjects effects of the ANOVAs were corrected by means of the Geisser–Greenhouse lower-bound adjustment (Geisser and Greenhouse 1958; Keselman and Rogan 1980; Berkovits et al. 2000) as calculated by the STATISTICA 6.0 (StatSoft, Inc.) software package. All post hoc comparisons were carried out with Bonferroni-corrected *t*-tests where appropriate.

## Results

### *Experiment 1*

None of the participants reported any adverse side effect from TMS. Two percent of trials were discarded because of pre-TMS EMG activity. Errors accounted for 4% of all trials. The analysis of RTs indicated that subjects were faster when responding with their index finger (main effect of MOVEMENT ($F_{1,17} = 28.8$, $P = 0.00005$)), as well as when they were responding to hands oriented leftward rather than rightward (main effect of ORIENTATION ($F_{1,17} = 30.6$, $P = 0.00004$)). Participants responded increasingly faster with increasing time between stimulus presentation and the GO-signal (main effect of ISI ($F_{5,85} = 94.2$, $P < 0.000001$)), which is illustrated in Figure 3. This main effect was investigated by comparing with multiple *t*-tests the RTs from consecutive ISIs in order to assess the advantage of increasing stimulus-processing time. The critical *P*-value was Bonferroni-corrected for 5 multiple comparisons to 0.01. Significant differences were found between all pairs of consecutive ISIs (all $P < 0.0005$) except for the last one ($P = 0.06$) (see asterisks in Fig. 3), indicating that the advantage of increasing ISI reached a floor level at 250–300 ms between stimulus onset and the GO-signal. Finally, a MOVEMENT\*ORIENTATION interaction ($F_{1,17} = 12.4$, $P = 0.003$) was found indicating that the advantage in responding to the leftward-oriented stimuli was present only when responses were given with the index finger. The mean values of RTs for each cell of the full factorial design are presented in Table 1.

The results of the ANOVA performed on normalized MEP amplitudes yielded a main effect of CONGRUENCE ($F_{1,17} = 5.1$, $P = 0.038$), an interaction of ORIENTATION\*CONGRUENCE ($F_{1,17} = 5.2$, $P = 0.036$) and an interaction of ORIENTATION\*MOVEMENT\*CONGRUENCE ($F_{1,17} = 4.6$, $P = 0.047$). These effects (reported in Supplementary Tables 1–3 and illustrated in Supplementary Figs 1–3) were not further investigated because, given our a priori experimental hypothesis, the results of interest were only those highlighting changes in the relation between congruent and incongruent MEPs at different ISIs, that is, accordingly, the only significant interaction of interest that was found is an ISI\*CONGRUENCE interaction ($F_{1,17} = 7.2$, $P = 0.016$) (see Fig. 4 and Table 2 for details). Post hoc comparisons were made between congruent and incongruent MEP amplitudes within each ISI and the critical *P* level was adjusted for 6 multiple comparisons to $P = 0.008$. At the ISI of 150 ms, the congruent MEPs were significantly larger ($P = 0.005$) than
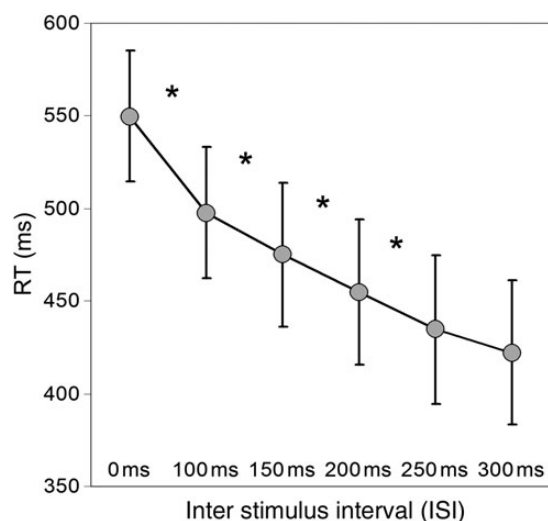


**Figure 3.** Experiment 1: mean response times given for each of the 6 ISIs. The *P* levels of the pairwise *t*-tests comparing data from consecutive ISIs are presented. Note that all comparisons were significant aside from the one between the 250- and the 300-ms ISIs. Error bars indicate 95% CI of the mean.

