Supplemental Data

Somatic and Motor Components of Action Simulation

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Supplemental Results

Effect of 1 Hz rTMS on Corticospinal Excitability

Low-frequency rTMS over dorsal premotor cortex and M1 hand areas is known to induce a transient suppression of MEP recorded from hand muscles [S1, S2]. Figure S2 shows the inhibitory effect of rTMS on MEP amplitude (main effect of session) in the three experiments for both the FDI (upper part of the figure) and the ADM (lower part of the figure) muscle. For each panel, the left column shows the average of MEP amplitude recorded during static, possible, and impossible conditions of the out-win session (outside the inhibitory effect of rTMS), and the right column shows mean MEP amplitude in the in-win session (within the time period of inhibitory influences of rTMS).

Inhibitory rTMS over vPMc (Figure S2A) brought about a strong and consistent reduction of MEP amplitude in the FDI (mean ± SEM: 61% ± 8% of the out-win baseline session, p < 0.001) and ADM (58% ± 7%, p < 0.0001) muscle. This finding is in keeping with a previous study demonstrating that TMS pulses over vPMc might interfere with execution of hand movements and induce electromyography (EMG) responses in precontracted hand muscles [S3] (see also Supplemental Experimental Procedures). Although the latter study suggests the presence of a hand representation in vPMc [S3], our study demonstrates that inhibition of this representation by rTMS can reduce corticospinal excitability in hand muscles, expanding previous knowledge on premotor-motor functional connectivity.

Low-frequency rTMS to M1 significantly reduced amplitude of MEPs recorded from the target muscles (FDI: 80% ± 8%, p < 0.049; ADM: 71% ± 7%, p = 0.003; Figure S2C) [S1, S2]. In keeping with previous studies [S2, S4], no significant modulation of corticospinal excitability was found after rTMS over S1 (Figure S2B) for either the FDI (91% ± 11%, p = 0.71) or for the ADM (75% ± 13%, p = 0.08) muscle.

Selective Disruption of Corticospinal Mapping in Experiments 1–3

We performed an additional analysis to estimate the amount of disruption of mirror corticospinal mapping of possible and biomechanically impossible movements. First, to assess the facilitation effect of the observation of movements independently from the effect of rTMS on corticospinal excitability, we normalized MEP amplitudes (raw values in possible and impossible conditions divided by raw values in static condition). Normalized MEPs were separately computed for each muscle (FDI and ADM), observed movement (possible and impossible), and session (in-win and out-win). Direct comparisons between these indices of facilitation are reported in the main text.

Then, we computed, for each muscle and type of movement, an action-observation MEP inhibition index obtained by subtracting out-win (MEP facilitations recorded outside the interventional effect of rTMS) from in-win (MEP facilitations recorded within the time period of interventional influences of rTMS) values. Negative values indicate disruption of mirror corticospinal facilitation due to virtual lesion. For each experiment, inhibition indices were entered into a two-way repeated-measure ANOVA with muscle (FDI, ADM) and movement condition (possible, impossible) as within factors.

In the first experiment (virtual lesion to vPMc, Figure S3A), we found a significant muscle × movement condition interaction (F[1, 12] = 6.18, p = 0.029), which was entirely accounted for by the higher MEP differences recorded from the FDI muscle during the possible condition with respect to the MEP differences recorded from the same muscle during the impossible condition (p = 0.0009) and the MEP differences recorded from the ADM muscle in the possible (p = 0.002) and impossible conditions (p = 0.0009). Thus, rTMS over vPMc selectively disrupted the mirror facilitation triggered by the observation of biomechanically possible movements (see Figure 1).

In the second experiment (virtual lesion to S1, Figure S3B), ANOVA on action-observation inhibition index revealed a significant muscle × movement condition interaction (F[1, 12] = 11.97, p = 0.005), which was entirely accounted for by the higher MEP differences recorded from the FDI muscle in the impossible condition with respect to the MEP differences recorded from the same muscle in the possible condition (p = 0.011) and the MEP differences recorded from the ADM muscle in the possible (p = 0.02) and impossible conditions (p = 0.002). This pattern of results would suggest that inhibition of S1 activity mainly disrupts simulation of impossible movements (see Figure 2).

The lack of changes in mirror corticospinal mapping after virtual lesion to M1 (third experiment, see Figure 3) was confirmed by ANOVA on the inhibition indices (Figure S3C). Importantly, no main effect of muscle, movement condition, or interaction were statistically significant, thus indicating that rTMS over M1, although effective in reducing motor excitability in the target muscles (Figure S2C), did not alter the pattern of mirror corticospinal facilitations induced by the observation of both biomechanically possible and impossible finger movements.

Supplemental Discussion

Cortical Architecture of Efferent and Afferent Components of Action Simulation

The “perturb and measure” TMS approach used in the present study allowed us to explore the causative functional connectivity of frontoparietal systems in the
corticospinal mapping of observed actions. Although it is possible that additional frontoparietal areas might have contributed to the observed effects (see main text), our data strongly suggest that vPMc and S1 play a fundamental role in mapping others’ possible and biomechanically impossible body movements onto the corticospinal system of an onlooker. In contrast, M1 is less directly involved in such mirror mapping. These findings suggest that separate cortical areas deal preferentially with afferent and efferent components of others’ actions.

