Cerebral Cortex March 2013;23:570–580
doi:10.1093/cercor/bhs040
Advance Access publication March 16, 2012

Compensatory Plasticity in the Action Observation Network: Virtual Lesions of STS Enhance Anticipatory Simulation of Seen Actions

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Observation of snapshots depicting ongoing motor acts increases corticospinal motor excitability. Such motor facilitation indexes the anticipatory simulation of observed (implied) actions and likely reflects computations occurring in the parietofrontal nodes of a cortical network subserving action perception (action observation network, AON). However, direct evidence for the active role of AON in simulating the future of seen actions is lacking. Using a perturb-and-measure transcranial magnetic stimulation (TMS) approach, we show that off-line TMS disruption of regions within (inferior frontal cortex, IFC) and upstream (superior temporal sulcus, STS) the parietofrontal AON transiently abolishes and enhances the motor facilitation to observed implied actions, respectively. Our findings highlight the critical role of IFC in anticipatory motor simulation. More importantly, they show that disruption of STS calls into play compensatory motor simulation activity, fundamental for counteracting the noisy visual processing induced by TMS. Thus, short-term plastic changes in the AON allow motor simulation to deal with any gap or ambiguity of ever-changing perceptual worlds. These findings support the active, compensatory, and predictive role of frontoparietal nodes of the AON in the perception and anticipatory simulation of implied actions.

Keywords: action prediction and simulation, functional connectivity, plasticity, superior temporal sulcus, transcranial magnetic stimulation

Introduction

Perceiving and understanding what other people do are crucial for effective social functioning. Mounting evidence suggests that this ability may be underpinned by frontal, parietal, and temporal areas that respond when seeing human actions (hereafter referred to as action observation network, AON) (Gazzola and Keysers 2009; Grafton 2009; Caspers et al. 2010; Van Overwalle and Baetens 2009). The inferior frontal (ventral premotor cortex and inferior frontal gyrus, hereafter referred to as "inferior frontal cortex," IFC) and parietal cortices are important nodes of the AON (Chong et al. 2008; Etzel et al. 2008; Kilner et al. 2009; Oosterhof et al. 2010) coupling action perception and execution. Monkey studies indicate that a proportion of neurons in these frontoparietal regions increase their firing rate during both action perception and execution (so called "mirror neurons") (di Pellegrino et al. 1992; Gallese et al. 1996; Fogassi et al. 2005) and may implement a mechanism that matches perceived actions with one's own motor representation of similar actions (Rizzolatti and Craighero 2004).

Strong evidence for a motor simulation of seen actions in humans comes from single-pulse transcranial magnetic stimulation (spTMS) studies showing that seeing others' actions increases the excitability of the corticospinal motor circuits involved in performing the same actions (Fadiga et al. 2005; Aglioti et al. 2008; Sartori et al. 2011). Relevant to the present study is that virtual lesions of IFC disrupt action observation–related motor facilitation (Avenanti et al. 2007) hinting at the crucial role of this structure in mediating action simulation in the motor cortex (M1).

Theoretical models of action perception have emphasized the predictive nature of the frontoparietal AON activity (Wilson and Knoblich 2005; Kilner et al. 2007; Schütz-Bosbach and Prinz 2007; Gazzola and Keysers 2009; Friston et al. 2011; Press et al. 2011; Schippers and Keysers 2011) and have suggested that action perception relies on forward internal models that predict the future course of others' motor acts. In keeping, neurophysiological studies have reported that M1 shows an anticipatory bias in the motor response to observed actions (Gangitano et al. 2004; Kilner et al. 2004; Borroni et al. 2005; Aglioti et al. 2008; Avenanti, Minio-Paluello, Sforza, et al. 2009).

Using motor-evoked potentials (MEPs) induced by spTMS, it has been demonstrated that M1 is activated during perception of static pictures of ongoing but incomplete human actions (implied actions, Urgesi et al. 2006; Candidi et al. 2010). Crucially, motor facilitation was greater for images depicting hand actions in their initial–middle phases than final phases (Urgesi et al. 2006, 2010). Thus, motor reactivity to implied actions likely reflects the anticipatory simulation of future phases of the observed implied action (Wilson and Knoblich 2005; Urgesi et al. 2010). While studies suggest that activation of M1 during action observation stems from activity within the frontoparietal AON (Avenanti et al. 2007; Koch et al. 2010; Catmur et al. 2011), direct evidence for the involvement of IFC in simulating the future of seen actions is lacking.

Moreover, no studies have addressed the issue of whether the anticipatory motor coding of the observed action 1) is linked to an active crucial role of frontoparietal AON (hypothesis A) (Wilson and Knoblich 2005; Kilner et al. 2007; Aglioti and Pazzaglia 2011; Friston et al. 2011) or 2) merely and passively reflects computations carried out in connected visual nodes of the AON (e.g., in the superior temporal sulcus, STS) as a consequence of learned Pavlovian-like visuomotor associations (Hickok 2009) (hypothesis B).

During action observation visual information is thought to reach the frontoparietal AON via the STS (Rizzolatti and Luppino 2001; Nishitani and Hari 2002; Nishitani et al. 2004;

Nelissen et al. 2011), a high-order visual area containing neurons that encode real or apparent biological motion stimuli (Keysers and Perrett 2004) and respond also to static images of body postures implying an action (Peigneux et al. 2000; Jellema and Perrett 2003). While neurons in STS may show anticipatory response to observed actions (Perrett et al. 2009), they do not respond to action execution and thus lack "classical" mirror properties.

One way of directly addressing the issue of the functional relation between the frontoparietal and the visual nodes of the AON in mediating action prediction is to test the motor facilitation to implied action after perturbation of neural processing either within (IFC) or upstream (STS) the frontoparietal AON. While both hypothesis A and B may predict that anticipatory action simulation in M1 can be disrupted by perturbation to IFC, they make opposite predictions regarding the effect of perturbation to STS.

If the AON is organized as a "passive" feed-forward system, where the frontoparietal AON nodes passively reflect computations carried out in STS due to sensory–motor pairing (hypothesis B), then suppression of STS should reduce the flow of information reaching the frontoparietal AON and thus decrease simulation activity in the network (and consequently in M1).

The alternative view (hypothesis A) predicts an "active" compensatory increase of action simulation after STS suppression. According to this hypothesis, the AON is organized as a dynamic control system where information initially flows from visual (STS) to visuomotor (frontoparietal) nodes and then back to visual regions (Schippers and Keysers 2011). In this vein, motor simulation activity occurring in frontoparietal regions is automatically called into play to solve fundamental computational challenges posed by action perception like completing missing information or making the best sense of ambiguous information (Wilson and Knoblich 2005; Schütz-Bosbach and Prinz 2007; Aglioti and Pazzaglia 2011; Avenanti and Urgesi 2011). An increment of noise in perceptual representation of actions would require the increase of filling-in function based on internal models of action (Kilner et al. 2007; Gazzola and Keysers 2009; D'Ausilio et al. 2011; Friston et al. 2011 Schippers and Keysers 2011). Thus, the disruption of visual processing in STS should trigger an increase of activity in the frontoparietal AON. This effect would be reflected in an increased M1 facilitation.

A direct test of these hypotheses would require to investigate how manipulation of neural activity in a given area (IFC or STS) influences responses in another (M1). Studies in the nonhuman primate have used such "perturb-and-measure" approach by showing that using a cooling procedure to inactivate temporarily a higher order visual area (middle temporal, MT) disrupted single-cell activity in the primary visual cortex (V1) and thus proved that the former area has a causal influence on the latter (Hupé et al. 1998). While the invasive nature of the direct interference approach limits its application to animal models, TMS allows to explore directly but noninvasively how transient inhibition of a target brain region (obtained by administration of repetitive TMS, rTMS) modifies neural responses in M1 (measured using spTMS) (Avenanti et al. 2007, 2012). Thus, thanks to this approach, it is possible to test directly in humans the causative connectivity between different nodes of a given neural network (Paus 2005).

Here, we used a perturb-and-measure TMS paradigm, which offers the unique possibility to 1) suppress neural activity in IFC or STS using low-frequency rTMS (to perturb and create "transient virtual lesions") and 2) assess the consequent functional modulation of corticospinal motor reactivity to observed actions via spTMS of M1 (Avenanti et al. 2007). Anticipatory action simulation processes in M1 were assessed by recording MEPs from the right hand during the observation of static pictures depicting a fine grasping performed with the index finger and the thumb (implied action stimuli). As a control, we presented images of a still hand and 2 nonbody static (icefall) and implied motion (waterfall) control visual stimuli.

Based on electromyography (EMG) recording performed during action execution (Urgesi et al. 2010), we expected that in normal physiological conditions watching a fine grasping would increase the cortical excitability of the first dorsal interosseous (FDI, controlling index finger movements) but not of the abductor digiti minimi (ADM) muscle that is not involved in fine grasping. To test the role of IFC and STS in anticipatory action simulation, functional modulation of M1 contingent upon the perception of still and implied motion stimuli was assessed in 3 different sessions that were collected either within (In-win) or outside (Out-win, baseline) the transient inhibitory window created by low-frequency rTMS over the left IFC or left STS.