**Table 1**

Mean response times (ms) from Experiment 1 (95% CI)

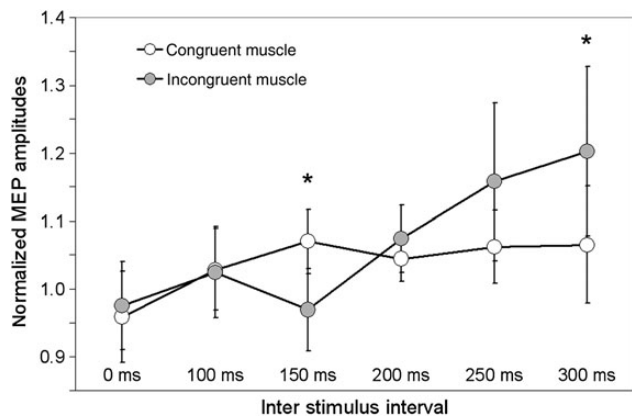| Observed movement | Index finger movement | | Little finger movement | |
|---|---|---|---|---|
| | Leftward | Rightward | Leftward | Rightward |
| Observed hand orientation (ms) | | | | |
| ISI 0 | 582.9 (38) | 585.1 (42) | 493.0 (41) | 538.4 (35) |
| ISI 100 | 536.5 (37) | 526.3 (44) | 437.7 (40) | 490.3 (38) |
| ISI 150 | 509.7 (39) | 512.5 (49) | 420.6 (41) | 457.7 (41) |
| ISI 200 | 497.0 (49) | 495.5 (47) | 402.7 (35) | 423.7 (41) |
| ISI 250 | 483.1 (52) | 477.4 (42) | 381.1 (38) | 397.5 (44) |
| ISI 300 | 457.4 (43) | 461.0 (47) | 377.6 (38) | 392.9 (45) |

**Figure 4.** Experiment 1: mean values of normalized congruent and incongruent MEP amplitudes. Error bars indicate 95% CI of the mean. Asterisks denote significant $t$-tests comparing congruent and incongruent MEPs within the same ISI (critical $P$ level corrected for multiple comparisons to 0.008).

**Table 2**

Mean values of normalized MEP amplitudes from Experiment 1 (95% CI)

| Recorded muscle | Congruent muscle | | Incongruent muscle | |
|---|---|---|---|---|
| | Leftward | Rightward | Leftward | Rightward |
| Observed hand orientation (ms) | | | | |
| ISI 0 | 0.912 (0.065) | 0.908 (0.073) | 0.905 (0.07) | 0.942 (0.063) |
| ISI 100 | 0.939 (0.056) | 1.018 (0.072) | 0.96 (0.07) | 0.99 (0.078) |
| ISI 150 | 1.009 (0.064) | 1.037 (0.061) | 0.921 (0.089) | 0.916 (0.056) |
| ISI 200 | 1.034 (0.04) | 0.957 (0.044) | 1.053 (0.045) | 0.992 (0.058) |
| ISI 250 | 1.053 (0.082) | 0.965 (0.057) | 1.066 (0.067) | 1.15 (0.159) |
| ISI 300 | 1.041 (0.093) | 0.987 (0.082) | 1.135 (0.144) | 1.164 (0.111) |

the incongruent ones. Conversely, at the ISI of 300 ms, the incongruent MEPs were significantly larger ($P = 0.003$) than the congruent ones. The remaining 4 comparisons were not significant (all $P$'s > 0.04). In summary, the main finding of Experiment 1 was that the reciprocal pattern of congruent and incongruent MEPs indicated a mirror response at 150 ms from stimulus onset and a counter-mirror response at 300 ms from stimulus onset.

### Experiment 2

None of the participants reported any adverse side effect from TMS. Three percent of trials were discarded because of pre-TMS EMG activity. Errors accounted for 4% of all trials. The ANOVA on RTs showed that participants responded faster at the ISI of 300 ms than at 150 ms as shown by the main effect of the ISI factor ($F_{1,26} = 174.40$, $P < 0.00001$). A main effect of ORIENTATION ($F_{1,26} = 7.9641$, $P = 0.009$) showed that participants were faster in response to leftward-oriented hands. Unlike in Experiment 1, no main effect of MOVEMENT was found ($P = 0.25$) but, as in Experiment 1 a significant MOVEMENT*ORIENTATION interaction ($F_{1,26} = 6.83$, $P = 0.015$) was found indicating that an advantage of responding with the index finger was indeed present but only in response to hands oriented rightward. Importantly, no effects of the BLOCK factor on RTs were found.

Experiment 2 was planned to highlight differential effects of effective and noneffective rTMS on the difference between congruent and incongruent MEPs at the 2 different ISIs in the 2 groups. Therefore, the a priori-defined effect of interest was any interaction involving all the TARGET, BLOCK, ISI, and
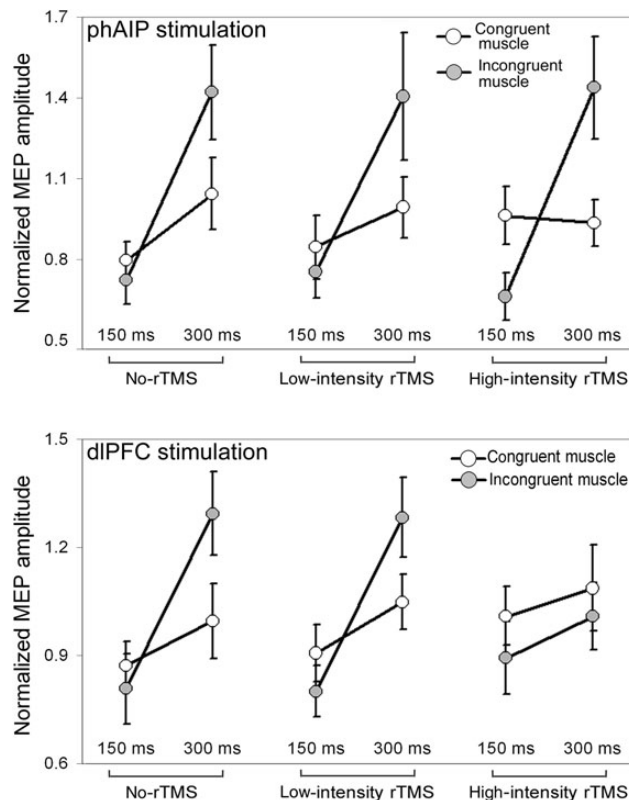




**Figure 5.** Experiment 2. Mean values of normalized congruent and incongruent MEP amplitudes collected at the 2 ISIs of 150 and 300 ms and represented separately for the 3 experimental blocks. The data from the phAIP group are shown in the upper panel and those from the dlPFC group are shown in the lower panel. Error bars indicate 95% CI of the mean.

CONGRUENCE. Effects that did not fall within the focus of interest were a main effect of CONGRUENCE ($F_{1,26} = 5.5670$, $P = 0.02609$), a main effect of ISI ($F_{1,26} = 48.763$, $P < 0.00001$), and a main effect of ORIENTATION ($F_{1,26} = 8.58$, $P = 0.007$). Additionally, we found an interaction of ISI*CONGRUENCE ($F_{1,26} = 44.67$, $P < 0.00001$), an ISI*CONGRUENCE*TARGET interaction ($F_{1,26} = 7.53$, $P = 0.01$), a CONGRUENCE*BLOCK interaction ($F_{2,52} = 4.73$, $P = 0.03$), and a CONGRUENCE* MOVEMENT*ORIENTATION interaction ($F_{1,26} = 6.17$, $P = 0.02$). These effects are reported in Supplementary Tables 4–10 and illustrated in Supplementary Figures 4–10.