Materials and Methods

Participants

Thirty-three participants took part to the study. Seventeen participants (8 females) aged between 22 and 29 years (mean: 25, standard deviation [SD]: 2.2) were tested in the TMS experiment. Sixteen participants were right handed and one participant was left handed according to a standard handedness inventory (Oldfield 1971). A group of additional 16 right-handed participants (8 females) aged between 20 and 33 years (mean: 24.8, SD: 4.0) were tested in the psychophysics study. Participants received University course credit for their participation and gave their written informed consent. None of them had neurological, psychiatric, or other medical problems or had any contraindication to TMS (Rossi et al. 2009). The protocol was approved by the local ethics committee at University of Bologna and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Visual Stimuli

Stimuli were color pictures taken with a digital camera and modified by means of the Adobe Photoshop software (Adobe Systems, San Jose, CA). Images subtended a 18.53° × 12.19° region and showed 1) a static hand laying on a table (still hand), 2) a right hand in the middle of a fine grasping movement involving the index finger and the thumb (implied motion hand), 3) a frozen waterfall (still object), and 4) a flowing waterfalls (implied motion object). To minimize habituation to the images and loss of attention, 2 different exemplars of body and nonbody stimuli were presented for each condition. Body stimuli represented the right hand of a male and a female actor during a pincer grip movement. To rule out that the mere observation of graspable objects would activate per se the motor system (Chao and Martin 2000; Nelissen et al. 2005), none of the action snapshots contained any object. For each body or nonbody category, corresponding still and motion stimuli were roughly matched for color, luminance, and viewing perspective. Stimuli were adapted from a previous study (Urgesi et al. 2006, experiment 3).

Study Design

The experiment included 3 spTMS sessions in which MEPs were recorded during the observation of the different snapshots (Fig. 1): 1) a baseline session outside the inhibitory influence of rTMS (Out-win); 2) a session immediately following inhibitory rTMS over the IFC ("In-win IFC"); and 3) a session immediately following inhibitory rTMS over the STS ("In-win STS"). The 3 sessions were separated by 90 min

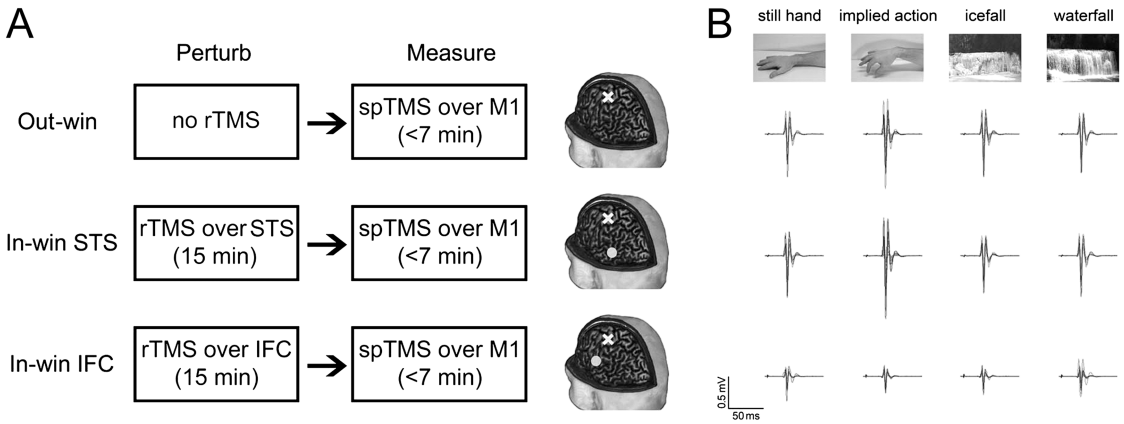

Figure 1. (*A*) Schematic representation of experimental design and TMS perturb-and-measure protocol. MEPs were recorded by means of spTMS during the observation of the visual stimuli. MEP recording was performed in 3 spTMS sessions, 1 outside (Out-win session, first row) and 2 within (In-win sessions, middle and lower rows) the influence of rTMS. In the In-win sessions, virtual lesions were applied using 1 Hz rTMS over the IFC or the STS. Talairach coordinates corresponding to the projection of the IFC or STS sites on brain surface were estimated through a neuronavigation system (IFC mean surface coordinates ± SEM: $x = -58.6 \pm 0.5$, $y = 9.4 \pm 0.5$, $z = 23.6 \pm 0.4$; STS: $x = -62.9 \pm 0.5$, $y = -52.5 \pm 0.1$, $z = 9.4 \pm 0.6$; white blobs in the head model). In all sessions, spTMS was performed by stimulating the hand representation in M1 (FDI OSP: $x = -38.2 \pm 2.9$, $y = -19.5 \pm 1.8$, $z = 56.9 \pm 2.0$; white crosses in the head model). (*B*) MEPs recorded from the FDI muscle of a representative subject during the observation of the 4 categories of stimuli. Top, middle, and low rows represent Out-win, In-win STS, and In-win IFC sessions, respectively.

(to minimize carryover effect of rTMS across sessions) and their order was counterbalanced across subjects. After the TMS sessions (at least 60 min from the last rTMS), participants provided subjective judgments about the stimuli.

Still hand and implied action stimuli depicted a right hand. Action simulation effects detected with TMS are largely contralateral with respect to the observed effectors (Aziz-Zadeh et al. 2002), thus, we hypothesized that stimulation of left M1 (with spTMS) and left IFC (with rTMS, in the In-win IFC session) would have been optimal to explore motor reactivity to right hand actions. Moreover, to avoid unwanted effects of hemispheric differences, in the In-win STS session, we stimulated the left STS. The choice of left STS was also based on a recent meta-analysis on 37 functional magnetic resonance imaging (fMRI) experiments that explored neural activity during observation of a right-hand action (Caspers et al. 2010). It was shown that while seeing right-hand actions activates a largely bilateral occipitotemporal network, the STS region was specifically active in the left and not in the right hemisphere.

EMG and spTMS Recordings

During visual stimuli presentation, MEPs induced by spTMS were recorded simultaneously from the right FDI and ADM muscles by means of a Biopac MP-150 (Biopac Corp, Goletta, CA) electromyograph. EMG signals were band-pass filtered (20 Hz–1.0 kHz, sampled at 5 kHz), digitized, and stored on a computer for off-line analysis. Pairs of silver/silver chloride surface electrodes were placed in a belly/tendon montage. Two ground electrodes were placed on the ventral surface of the right wrist.

TMS was performed with a figure-of-8 coil connected to a Magstim Rapid^2 stimulator (Magstim, Whitland, Dyfed, UK) placed over subjects' left M1. The coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline. In this way, the current induced in the underlying neural tissue was directed approximately perpendicular to the line of the central sulcus and was optimal for trans-synaptic activation of the corticospinal pathways (Brasil-Neto et al. 1992). By using a slightly suprathreshold stimulus intensity, the coil was moved over the left hemisphere to determine the optimal scalp position (OSP) from which MEPs of maximal amplitude were recorded from FDI. The OSP was then marked on a bathing cap worn by subjects to ensure correct coil placement throughout the experiment. During the experimental spTMS sessions, the intensity of magnetic pulses was set at 120% of the individual resting motor threshold (rMT), defined as the minimal intensity of the stimulator output that produces MEPs with amplitudes of at least 50 μV with 50% probability in the muscle with the higher threshold (Rossini et al. 1994). This way a stable signal could be obtained in both muscles. Mean values (% of maximum stimulator output ± SDs) of rMT were 58.5 ± 9.2%. The absence of muscle contractions was continuously verified online by visually monitoring the EMG signal.

Each spTMS session (Out-win, In-win IFC, In-win STS) included 16 trials for each condition (64 trials in total per session) presented in a randomized order. In each session, a central cross (1000 ms) indicated the beginning of a trial. On each trial, a magnetic pulse was randomly delivered between 800 and 100 ms before the end of the visual stimulus (lasting 1500 ms) to avoid any priming effects that could affect MEP size. A blank screen was shown for 3500 ms in the intertrial intervals. Each spTMS session lasted 6.4 min each. The 2 In-win spTMS sessions started 1 min after the cessation of the rTMS, and thus, in the In-win sessions, all MEPs were recorded within 7.4 min after the end of rTMS. The 1 min pause between rTMS and spTMS allowed changing the stimulating coil and setting the TMS pulse intensity. The experiment was programmed using a C++ software to control sequence and duration of images and to trigger TMS and EMG recording.

rTMS and Neuronavigation

The 2 In-win sessions were preceded by 15 min of 1 Hz rTMS (900 stimuli in total) over the target area (either left IFC or left STS). This low-frequency rTMS protocol is known to reduce the excitability and disrupt the functions related to the target area for at least 50% of the time of stimulation (Walsh and Pascual-Leone 2003; O'Shea et al. 2007; Serino et al. 2011; Avenanti et al. 2012). Since the entire In-win sessions were performed within 7.4 min after the end of rTMS, all MEPs in such sessions were recorded well within the temporal window of reduced excitability created by 1 Hz rTMS. A subthreshold stimulation intensity was used (90% of rMT), and subjects were asked to keep their muscles as relaxed as possible during the rTMS as contraction may reduce the inhibitory effect of rTMS on motor excitability (Touge et al. 2001).