The only effect of interest that emerged from the ANOVA was a TARGET*BLOCK*ISI*CONGRUENCE interaction ($F_{2,52} = 10.73$, $P = 0.0029$), which is illustrated in Figure 5 and detailed in Table 3. The interaction was further explored by 2 TARGET*BLOCK*CONGRUENCE ANOVAs, 1 for each of the 2 ISIs, which both resulted in significant TARGET*BLOCK* CONGRUENCE interactions (($F_{2,52} = 4.41$, $P = 0.045$) for the 150-ms ISI and ($F_{2,52} = 5.69$, $P = 0.024$) for the 300-ms ISI).

The data from the 150-ms ISI were further analyzed by means of 2 BLOCK*CONGRUENCE ANOVAs, separately for each of the 2 groups, which yielded only a main effect of CONGRUENCE ($F_{1,13} = 21.43$, $P = 0.0005$) in the prefrontal group and a main effect of CONGRUENCE ($F_{1,13} = 30.90$, $P = 0.00009$) in the parietal group. The main effects were due in both groups to congruent MEPs being larger than incongruent ones. It is important to note that the present results strongly replicate the finding of a congruency effect at 150 ms that is

**Table 3**
Mean values of normalized MEP amplitudes from Experiment 2 (95% CI)

| Target | Block | Congruent muscle | | Incongruent muscle | |
|---|---|---|---|---|---|
| | | 150 ms | 300 ms | 150 ms | 300 ms |
| phAIP | No-rTMS | 0.801 (0.07) | 1.047 (0.133) | 0.725 (0.086) | 1.426 (0.177) |
| | Low-intensity rTMS | 0.847 (0.12) | 0.994 (0.114) | 0.752 (0.096) | 1.411 (0.24) |
| | High-intensity rTMS | 0.964 (0.108) | 0.937 (0.085) | 0.664 (0.088) | 1.439 (0.191) |
| dlPFC | No-rTMS | 0.873 (0.068) | 0.996 (0.104) | 0.808 (0.097) | 1.293 (0.116) |
| | Low-intensity rTMS | 0.907 (0.078) | 1.05 (0.077) | 0.801 (0.07) | 1.283 (0.111) |
| | High-intensity rTMS | 1.009 (0.082) | 1.087 (0.12) | 0.892 (0.1) | 1.009 (0.093) |

reported in Experiment 1. However, to fully demonstrate that the results of Experiment 2 replicate those of Experiment 1, we also compared congruent and incongruent trials in the no-rTMS condition of the 150 ISI conditions in the parietal group, since, in these conditions, we found a significant BLOCK*CONGRUENCE interaction. The comparison yielded a significant result showing that congruent MEPs were larger than incongruent MEPs ($P = 0.019$). Regarding all other conditions, the individual values of normalized MEP amplitudes and a full set of comparisons between congruent and incongruent MEPs is provided in Supplementary Table 12.

More interestingly in the light of interpreting the complex interactions, only in the parietal group a BLOCK*CONGRUENCE interaction ($F_{2,26} = 9.04$, $P = 0.01$) was found. This interaction was finally explored by comparing separately, for each MEP type (congruent or incongruent), the mean values of normalized MEP amplitudes between each of the 3 blocks, corresponding to a total number of 6 multiple comparisons. The critical $P$ level was therefore adjusted to $P = 0.008$. The results showed that congruent MEPs in the high-intensity rTMS block were significantly larger than those in the no-rTMS block ($P = 0.002$) and than those in the low-intensity rTMS block ($P = 0.005$). No significant differences were found between blocks in the incongruent MEPs (all $P$'s $< 0.16$).

Also, the data from the 300-ms ISI were analyzed by means of 2 BLOCK*CONGRUENCE ANOVAs, separately for each of the 2 groups, which yielded a main effect of CONGRUENCE in both the parietal group ($F_{1,13} = 12.75$, $P = 0.003$) and in the prefrontal group ($F_{1,13} = 13.451$, $P = 0.003$). Also at this ISI, these findings represent a replica of the data from Experiment 1, namely of the counter-imitative tendency was observed at the 300-ms ISI. However, similarly to the 150-ms ISI condition, the finding of a significant BLOCK*CONGRUENCE interaction required that we perform a direct comparison between the congruent and the incongruent MEPs in the prefrontal group for the no-rTMS block. A paired-samples t-test indicated a significant difference ($P = 0.022$) between the 2 conditions, with congruent MEPs more ample than incongruent MEPs. Additionally, Supplementary Table 12 reports all other comparisons between congruent and incongruent MEPs in all other conditions.

Interestingly, only in the prefrontal group a BLOCK*CONGRUENCE ($F_{2,26} = 8.95$, $P = 0.01$) interaction was found. This interaction was explored within the prefrontal group by comparing separately for each MEP type (congruent or incongruent) the mean values of normalized MEP amplitudes between each of the 3 blocks, corresponding to a total number of 6 multiple comparisons. The critical $P$ level was therefore

adjusted to $P = 0.008$. Congruent MEPs showed no difference between blocks (all $P$'s $> 0.23$). On the contrary, incongruent MEPs in the high-intensity rTMS block resulted to be significantly smaller than those in the low-intensity rTMS block ($P = 0.003$) and from those in the no-rTMS block ($P = 0.005$).

In summary, the main result of Experiment 2 indicated that (A) high-intensity rTMS applied to the prefrontal cortex produced effects only at the 300-ms ISI and that these effects consisted in a lesser increase of incongruent MEPs and (B) high-intensity rTMS applied to the parietal cortex produced effects limited to the 150-ms ISI and these consisted in a more marked increase in amplitude of congruent MEPs. Additionally, it should be noted that the results validated the original experimental assumption that low-intensity rTMS was equivalent to the no-rTMS condition.

## Discussion

We show here that during a simple social interaction, such as producing a rule-based motor response to the movements of another individual, 2 distinct processes occur in the motor system of the participant, in the critical period between the onset of the cue movement and completion of the task. The earliest phenomenon that can be read in the motor output of the participant appears around 150 ms from the onset of the cue movement (Fig. 4). It is a visuomotor mapping that specifically imitates of the observed motor act and does not depend on the arbitrary rule to be implemented. Two basic properties of this early response appear to be particularly relevant to identify it as a pure "mirror" response. First, the response is specific for the observed effector's identity, because it mirrors equally the cue-movements of the little finger or the index finger. Second, the early "mirror" effect is independent from the spatial relations between the participant's effector and the observed one because (Fig. 2) visual stimuli were orthogonal to the participant's hand and randomly oriented leftward or rightward. Furthermore, this early phenomenon can be defined as automatic, if "the pivotal property that distinguishes automatic from controlled processes is that an automatic process is triggered without the actor's intending to do so and cannot be stopped even when the actor intends to and it is in that actor's best interests to do so" (Kornblum et al. 1990).

The second phenomenon becomes evident around 300 ms from the onset of the cue-movement and is specifically following the rule of the arbitrary visuomotor task. As for the early mirror response, also this "executive" response is body part-specific and devoid of stimulus-response spatial features. This component reflects therefore the output of the executive processes that transform the visual cue into the rule-based response. We propose that the early mirror response is produced by a bottom-up stimulus-driven process and the later one is produced by a top-down goal-driven process. The results of the first experiment of the present work validate and extend the results of our previous work (Barchiesi and Cattaneo 2013) showing biphasic responses to others' actions in conflictual stimulus-response behavior. The latency of the early response observed in Barchiesi and Cattaneo (2013) was of 250 ms from stimulus onset, while, in the present work, it was confined at 150 ms from movement onset. Though the biphasic pattern is preserved in the 2 experiments, the difference in timing of the early response is due, in our view, to 2 separate phenomena. First, the mirror response is highlighted earlier in the present experiment because it is recorded during

movement preparation compared with the passive viewing condition of Barchiesi and Cattaneo (2013), thus decreasing the excitability threshold of the motor system. Second, one possible explanation why the mirror response is no longer seen at 250 ms could be that the task stresses the speed of response and the counter-mirror effect is anticipated and therefore it overrides the mirror response at an earlier time than during passive observation. Also indirect evidence from the electroencephalographic literature indicates that body-related visual information accesses the visuomotor system well before 200 ms from stimulus onset (van Schie et al. 2008; Bortoletto et al. 2011).