Coil position was identified on each participant's scalp with the SofTaxic Navigator system (EMS, Italy) as in our previous TMS research (Avenanti et al. 2007; Urgesi et al. 2007; Bertini et al. 2010; Serino et al. 2011). Skull landmarks (nasion, inion, and 2 preauricular points) and about 60 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra Optical Tracking System (NDI, Canada). Coordinates in Talairach space were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template. The IFC was targeted in the anterior ventral aspect of the precentral gyrus (ventral premotor cortex) at the border with the pars opercularis of the inferior frontal gyrus (coordinates: x = –52, y = 10, z = 24), corresponding to Brodmann's area 6/44 (Mayka et al. 2006; Avenanti et al. 2007; Gazzola et al. 2007; Urgesi et al. 2007; Van Overwalle et al.

2009; Caspers et al. 2010). The STS was targeted in its posterior aspect (x = −52, y = −53, z = 9, corresponding to Brodmann's area 21; Van Overwalle and Baetens 2009; Caspers et al. 2010). Scalp positions were identified by means of the SofTaxic Navigator system and marked on the bathing cap with a pen. Moreover, the neuronavigation system was used to estimate the projections of the TMS sites (IFC, STS, M1) on the brain surface (Fig. 1). No adverse effects during (subthreshold) 1 Hz rTMS were reported or noticed in any subjects.

Psychophysical Testing

At least 1 h after the last TMS session (thus outside the influence of rTMS), all the experimental stimuli were presented in a randomized order, and participants were asked to rate the strength of the implied motion sensation induced by each image. The 1-h interval was adopted to be sure that rTMS effects had faded away and could not influence subjective ratings. Subjects rated the stimuli by marking a vertical 10 cm visual analogue scale (VAS) with 0 cm indicating "no effect" and 10 cm "maximal effect imaginable." Stimuli were presented for 1.5 s each on the same monitor as in the TMS experiment.

To further assess implied motion in the absence of any rTMS, an additional group of 16 healthy subjects not participating to the TMS experiment was asked to rate along a VAS the strength of the implied motion sensation induced by the visual stimuli.

Data Analysis

Neurophysiological data were processed off-line. Trials with EMG activity exceeding 50 μV in a window of 100 ms prior to the TMS pulse were discarded from the analysis (<4%). One subject was removed from the analysis due to a high number of precontraction artifacts (~40%); thus all the analyses were carried out on a sample of 16 subjects. The removal of the left-handed subject from this sample did not change the pattern of results (not shown in the paper). Mean MEP amplitude values in each condition were measured peak-to-peak (in millivolts). For each muscle and each condition, MEPs with amplitude deviating from the mean by more than 2.0 SD were removed from the analysis (<2%).

Raw MEPs values were analyzed by means of a four-way repeated measures analysis of variance (ANOVA) with Session (Out-win, In-win STS, In-win IFC), Muscle (FDI, ADM), Object (Hand, Fall), and Motion (Still, Implied Motion) as within-subjects factors. To quantify the amount of "resonant" facilitation in the Out-win and In-win sessions, an action observation facilitation index was computed [(implied action − static hand)/(static hand)] for each session and muscle, separately. To assess how rTMS perturbation affected corticospinal responses to implied actions, a Session × Muscle ANOVA on the action facilitation index was performed. VAS measures were submitted to Object × Motion ANOVAs. In all ANOVAs, post hoc analysis was carried out using Duncan test correction for multiple comparisons. A correlational analysis was performed between action facilitation indices and VAS judgments (implied action − static hand) in the 3 different sessions using the Pearson's r coefficient.

Results

Suppression of IFC, but Not of STS Activity, Reduces Corticospinal Excitability

In 3 spTMS sessions (Out-win, In-win STS, In-win IFC), participants were asked to observe still hand, implied action (fine grasping), icefall, and waterfall visual stimuli, and MEPs were simultaneously recorded from the right FDI and the ADM muscle (see Fig. 1*A*).

The Session × Muscle × Object × Motion ANOVA on MEP amplitudes revealed a main effect of Muscle ($F_{1,15}$ = 6.92, P = 0.02; higher amplitudes in the FDI than in the ADM, mean ± standard error of the mean [SEM]: 0.93 mV ± 0.16 vs. 0.60 mV ± 0.12). Importantly, a significant main effect of Session ($F_{2,30}$ = 5.84, P = 0.007) was also found. This effect was accounted for by the lower MEP amplitude recorded in the In-win IFC (0.59 mV ± 0.09) than in the Out-win (0.83 mV ± 0.15; P = 0.02) and the In-win STS sessions (0.89 mV ± 0.16; P = 0.008), which in turn did not differ from one another (P = 0.5; see Table 1). Thus, overall, rTMS over IFC induced a reduction of M1 excitability. This inhibitory effect was equally present in the FDI and the ADM since the interaction Session × Muscle was not significant (P = 0.9). These findings confirm that suppression of IFC reduces the excitability of hand representation in M1 (Avenanti et al. 2007) and suggest that at rest, the IFC may exert a facilitatory influence on M1 (Shimazu et al. 2004).

Table 1
Effect of rTMS on corticospinal excitability (across visual conditions)

	Out-win	In-win STS	In-win IFC
FDI	1.00 ± 0.20	1.07 ± 0.21	0.73 ± 0.10
ADM	0.65 ± 0.14	0.71 ± 0.16	0.44 ± 0.11

Note: MEP amplitudes (in millivolts) ± SEM recorded from the 2 muscles in the 3 different sessions. In both muscles, MEPs recorded in the In-win IFC sessions were lower than MEPs recorded in the other 2 sessions indicating that suppression of IFC brought about a reduction of hand corticospinal excitability.

Effect of rTMS on Motor Reactivity to Visual Input

The ANOVA also showed higher order interactions, including the quadruple Session × Muscle × Object × Motion interaction ($F_{2,30}$ = 6.00, P = 0.006). To further analyze this interaction, 2 follow-up Session × Object × Motion ANOVAs were carried out separately for the 2 muscles.

The ANOVA performed on MEPs recorded from the ADM muscle (control) revealed only a main effect of Session ($F_{2,30}$ = 3.42, P = 0.05; Table 1) but no other main effects or interactions (all P > 0.2), indicating a lack of modulation due to the different observational conditions.

In contrast, the ANOVA on MEPs recorded from the FDI muscle (target) showed the main effect of Session ($F_{2,30}$ = 3.39, P = 0.05; Table 1) and Motion ($F_{1,15}$ = 8.47, P = 0.01). Crucially, the triple interaction Session × Object × Motion was significant ($F_{2,30}$ = 9.04, P = 0.0008; Fig. 1*B*). Post hoc analysis showed that in the Out-win (Baseline) session (Fig. 2*A*), MEPs recorded from the FDI muscle were higher during observation of implied action than when watching static hand (P = 0.02), icefall (P = 0.05), and waterfall (P = 0.02) stimuli, which in turn did not differ from one another (all P > 0.6).

Similar but stronger modulations were found in the In-win STS session (Fig. 2*B*): MEPs from the FDI were higher during observation of implied actions than during observation of static hand (P < 0.0001), icefall (P = 0.0002), and waterfall stimuli (P = 0.0001), which in turn did not differ from one another (all P > 0.4). Notably, pairwise comparisons between the Out-win and the In-win STS sessions revealed that MEPs during implied actions were greater after suppression of STS than in the baseline session (all P < 0.004); MEPs in the 2 sessions were comparable for the other 3 control conditions (all P > 0.3).

In the In-win IFC sessions (Fig. 2*C*), MEPs from the FDI were in general lower than in the other 2 sessions (for all pairwise comparisons, P < 0.002), and, importantly, they were not modulated by the different observational conditions (all P > 0.2).

In sum, as expected, the observation of implied body actions in the absence of any rTMS interference with the activity of IFC or STS (Out-win baseline session), selectively facilitated the

corticospinal representation of the muscle (FDI) that would be recruited during performance of the observed motor act but not of a hand muscle (ADM) that was not involved in the observed motor act (Urgesi et al. 2010). Importantly, suppression of STS induced a motor facilitation greater than in the baseline session, which strikingly contrasts with the lack of motor facilitation induced by suppression of IFC. No modulation was found during the observation of static or implied motion nonbody stimuli either in the Out-win or in the In-win sessions.