We performed a second experiment to explore the anatomical substrates of this model. With 1-Hz rTMS, we produced neuromodulation in 2 cortical regions that are related to bottom-up mirror responses, that is, the phAIP, and top-down control of arbitrary visuomotor associations, that is, the dlPFC. The phAIP is robustly activated by action observation (Molenberghs et al. 2012) and the dlPFC is crucial in implementing visuomotor rules (Passingham et al. 2000). The results showed a clear-cut double dissociation of the effects of rTMS on early and late motor responses. As expected, interference with the dlPFC only reduced the size of the late response (Fig. 5) and rTMS over the phAIP produced effects exclusively on the early response consisting in an increase of the mirror phenomenon.

These findings favor our experimental hypothesis that the 2 sensorimotor phenomena, the early mirror and the late executive response, are mediated by 2 different neural systems. The early responses are conveyed along the dorsal visual stream and are unequivocally bottom-up responses. The late responses are mediated by the prefrontal cortex and are top-down responses. The present double dissociation also provides information on the feeding of visual information to the prefrontal cortex. The fact that targeting phAIP did not influence the late response as well as the early one indicates that the prefrontal cortex can gain visual information on the cue-movement by pathways other than the ones producing automatic mirror responses. The present interpretation of the data by no means excludes the possibility of a cross-talk between bottom-up and top-down processes, which are known to influence each other at several levels of response processing. This datum actually fits well with models proposed in nonhuman primates (Boussaoud et al. 1995; Lebedev and Wise 2002) and in humans (Passingham and Toni 2001; Toni, Rushworth, et al. 2001; Vry et al. 2012) describing 2 routes to action that rely on the dorsal and the ventral visual streams. An additional dissociated effect of rTMS over the 2 target areas is that parietal stimulation produced an effect on the representation of the congruent movement, that is, on the movement that was seen in the visual stimulus and, on the contrary, prefrontal stimulation produced an effect limited to the incongruent movement, that is, to the movement that was NOT seen in the visual stimulus but that had to be implemented in the counter-mirror task. This result further supports the association of the parietal cortex with true mirror responses, related to the observed movement, and of the prefrontal cortex with the counter-mirror response, based on an arbitrary motor mapping. The present experiment was not designed to test motor efficiency in counter-imitative tasks and this is possibly the reason why we did not find in Experiment 2 an effect of rTMS on RTs. In particular, the presence of a delay between the cue and the response which was necessary to apply TMS during the trials may have reduced potential effects of rTMS on motor performance.

It is interesting to note that the remote effects of rTMS on the 2 targets were of different polarity, that is, a reduction of the specific physiological response pattern in the case of prefrontal rTMS and an increase in the physiological pattern in the case of parietal rTMS. When investigating the effects of 1-Hz rTMS on distant cortical areas, it is difficult to make a priori assumptions on what the net effect will be on the remote region. This is clearly observed when coupling 1-Hz rTMS with a whole-brain measure such as fMRI. As reviewed in Arfeller et al. (2013), the hemodynamic effects of 1-Hz rTMS on regions distant from the stimulated one are strictly task-dependent and can be, unpredictably, inhibitory or excitatory. Also in the specific field of brain responses to action observation, remote effects of 1-Hz rTMS on the mirror neuron circuit can be facilitatory after rTMS of the posterior superior temporal sulcus (Arfeller et al. 2013; Avenanti et al. 2013) or inhibitory after stimulation of the premotor cortex (Avenanti et al. 2007). The occurrence of facilitatory remote effects of 1-Hz rTMS is generally attributed to compensatory increase of the activity within a network in face of reduced functioning of the stimulated target (Avenanti et al. 2013). More generally, the traditional view of the effects of 1-Hz rTMS being considered "inhibitory" is no longer supported. The current view is that rTMS has effects which are not predictable a priori on the basis of the sole stimulation frequency, but that can be excitatory or inhibitory according to different experimental conditions (Silvanto et al. 2008) as reviewed in (Silvanto and Pascual-Leone 2008; Miniussi et al. 2013). Another possible explanation for the facilitatory effect of phAIP stimulation is that AIP is not involved in mirroring, but rather in the prevention of mirroring, for example, by mapping the observed stimulus onto the nonmirror response required by the task and, therefore, the increase in mirroring takes place because disruption of AIP releases the automatic mirror response, as proposed by Corbetta and Shulman (2002). In summary, the 3 possible hypotheses accounting for the facilitatory effects of phAIP stimulation include 1) phAIP has been disrupted and compensatory overactivity is observed in the mirror system; 2) rTMS produced facilitation of physiological phAIP activity rather than inhibition; 3) phAIP is disrupted by rTMS but it is located in parallel to the pathway conveying automatic action mirroring which it actively inhibits. It is worth noting that all 3 hypotheses are consistent with a separate genesis of the early and the late motor responses, thus validating at least in part our initial hypothesis.