Effect of rTMS on Anticipatory Action Simulation

The main analysis indicates that STS disruption increases the motor facilitation to implied actions. To quantify the amount of

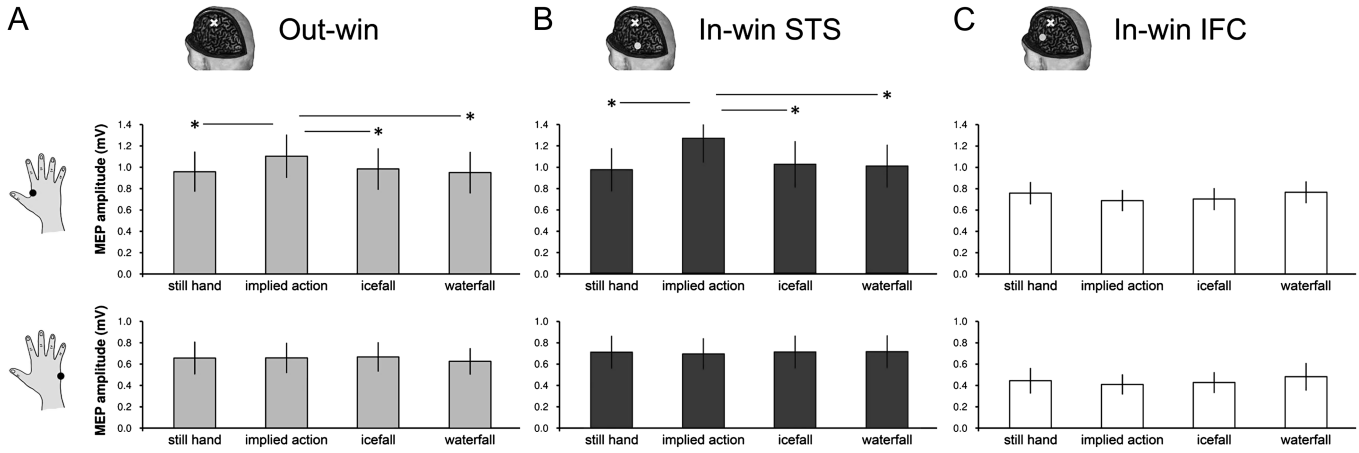

Figure 2. MEPs recorded from the FDI (top) and the ADM (bottom) muscle in the 3 different spTMS sessions. (*A*) Out-win, (*B*) In-win STS, and (*C*) In-win IFC. Asterisks indicate significant post hoc comparisons. Only within sessions, comparisons are represented, see main text for further pairwise comparisons between sessions. Error bars denote SEM.

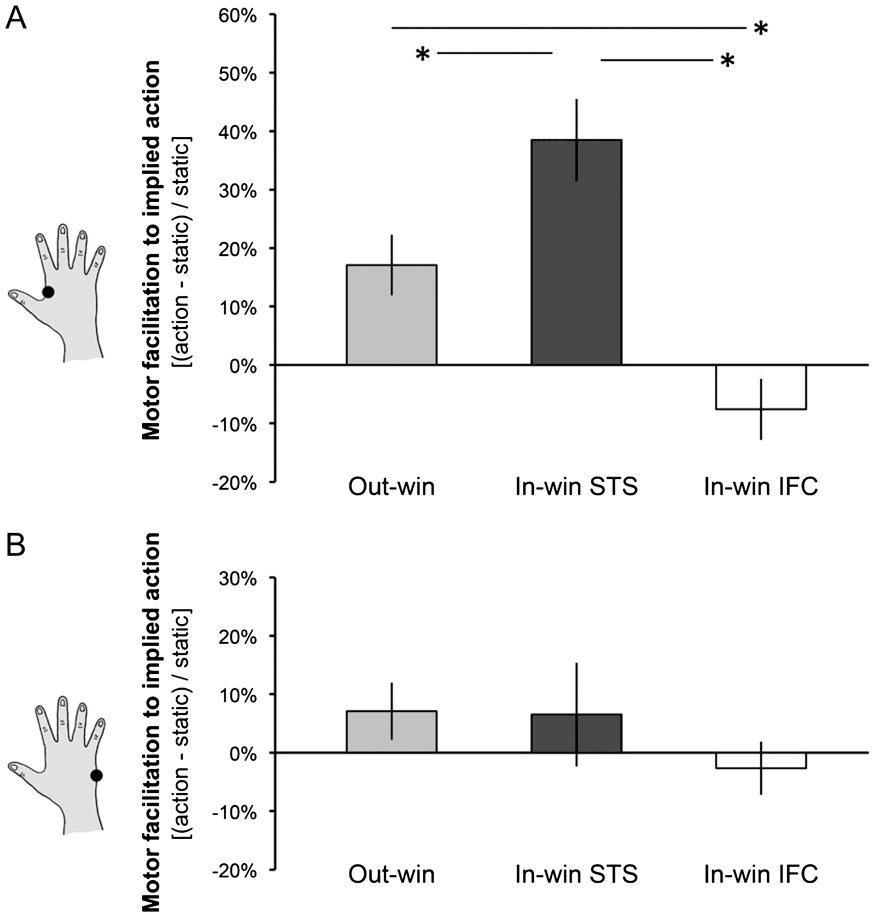

Figure 3. Motor facilitation to implied action stimuli recorded from the (*A*) FDI and (*B*) ADM muscle in the 3 different sessions. Asterisks indicate significant post hoc comparisons. Error bars denote SEM.

changes in motor facilitation due to IFC and STS perturbation, a further analysis was conducted on facilitation ratios [(implied action – still hand)/still hand] computed in the 3 sessions. Facilitation ratios were calculated for the FDI (target) and, to test muscle specificity, for the ADM muscle (control). These indices were entered into a repeated measure Muscle × Session ANOVA (Fig. 3). The analysis showed a main effect of Session ($F_{2,30}$ = 10.43, P = 0.0004), a main effect of Muscle ($F_{1,15}$ = 9.09, P = 0.009), and, importantly, a significant Muscle × Session interaction ($F_{2,30}$ = 6.20, P = 0.006). The facilitation of the FDI muscle (Fig. 3*A*) in the Out-win session (mean facilitation ratio ± SEM: 17% ± 5) was greater than in the In-win IFC session (–8% ± 5; P = 0.02). Crucially, in the In-win STS session, the facilitation (38% ± 6) was greater than in the Out-win (P = 0.02) and In-win IFC (P < 0.0001) sessions. Thus, disruption of IFC neural activity reduced motor facilitation more than 1 SD as compared to its baseline level (large effect size, d = 1.27), while STS activity increased motor facilitation more than 1 SD than its baseline level (large effect size, d = 0.90). No modulation was found in the facilitation index computed on the ADM muscle (P > 0.3; Fig. 3*B*).

Subjective Data

At least 1 h after the last TMS session (thus outside the influence of rTMS), participants used VAS to rate the strength of the movement sensation induced by the visual stimuli. The Object × Motion ANOVA on VAS ratings of implied motion sensation showed a significant main effect of Motion ($F_{1,15}$ = 132.00, P < 0.0001) indicating that implied motion stimuli (mean VAS rating ± SEM: 6.93 cm ± 0.37) were rated as more "dynamic" than still stimuli (1.47 cm ± 0.25); this effect was present for both the hand and the fall stimuli as evinced by the nonsignificant Object × Motion interaction (P = 0.9). The main effect of Object was not significant (P = 0.09; Table 2).

These findings were replicated in a further psychophysical experiment conducted on an additional group of 16 subjects who did not participate in the TMS experiment (Main effect of Motion: $F_{1,15}$ = 263.59, P < 0.0001; no main effect or interaction with factor Object: P > 0.3; Table 2). Moreover, a further mixed-model Group × Object × Motion ANOVA (including the group of subjects tested after TMS and the one tested only in the psychophysical experiment) revealed only a main effect of Motion ($F_{1,30}$ = 349.81, P < 0.0001) but no main effect or interaction with factor Group (P > 0.3). This rules out that subjective ratings in the TMS experiment were the results of the long exposure to the visual stimuli or of brain stimulation.

In the TMS experiment, we also investigated the relation between motor response to observed pictures of implied actions and the strength of the movement sensation induced by such images. Correlations between action simulation indices (facilitation ratios computed separately for each session and muscle) and VAS ratings of implied motion were not significant (–0.04 < r < 0.39, P > 0.1). However, after the removal of one outlier (with standard residuals > 2 sigma), we found a significant positive relation between action simulation index (FDI facilitation ratios) and subjective ratings. In the Out-win session, stronger FDI facilitation was found for those subjects who attributed more implied motion to hand stimuli (r = 0.72, P = 0.003; Fig. 4*A*). A similar relation was found in the In-win STS session (r = 0.56, P = 0.03; Fig. 4*B*) but not in the In-win IFC session (r = 0.22, P = 0.4; Fig. 4*C*). No significant correlations were found between ADM modulations and subjective ratings of implied motion (–0.11 < r < 0.28, P > 0.3).

Table 2
Subjective report of implied motion

	Still hand (body static)	Implied action (body implied motion)	Icefalls (nonbody static)	Waterfall (nonbody implied motion)
TMS experiment	0.94 ± 0.29	6.45 ± 0.47	2.00 ± 0.53	7.41 ± 0.60
Psychophysical experiment	1.44 ± 0.36	6.34 ± 0.52	1.55 ± 0.54	7.37 ± 0.45

Note: Mean VAS ratings (in centimeters) ± SEM. The top row reports data collected in the TMS experiment (1 h after the end of the last TMS session). The bottom row reports data collected in the psychophysical experiment.

Discussion

Frontal and parietal cortices are activated during both action observation and execution. Unlike what happens during action execution, observing actions activates neurons in the temporal region, STS, thought to be crucial for biological motion perception and for providing the frontoparietal AON with high-order visual representations of the observed actions (Keysers and Perrett 2004; Rizzolatti and Craighero 2004; Nelissen et al. 2011). While previous "virtual" or real lesion studies have shown that both IFC (Pobric and Hamilton 2006; Avenanti et al. 2007; Urgesi et al. 2007; Moro et al. 2008; Pazzaglia et al. 2008;

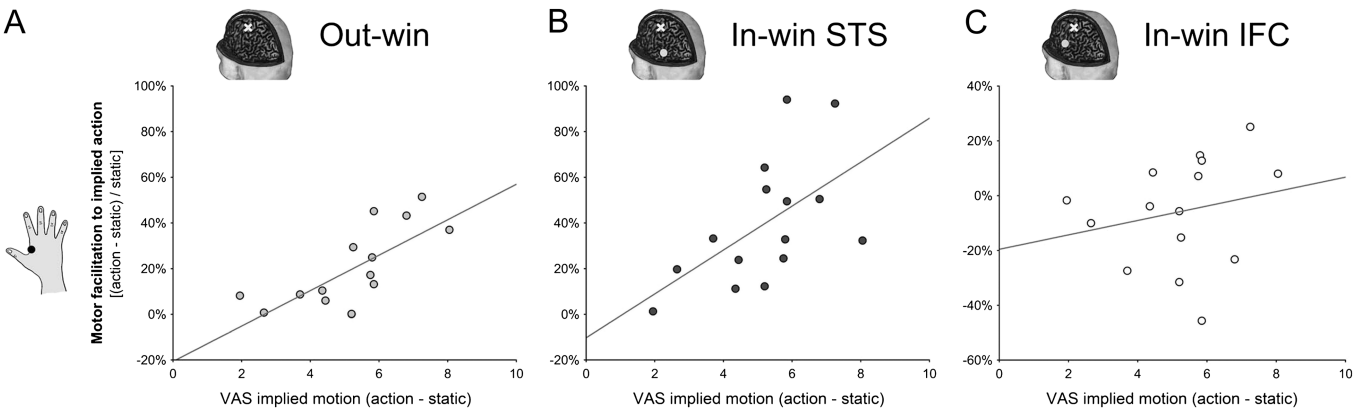

Figure 4. Relation between FDI motor facilitation to implied action and subjective perception of implied motion. Facilitation index computed in (*A*) Out-win, (*B*) In-win STS, and (*C*) In-win IFC sessions.

Tidoni et al. 2012) and STS (Grossman et al. 2005; Saygin 2007; Candidi et al. 2011) are essential in observed action representation, the specific role of the frontal and temporal areas in the process of implied action simulation remains unclear.

We explored this issue by using a perturb-and-measure paradigm based on the combination of rTMS and spTMS. Low-frequency rTMS was applied to transiently suppress cortical activity either within (IFC) or upstream (STS) the frontoparietal AON. SpTMS was used to assess the reactivity of the corticospinal system during observation of implied action stimuli either within (In-win sessions) or outside (Out-win) the influence of the "virtual lesions" induced by rTMS. We found that the motor facilitation contingent upon observation of implied stimuli was disrupted by the suppression of IFC, demonstrating that the anticipatory simulation in M1 is critically linked to the activity of the anterior node of the AON. Importantly, our paradigm allowed testing 2 alternative hypotheses about the functional architecture of the AON. In striking contrast to a passive feed-forward architecture model (hypothesis B in the Introduction), we found that the disruption of STS region resulted in an enhanced motor simulation, which clearly hints at an active role of the frontoparietal AON in action simulation (hypothesis A in the Introduction). Thus, we provide direct causative evidence of a functional interplay between IFC/STS and M1 during extrapolation of dynamic action-related information from static images.

These findings provide neurophysiological support to the predictive theories of action perception (Wilson and Knoblich 2005; Kilner et al. 2007; Schubotz 2007; Schütz-Bosbach and Prinz 2007; Gazzola and Keysers 2009; Friston et al. 2011; Press et al. 2011; Schippers and Keysers 2011) according to which the AON is organized as a dynamic control system where information can flow not only from visual (STS) to visuomotor (frontoparietal) nodes but also in the opposite direction, that is, from IFC to STS. In this vein, watching an action activates stored motor representations (in frontoparietal nodes) that provide an internal forward model of the ongoing action. These representations are likely used for predicting the future course of the observed action and for achieving a degree of perceptual stability sufficient to deal with any perceptual ambiguity derived from discontinuities in the sensory input. These theories predict that a gap of visual information would require increased activity in the motor system in order to guarantee stable action perception (Wilson and Knoblich 2005; Aglioti and Pazzaglia 2011; Avenanti and Urgesi 2011; Friston et al. 2011; Schippers and Keysers 2011).

Perception of Implied Actions Triggers the Simulation of Their Future

Influential theoretical models suggest that the human motor system is designed to work as an "anticipation device" and that humans predict forthcoming actions by using their own motor system as an internal forward model (Wolpert et al. 2003; Schütz-Bosbach and Prinz 2007; Gazzola and Keysers 2009). In keeping, human and monkey evidence suggests activations of the motor system contingent upon action observation may 1) occur prior to the observation of a predictable motor act (Umiltà et al. 2001; Kilner et al. 2004; Fogassi et al. 2005; Aglioti et al. 2008; Avenanti, Minio-Paluello, Sforza et al. 2009) and 2) show an anticipatory bias in the simulation of the upcoming phases of observed actions (Gangitano et al. 2004; Borroni et al. 2005). Anticipatory simulation is particularly evident during processing of implied actions where muscle-specific motor facilitation is maximal for static images depicting initial and middle phases of a given action (that correspond to the initial muscular involvement during the actual execution of the action) and reduced for its final posture (that corresponds to the maximal muscular involvement during execution) (Urgesi et al. 2006; Urgesi et al. 2010). These findings indicate that motor facilitation is maximal during extrapolation of dynamic information about the upcoming action phases and suggest that M1 is preferentially activated by the anticipatory simulation of future action phases.

In keeping, the Out-win session of the present study (outside the inhibitory effect of rTMS) shows that watching static pictures of an ongoing fine grasping increased the amplitude of MEPs recorded from the FDI muscle, which is recruited during execution of the very same action (Fadiga et al. 2005; Urgesi et al. 2010). Importantly, greater muscle-specific motor facilitation was found in participants who provided greater ratings of implied motion, suggesting a link between neurophysiological markers of action simulation and the subjective perception of implied motion. Tellingly, no motor modulation was found when observing static (icefall) or implied motion (waterfall) nonbody stimuli, although a comparable modulation of implied motion ratings was found for nonbody and hand stimuli. This suggests that the recruitment of the motor system during implied action perception does not reflect a nonspecific response to the presence of implied motion in the scene (i.e., in nonhuman entities), but the process of deriving dynamic information from static images that imply ongoing human body actions. Our perturb-and-measure paradigm highlights the IFC as a critical neural locus for this selective processing, as outlined in the next paragraph.

Suppression of IFC Disrupts Anticipatory Action Simulation

Monkeys' premotor cortices are known to modulate corticospinal activity through indirect corticocortical connections (Shimazu et al. 2004) as well as direct corticospinal connections (Dum and Strick 1991; Kraskov et al. 2009). In humans, the functional contribution of the IFC on M1 activity is evident during action preparation and execution (Uozumi et al. 2004; Davare et al. 2009); moreover, studies suggest that during precision grasping the IFC sends muscle-specific signals to M1 in order to execute the grasp (Cattaneo et al. 2005; Davare et al. 2009). Similar corticocortical neural interactions are thought to be at play during covert motor simulation (Fadiga et al. 2005; Fourkas et al. 2008; Avenanti, Minio-Paluello, Bufalari, et al. 2009; Koch et al. 2010; Catmur et al. 2011). It is also worth noting that action observation, execution, and imitation bring about a comparable sequential activation of IFC and M1 (Nishitani and Hari 2002; Nishitani et al. 2004). Importantly, real (Saygin 2007; Moro et al. 2008; Pazzaglia et al. 2008; Fazio et al. 2009) or virtual lesions (Pobric and Hamilton 2006; Urgesi et al. 2007; Tidoni et al. 2012) of the IFC have been shown to disrupt action recognition (Avenanti and Urgesi 2011) and imitation (Heiser et al. 2003), highlighting the critical role of the frontal node of the AON in the internal representation of observed actions. While providing evidence for a clear role of motor regions in visual action perception and imitation, the above studies do not clarify the specific functional influence of IFC on the motor mapping of implied actions.