It is worth putting the present data in the perspective of current knowledge of bottom-up and top-down processes controlling the production of actions in other domains, where a biphasic time-course of visuomotor behavior has been described. In the case of object affordance, when implementing a visuomotor rule, which conflicts with the affordance of an object, an early automatic stimulus-response mapping occurs, between 100 and 200 ms from stimulus onset (Goslin et al. 2012). In this context also "partial errors" are frequently produced (McBride, Sumner, et al. 2012), consisting in an early tendency to produce an automatic motor response, later substituted by the rule-based response, which further suggest the occurrence of a fast automatic affordance-driven response and a subsequent slower task-driven motor response. In tasks in which spatial stimulus-response conflicts are present (Simon task), automatic stimulus-driven responses measured with EMG appear around 200 ms and disappear around 300 ms from stimulus onset (Burle et al. 2002; Hasbroucq et al.

2009). A recent study investigated the time-course of MEP modulation during the performance of an Eriksen flanker task and found for incongruent trials, a time-course of MEP amplitude changes strikingly similar to the ones presented here (Michelet et al. 2010), with stimulus-driven responses appearing between 160 and 240 ms after stimulus onset. Also in the case of visual search tasks, it was found that independent stimulus-driven and goal-driven mechanisms coexist but with different timing. Saccades produced in an early time-window between 150 and 250 ms are more frequently directed toward a distracter rather than toward the arbitrary target (Muller and Rabbitt 1989; van Zoest and Donk 2006; Siebold et al. 2011). These data, taken together with our findings, indicate that the relative timing between early stimulus-driven and late goal-driven responses is robustly preserved across domains in the visual modality, with small task-dependent variations in the absolute timing of the early responses.

Finally, a recent paper (Cavallo et al. 2013) investigated during passive viewing an experimental paradigm similar to the present one and failed to find an early mirror response after counter-imitative training. These results are not in conflict with those of the present experiment because of a fundamental methodological difference between them. In the experiment described by Cavallo et al. (2013), the observed movement was unambiguously spatially oriented, that is, the observed little finger moved always upward and the observed index finger moved always downward. The counter-imitative task could therefore be solved simply by a spatial strategy (when anything move upward move your index finger and when anything moves downward move your little finger). As stated by the authors, it is possible that, during training, participants learned to associate also the location of the observed action with the relevant response. On the contrary, the task in the present experiment is much more difficult to be approached with any efficient spatial strategy because movement and spatial direction are dissociated. The present task is more likely to be solved by taking into account which body part is moving rather than the direction of observed motion and, therefore, it is much more powerful to detect genuine mirror responses rather than any other visuomotor association.

In conclusion, the present experiment adds important information on the mechanisms by which the brain is both tuned to produce imitative responses in a fast automatic way but is also capable of overriding them by means of a parallel, more flexible, visuomotor coupling that follows arbitrary visuomotor associations. The fast imitative tendencies seemingly persist also during the online performance of a counter-imitative task. The 2 processes access the motor output by 2 partially independent neural substrates.

## Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

## Funding

## Notes

*Conflict of Interest:* None declared

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