Based on the notion that IFC and other motor regions are activated by implied action observation (Nishitani and Hari 2002; Johnson-Frey et al. 2003; Proverbio et al. 2009), in the present study, we applied low-frequency rTMS to IFC and tested any modulation of corticospinal motor reactivity consequent to implied action stimuli. We found that motor facilitation occurring during observation of static images of hand conveying action information was abolished by rTMS over IFC. Moreover, after IFC-rTMS, motor response to implied actions was not correlated to the perceived sensation of motion implied in such stimuli. The lack of MEP modulation after suppression of IFC shows that the activity of the frontal node of the AON is crucial for encoding implied action stimuli in the observers' motor system. This result complements and extends previous studies showing that IFC is selectively involved in visual discrimination of biological dynamic (Pobric and Hamilton 2006; Saygin 2007; Tidoni et al. 2012) and implied actions (Urgesi et al. 2007; Moro et al. 2008) and indicates that the anterior node of the AON plays a critical role in the basic visuomotor encoding of action information extrapolated from static body postures. It is likely that other neural regions coupling action perception and execution (e.g., parietal regions) may participate to this predictive motor coding and further perturb-and-measure studies would directly test this hypothesis.

It should be noted that suppression of IFC but not of STS also induced a general reduction of MEP amplitude from both the FDI and the ADM muscles, in keeping with evidence that the former but not the latter region contains a hand motor representation functionally related to M1 (Rizzolatti and Luppino 2001; Uozumi et al. 2004; Davare et al. 2009). These findings support the notion that inhibiting hand representations in premotor regions reduces hand corticospinal excitability (Gerschlager et al. 2001; O'Shea et al. 2007) and further establish the facilitatory functional connectivity between IFC and M1 (Shimazu et al. 2004; Avenanti et al. 2007). The disruption of action simulation observed after IFC-rTMS, however, is unlikely to be due to the indirect inhibitory effect of IFC-rTMS on M1 activity. Indeed, we have previously shown that although both IFC-rTMS and M1-rTMS induce a reduction of corticospinal excitability, suppression of IFC but not of M1 disrupts the action observation motor facilitation (Avenanti et al. 2007). Moreover, stimulation of IFC, but not of M1, may influence action perception (Avenanti and Urgesi 2011; Cattaneo et al. 2011). Taken together, these findings provide direct causative evidence for the notion that action simulation mechanisms in M1 passively reflect computations carried out in the AON and in particular in its frontal node (Fadiga et al. 2005; Avenanti et al. 2007; Schütz-Bosbach et al. 2009).

Suppression of STS Enhances Anticipatory Action Simulation

A major point of novelty of the present study concerns the functional interplay between frontotemporal brain regions involved in action perception and motor simulation in M1.

Middle/superior temporal cortices are typically activated during the visual experience of real, illusory, or implied motion of animate as well as inanimate entities (Tootell et al. 1995; Kourtzi and Kanwisher 2000; Senior et al. 2000). In particular, the activity of STS has been selectively associated to the processing of biological motion (Grossman et al. 2000; Keysers and Perrett 2004; Peelen et al. 2006) and of implied body movements (Peigneux et al. 2000; Jellema and Perrett 2003). Studies suggest that STS integrates body form and motion information from ventral and dorsal pathways (Vaina et al. 2001; Giese and Poggio 2003) to create a high-order visual representation of others' actions. This representation is visual in nature as neurons in STS do not respond to action execution (Keysers and Perrett 2004; Rizzolatti and Craighero 2004). Importantly neurons in STS seem to be able to compute action anticipation based on visual information alone (Perrett et al. 2009).

A plausible scenario is that during action observation, visually derived movement-related information is sent from STS to parietal and IFC regions where visuomotor coupling takes place. The output of such computational process is then sent to M1 (Nishitani and Hari 2002; Nishitani et al. 2004) and can feed back in perceptual systems (Wilson and Knoblich 2005; Schippers and Keysers 2011). While it is held that the frontoparietal AON receives action-related visual information processed in STS, no previous studies have directly explored action simulation in M1 (reflecting the anticipatory activity of frontoparietal AON) after the inhibition of STS.

Our findings speak against the hypothesis that the AON is organized as a pure feed-forward system where frontoparietal regions passively reflect computations occurring in STS (hypothesis B; Hickok 2009) and rather support the notion that the AON is a dynamic control system (hypothesis A) where the frontoparietal nodes actively compute anticipatory action simulations de novo. We found that disruption of STS leads to an increase of corticospinal reactivity to implied actions, in keeping with the notions that involvement of motor system is greater when perceptual information is noisy (D'Ausilio et al. 2011), and internal models of action may contribute to filling-in missing or ambiguous perceptual information (Kilner et al. 2007; Gazzola and Keysers 2009; Friston et al. 2011; Schippers and Keysers 2011).

This result suggests that, given the rTMS induced noise in STS, the frontal node of AON compensates for any gap of implied action–related visual information by enhancing its anticipatory simulative properties. Such an active, compensatory function indicates that visual perception of actions may be sustained by the simulative computations likely occurring in the frontal node of the AON (Wilson and Knoblich 2005; Schütz-Bosbach and Prinz 2007; Aglioti and Pazzaglia 2011; Avenanti and Urgesi 2011). In keeping, while neuromagnetic studies have reported that during action observation, there is a sequential cortical activation from STS to parietal and frontal regions (Nishitani and Hari 2002; Nishitani et al. 2004), a recent fMRI study suggests that information within the AON may also flow from IFC to parietal and STS regions (Schippers and Keysers 2011). Such action-related information flow may be particularly relevant for compensating the noisy STS processing induced by rTMS and reflect the predictive information flow from premotor to STS regions hypothesized by forward models.

Before accepting this interpretation, a critical methodological issue needs to be discussed. Suprathreshold TMS over STS can activate the temporal fascia muscle and may induce discomfort, at least in some subjects (Cattaneo et al. 2010). It may thus be that unspecific factor (e.g., increased vigilance due to STS stimulation) may explain the increase motor response to action stimuli in the In-win STS session. We find this alternative hypothesis unlikely. First, off-line rTMS is thought to minimize unspecific effects due to scalp sensations (Walsh and Pascual-Leone 2003), and in our study, MEPs were collected after 1 min from the end of rTMS. Second, no discomfort or aversive effects

of stimulation were reported or noticed in any subjects during rTMS, likely due to our subthreshold simulation intensity. Critically, also IFC stimulation may activate (facial) muscles and in principle result in increased vigilance. However, in the In-win IFC session, we found a disruption, not an enhancement, in the MEP facilitation to implied action. Moreover, in a previous perturb-and-measure TMS study, we found that 1 Hz rTMS over IFC (using even higher stimulation intensity) disrupted MEP facilitation to biomechanically possible actions (i.e., actions that could be performed by the observers, like those used in the present study) but did not affect the MEP facilitation to actions representing extreme stretching movements (biomechanically impossible actions) (Avenanti et al. 2007) whose facilitation relied on the somatosensory cortex. These findings speak against the possibility that potentially discomforting scalp sensations due to rTMS result in an increase in motor reactivity and suggest that the enhancement of action simulation observed in the present experiment was specifically due to disruption of neural processing in STS.

The Future of Seen Action in the AON

While we focused on 2 key nodes of the AON, other regions of the network may contribute to anticipatory action simulation. Low-frequency rTMS can modulate activity in remote interconnected regions (Gerschlager et al. 2001; Paus 2005; O'Shea et al. 2007; Avenanti et al. 2012). Thus, it is possible that rTMS over STS or IFC modulated activity in other visual (e.g., area MT) or visuomotor (e.g., intraparietal) interconnected regions and that these regions contributed to the observed effects. At any rate, our data demonstrate a clear dissociation in action simulation when virtual lesions are applied to the STS or IFC sites that are typically active during action observation (as indicated by brain imaging meta-analyses, Van Overwalle and Baetens 2009; Caspers et al. 2010). Interestingly, a recent TMS study has suggested that also a more anterior sector of STS may be critically involved in action perception (Cattaneo et al. 2010). Future perturb-and-measure studies are needed to test whether disruption of other sectors of STS (or IFC) may induce changes in action simulation similar to those observed in the present experiment.

Our study supports the notion that the functional role of motor activation during action perception is based on predictive coding. This process may allow to understand the goal of an action and ultimately to perform an anticipatory readout of the intention behind the action (Rizzolatti and Craighero 2004; Fogassi et al. 2005; Friston et al. 2011; Press et al. 2011) as well as to anticipate the future phases of upcoming actions of others (Wilson and Knoblich 2005; Schütz-Bosbach and Prinz 2007; Aglioti and Pazzaglia 2011; Avenanti and Urgesi 2011).

Predictive theories of action perception propose that the observer's motor system generates anticipatory representations of others' actions by projecting the course of ongoing movements into the future. These predictions are then fed back into perceptual systems (e.g., in STS) that create top-down expectations and constrain visual perception. According to this view, action simulation mechanisms are called into play to solve the computational challenges posed by action perception, that is, to fill-in missing or ambiguous visual information and to provide an anticipatory representation of ongoing actions ahead of their realization (Wilson and Knoblich 2005; Schütz-Bosbach and Prinz 2007; Aglioti and Pazzaglia 2011; Avenanti and Urgesi 2011; Friston et al. 2011; Schippers and Keysers 2011). By showing enhanced action simulation after suppression of visual processing in STS our study provides neurophysiological evidence for a role of frontoparietal AON in implementing compensatory action simulation mechanisms that may be fundamental for perceiving and predicting others' actions.

Our study shows that dynamic action–related information is extracted from static images and mapped onto the motor system to provide forward anticipatory representations of ongoing actions. Moreover, the study highlights the active, compensatory, and predictive nature of the simulation triggered by perception of implied actions.

Funding

A.A., C.U., and S.M.A. are funded by the Istituto Italiano di Tecnologia SEED 2009 (Protocol Number 21538); A.A. is also funded by grants from Ministero Istruzione Università e Ricerca (Progetti di Ricerca di Interesse Nazionale, PRIN 2008) and University of Bologna (Ricerca Fondamentale Orientata); S.M.A and C.U. are also funded by grants from the Ministero Istruzione Università e Ricerca (Progetti di Ricerca di Interesse Nazionale, PRIN 2009)

Notes

Conflict of Interest: None declared.

References

Aglioti SM, Cesari P, Romani M, Urgesi C. 2008. Action anticipation and motor resonance in elite basketball players. Nat Neurosci. 11:1109–1116.

Aglioti SM, Pazzaglia M. 2011. Sounds and scents in social action. Trends Cogn Sci. 15:47–55.

Avenanti A, Bolognini N, Maravita A, Aglioti SM. 2007. Somatic and motor components of action simulation. Curr Biol. 17:2129–2135.

Avenanti A, Coccia M, Ladavas E, Provinciali L, Ceravolo MG. 2012. Low-frequency rTMS promotes use-dependent motor plasticity in chronic stroke: a randomized trial. Neurology. 78:256–264.

Avenanti A, Minio-Paluello I, Bufalari I, Aglioti SM. 2009. The pain of a model in the personality of an onlooker: influence of state-reactivity and personality traits on embodied empathy for pain. Neuroimage. 44:275–283.

Avenanti A, Minio-Paluello I, Sforza A, Aglioti SM. 2009. Freezing or escaping? Opposite modulations of empathic reactivity to the pain of others. Cortex. 45:1072–1077.

Avenanti A, Urgesi C. 2011. Understanding 'what' others do: mirror mechanisms play a crucial role in action perception. Soc Cogn Affect Neurosci. 6:257–259.

Aziz-Zadeh L, Maeda F, Zaidel E, Mazziotta J, Iacoboni M. 2002. Lateralization in motor facilitation during action observation: a TMS study. Exp Brain Res. 144:127–131.

Bertini C, Leo F, Avenanti A, Làdavas E. 2010. Independent mechanisms for ventriloquism and multisensory integration as revealed by theta-burst stimulation. Eur J Neurosci. 31:1791–1799.

Borroni P, Montagna M, Cerri G, Baldissera F. 2005. Cyclic time course of motor excitability modulation during the observation of a cyclic hand movement. Brain Res. 1065:115–124.

Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M. 1992. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J Clin Neurophysiol. 9:132–136.

Candidi M, Stienen BM, Aglioti SM, de Gelder B. 2011. Event-related repetitive transcranial magnetic stimulation of posterior superior temporal sulcus improves the detection of threatening postural changes in human bodies. J Neurosci. 31:17547–17554.

Candidi M, Vicario CM, Abreu AM, Aglioti SM. 2010. Competing mechanisms for mapping action-related categorical knowledge and observed actions. Cereb Cortex. 20:1018–1022.

Caspers S, Zilles K, Laird AR, Eickhoff SB. 2010. ALE meta-analysis of action observation and imitation in the human brain. Neuroimage. 50:1148–1167.
Catmur C, Mars RB, Rushworth MF, Heyes C. 2011. Making mirrors: premotor cortex stimulation enhances mirror and counter-mirror motor facilitation. J Cogn Neurosci. 23:2352–2362.
Cattaneo L, Barchiesi G, Tabarelli D, Arfeller C, Sato M, Glenberg AM. 2011. One's motor performance predictably modulates the understanding of others' actions through adaptation of premotor visuo-motor neurons. Soc Cogn Affect Neurosci. 6:301–310.
Cattaneo L, Sandrini M, Schwarzbach J. 2010. State-dependent TMS reveals a hierarchical representation of observed acts in the temporal, parietal, and premotor cortices. Cereb Cortex. 20:2252–2258.
Cattaneo L, Voss M, Brochier T, Prabhu G, Wolpert DM, Lemon RN. 2005. A cortico-cortical mechanism mediating object-driven grasp in humans. Proc Natl Acad Sci U S A. 102:898–903.
Chao LL, Martin A. 2000. Representation of manipulable man-made objects in the dorsal stream. Neuroimage. 12:478–484.
Chong TT, Cunnington R, Williams MA, Kanwisher N, Mattingley JB. 2008. fMRI adaptation reveals mirror neurons in human inferior parietal cortex. Curr Biol. 18:1576–1580.
Davare M, Montague K, Olivier E, Rothwell JC, Lemon RN. 2009. Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. Cortex. 45:1050–1057.
D'Ausilio A, Bufalari I, Salmas P, Fadiga L. 2011. The role of the motor system in discriminating normal and degraded speech sounds. Cortex. doi: 10.1016/j.cortex.2011.05.017.
di Pellegrino G, Fadiga L, Fogassi L, Gallese V, Rizzolatti G. 1992. Understanding motor events: a neurophysiological study. Exp Brain Res. 91:176–180.
Dum RP, Strick PL. 1991. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci. 11:667–689.
Etzel JA, Gazzola V, Keysers C. 2008. Testing simulation theory with cross-modal multivariate classification of fMRI data. PLoS One. 311:e3690.
Fadiga L, Craighero L, Olivier E. 2005. Human motor cortex excitability during the perception of others' action. Curr Opin Neurobiol. 15:213–218.
Fazio P, Cantagallo A, Craighero L, D'Ausilio A, Roy AC, Pozzo T, Calzolari F, Granieri E, Fadiga L. 2009. Encoding of human action in Broca's area. Brain. 132:1980–1988.
Fogassi L, Ferrari PF, Gesierich B, Rozzi S, Chersi F, Rizzolatti G. 2005. Parietal lobe: from action organization to intention understanding. Science. 308:662–667.
Fourkas AD, Bonavolontà V, Avenanti A, Aglioti SM. 2008. Kinesthetic imagery and tool-specific modulation of corticospinal representations in expert tennis players. Cereb Cortex. 18:2382–2390.
Friston K, Mattout J, Kilner J. 2011. Action understanding and active inference. Biol Cybern. 104:137–160.
Gallese V, Fadiga L, Fogassi L, Rizzolatti G. 1996. Action recognition in the premotor cortex. Brain. 119:593–609.
Gangitano M, Mottaghy FM, Pascual-Leone A. 2004. Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements. Eur J Neurosci. 20:2193–2202.
Gazzola V, Keysers C. 2009. The observation and execution of actions share motor and somatosensory voxels in all tested subjects: single-subject analyses of unsmoothed fMRI data. Cereb Cortex. 19:1239–1255.
Gazzola V, van der Worp H, Mulder T, Wicker B, Rizzolatti G, Keysers C. 2007. Aplasics born without hands mirror the goal of hand actions with their feet. Curr Biol. 17:1235–1240.
Gerschlager W, Siebner HR, Rothwell JC. 2001. Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. Neurology. 57:449–455.
Giese MA, Poggio T. 2003. Neural mechanisms for the recognition of biological movements. Nat Rev Neurosci. 4:179–192.
Grafton ST. 2009. Embodied cognition and the simulation of action to understand others. Ann N Y Acad Sci. 1156:97–117.
Grossman E, Donnelly M, Price R, Pickens D, Morgan V, Neighbor G, Blake R. 2000. Brain areas involved in perception of biological motion. J Cogn Neurosci. 12:711–720.
Grossman ED, Battelli L, Pascual-Leone A. 2005. Repetitive TMS over posterior STS disrupts perception of biological motion. Vision Res. 45:2847–2853.
Heiser M, Iacoboni M, Maeda F, Marcus J, Mazziotta JC. 2003. The essential role of Broca's area in imitation. Eur J Neurosci. 17:1123–1128.
Hickok G. 2009. Eight problems for the mirror neuron theory of action understanding in monkeys and humans. J Cogn Neurosci. 21:1229–1243.
Hupé JM, James AC, Payne BR, Lomber SG, Girard P, Bullier J. 1998. Cortical feedback improves discrimination between figure and background by V1, V2 and V3 neurons. Nature. 394:784–787.
Jellema T, Perrett DI. 2003. Cells in monkey STS responsive to articulated body motions and consequent static posture: a case of implied motion? Neuropsychologia. 41:1728–1737.
Johnson-Frey SH, Maloof FR, Newman-Norlund R, Farrer C, Inati S, Grafton ST. 2003. Actions or hand-object interactions? Human inferior frontal cortex and action observation. Neuron. 39:1053–1058.
Keysers C, Perrett DI. 2004. Demystifying social cognition: a Hebbian perspective. Trends Cogn Sci. 8:501–507.
Kilner JM, Friston KJ, Frith CD. 2007. Predictive coding: an account of the mirror neuron system. Cogn Process. 8:159–166.
Kilner JM, Neal A, Weiskopf N, Friston KJ, Frith CD. 2009. Evidence of mirror neurons in human inferior frontal gyrus. J Neurosci. 29:10153–10159.
Kilner JM, Vargas C, Duval S, Blakemore SJ, Sirigu A. 2004. Motor activation prior to observation of a predicted movement. Nat Neurosci. 7:1299–1301.
Koch G, Versace V, Bonnì S, Lupo F, Lo Gerfo E, Oliveri M, Caltagirone C. 2010. Resonance of cortico-cortical connections of the motor system with the observation of goal directed grasping movements. Neuropsychologia. 48:3513–3520.
Kourtzi Z, Kanwisher N. 2000. Activation in human MT/MST by static images with implied motion. J Cogn Neurosci. 12:48–55.
Kraskov A, Dancause N, Quallo MM, Shepherd S, Lemon RN. 2009. Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? Neuron. 64:922–930.
Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. 2006. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. Neuroimage. 31:1453–1474.
Moro V, Urgesi C, Pernigo S, Lanteri P, Pazzaglia M, Aglioti SM. 2008. The neural basis of body form and body action agnosia. Neuron. 60:235–246.
Nelissen K, Borra E, Gerbella M, Rozzi S, Luppino G, Vanduffel W, Rizzolatti G, Orban GA. 2011. Action observation circuits in the macaque monkey cortex. J Neurosci. 31:3743–3756.
Nelissen K, Luppino G, Vanduffel W, Rizzolatti G, Orban GA. 2005. Observing others: multiple action representation in the frontal lobe. Science. 310:332–336.
Nishitani N, Avikainen S, Hari R. 2004. Abnormal imitation-related cortical activation sequences in Asperger's syndrome. Ann Neurol. 55:558–562.
Nishitani N, Hari R. 2002. Viewing lip forms: cortical dynamics. Neuron. 36:1211–1220.
Oldfield RC. 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 9:97–113.
Oosterhof NN, Wiggett AJ, Diedrichsen J, Tipper SP, Downing PE. 2010. Surface-based information mapping reveals crossmodal vision-action representations in human parietal and occipitotemporal cortex. J Neurophysiol. 104:1077–1089.
O'Shea J, Johansen-Berg H, Trief D, Gobel S, Rushworth MFS. 2007. Functionally specific reorganization in human premotor cortex. Neuron. 54:479–490.
Paus T. 2005. Inferring causality in brain images: a perturbation approach. Philos Trans R Soc Lond B Biol Sci. 360:1109–1114.
Pazzaglia M, Smania N, Corato E, Aglioti SM. 2008. Neural underpinnings of gesture discrimination in patients with limb apraxia. J Neurosci. 28:3030–3041.

Peelen MV, Wiggett AJ, Downing PE. 2006. Patterns of fMRI activity dissociate. overlapping functional brain areas that respond to biological motion. Neuron. 49:815–822.
Peigneux P, Salmon E, van der Linden M, Garraux G, Aerts J, Delfiore G, Degueldre C, Luxen A, Orban G, Franck G. 2000. The role of lateral occipitotemporal junction and area MT/V5 in the visual analysis of upper-limb postures. Neuroimage. 11:644–655.
Perrett DI, Xiao D, Barraclough NE, Keysers C, Oram MW. 2009. Seeing the future: natural image sequences produce "anticipatory" neuronal activity and bias perceptual report. Q J Exp Psychol (Colchester). 62:2081–2104.
Pobric G, Hamilton AF. 2006. Action understanding requires the left inferior frontal cortex. Curr Biol. 16:524–529.
Press C, Heyes C, Kilner JM. 2011. Learning to understand others' actions. Biol Lett. 7:457–460.
Proverbio AM, Riva F, Zani A. 2009. Observation of static pictures of dynamic actions enhances the activity of movement-related brain areas. PLoS One. 4:e5389.
Rizzolatti G, Craighero L. 2004. The mirror-neuron system. Annu Rev Neurosci. 27:169–192.
Rizzolatti G, Luppino G. 2001. The cortical motor system. Neuron. 31:889–890.
Rossi S, Hallett M, Rossini PM, Pascual-Leone A. The Safety of TMS Consensus Group 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 120:2008–2039.
Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijević MR, Hallett M, Katayama Y, Lücking CH, et al. 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol. 91:79–92.
Sartori L, Cavallo A, Bucchioni G, Castiello U. 2011. Corticospinal excitability is specifically modulated by the social dimension of observed actions. Exp Brain Res. 211:557–568.
Saygin AP. 2007. Superior temporal and premotor brain areas necessary for biological motion perception. Brain. 130:2452–2461.
Schippers MB, Keysers C. 2011. Mapping the flow of information within the putative mirror neuron system during gesture observation. Neuroimage. 57:37–44.
Schubotz RI. 2007. Prediction of external events with our motor system: towards a new framework. Trends Cogn Sci. 11:211–218.
Schütz-Bosbach S, Avenanti A, Aglioti SM, Haggard P. 2009. Don't do it! Cortical inhibition and self-attribution during action observation. J Cogn Neurosci. 21:1215–1227.
Schütz-Bosbach S, Prinz W. 2007. Prospective coding in event representation. Cogn Process. 8:93–102.
Senior C, Barnes J, Giampietro V, Simmons A, Bullmore ET, Brammer M, David AS. 2000. The functional neuroanatomy of implicit-motion perception or representational momentum. Curr Biol. 10:16–22.
Serino A, Canzoneri E, Avenanti A. 2011. Fronto-parietal areas necessary for a multisensory representation of peripersonal space in humans: an rTMS study. J Cogn Neurosci. 23:2956–2967.
Shimazu H, Maier MA, Cerri G, Kirkwood PA, Lemon RN. 2004. Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. J Neurosci. 24:1200–1211.
Tidoni E, Borgomaneri S, di Pellegrino G, Avenanti A. 2012. Action simulation plays a critical role in deceptive action recognition. In revision.
Tootell RB, Reppas JB, Kwong KK, Malach R, Born RT, Brady TJ, Rosen BR, Belliveau JW. 1995. Functional analysis of human MT and related visual cortical areas using magnetic resonance imaging. J Neurosci. 15:3215–3230.
Touge T, Gerschlager W, Brown P, Rothwell JC. 2001. Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clin Neurophysiol. 112:2138–2145.
Umiltà MA, Kohler E, Gallese V, Fogassi L, Fadiga L, Keysers C, Rizzolatti G. 2001. I know what you are doing. a neurophysiological study. Neuron. 31:155–165.
Uozumi T, Tamagawa A, Hashimoto T, Tsuji S. 2004. Motor hand representation in cortical area 44. Neurology. 62:757–761.
Urgesi C, Candidi M, Ionta S, Aglioti SM. 2007. Representation of body identity and body actions in extrastriate body area and ventral premotor cortex. Nat Neurosci. 10:30–31.
Urgesi C, Maieron M, Avenanti A, Tidoni E, Fabbro F, Aglioti SM. 2010. Simulating the future of actions in the human corticospinal system. Cereb Cortex. 20:2511–2521.
Urgesi C, Moro V, Candidi M, Aglioti SM. 2006. Mapping implied body actions in the human motor system. J Neurosci. 26: 7942–7949.
Vaina LM, Solomon J, Chowdhury S, Sinha P, Belliveau JW. 2001. Functional neuroanatomy of biological motion perception in humans. Proc Natl Acad Sci U S A. 98:11656–11661.
Van Overwalle F, Baetens K. 2009. Understanding others' actions and goals by mirror and mentalizing systems: a meta-analysis. Neuroimage. 48:564–584.
Walsh V, Pascual-Leone A. 2003. Transcranial magnetic stimulation. A neurochronometrics of mind. Cambridge (MA): MIT Press.
Wilson M, Knoblich G. 2005. The case of motor involvement in perceiving conspecifics. Psychol Bull. 131:460–473.
Wolpert DM, Doya K, Kawato M. 2003. A unifying computational framework for motor control and social interaction. Philos Trans R Soc Lond B Biol Sci. 2358:593–602.