On the basis of these results, we propose that the human frontoparietal mirror system contains two dissociable and somewhat independent nodes that are specialized for simulating efferent and afferent components of observed actions. One frontal node likely processes the efferent components of observed actions that are mapped onto the observer’s vPMc. Neurophysiological
and neuroanatomical evidence strongly suggest that this frontal node is functionally coupled with regions of the inferior parietal areas in which cells with mirror properties have been discovered [S5, S6].

A second node might specialize in processing afferent components of observed actions like, for example, the somatic feelings that arise from the observation of impossible actions. Although in this study we tested only the role of S1, it is entirely plausible that other anterior parietal areas concerned with merging vision, touch, and proprioception are involved in this same type of mapping [S7].

The action simulation computations performed by these two areas are conveyed to the spinal motoneurons that ultimately control the contraction of specific muscles. The vPMc computations necessary for mapping biomechanically possible actions might be relayed to the motor cortex. This would be in keeping with a MEG study that investigated temporal dynamics of action simulation in which observing an action induced two subsequent peaks of activity in premotor cortex and then M1, suggesting that premotor activity might trigger modulation of the motor cortex [S8]. Moreover, the premotor cortex also sends projections to primary sensorimotor cortices [S9, S10]. Finally, anatomical and functional studies in monkeys demonstrate that the vPMc sends direct connections to spinal cord motoneurons that control not only proximal but also distal muscles [S11, S12]. Therefore, in principle, vPMc might exert its influence on the mirror corticospinal mapping through the three above outlined different anatomical pathways. Whatever direct or indirect pathways might mediate the observational action-related corticospinal mapping, the causative evidence that vPMc (experiment 1) but not M1 (experiment 3) or S1 (experiment 2) is crucial in the mirroring of biomechanically possible actions suggests that the motor properties of the observed possible actions are encoded into the ventral premotor cortex and transferred to the spinal cord without any specific computations carried out in primary sensorimotor cortices.

Although it is well known that M1 activity can be enhanced by action observation [S7, S8, S13–S16], whether such modulation is functionally relevant, as during action execution [S17], or is simply a consequence of the strong reciprocal corticocortical vPMc-M1 connections [S9–S12] is still under debate [S16]. Our data support the latter hypothesis and are in keeping with the absence (or possibly the scarcity) of mirror neurons in M1 [S6, S18]. Note however that there is also evidence that observation of hand actions might reduce BOLD signal in M1 hand representation [S19]. Although the interpretation of fMRI deactivations is not straightforward, this result would suggest that the role of M1 (if any) in action simulation is distinct from the one played by the vPMc.

S1 seems to play a crucial role in the stimulation of afferent components of actions. This might indicate

<table>
<thead>
<tr>
<th>Stimulus Qualities</th>
<th>VAS Ratings (0–10)</th>
<th>VAS Score Comparisons</th>
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<tbody>
<tr>
<td></td>
<td>Possible Mean (SD)</td>
<td>Impossible Mean (SD)</td>
</tr>
<tr>
<td>Joint stretch</td>
<td>2.26 (2.22)</td>
<td>8.44 (1.92)</td>
</tr>
<tr>
<td>Touch</td>
<td>0.96 (1.51)</td>
<td>2.50 (3.49)</td>
</tr>
<tr>
<td>Pain</td>
<td>0.81 (1.19)</td>
<td>7.03 (3.47)</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>0.91 (1.44)</td>
<td>6.63 (2.97)</td>
</tr>
<tr>
<td>Biomechanical</td>
<td>0.69 (0.95)</td>
<td>9.81 (0.29)</td>
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Mean (SD) visual analog scale (VAS) scores concerning somatic feelings induced by observation of biomechanically possible or impossible index-finger abduction-adduction movements and evaluation scores of unpleasantness and biomechanical implausibility of the stimuli. All of the explored somatic features (joint stretch, touch, and pain) were significantly higher during observation of impossible than possible videos, thus suggesting that the former type of video elicits simulation of afferent components of observed actions. Moreover, impossible movements were judged as emotionally more unpleasant and violating the normal biomechanical constraints of the relative joint than possible movements.
that the neural activity in premotor or motor areas and corticospinal system detected by means of fMRI [S7] and spTMS [S20] during observation of impossible movements is mediated via facilitatory modulation from parietal somatic areas. Although motor systems might be influenced by activity originating in somatic areas, our data strongly suggest that motor or premotor areas do not play an important role in modulating MEPs during the observation of biomechanically impossible actions. That observed impossible actions might be encoded in somatic areas and transferred to the spinal cord either directly or via M1.

Note that modulation of somatic cortices during action perception has often been attributed to the activity originating from premotor-parietal action mirror system [S21, S22]. Our results suggest that, at least in some cases (i.e., during the observation of biomechanically impossible movements, when the afferent component of action might be extremely salient), the relation between motor and somatic areas might be the reverse, suggesting that neural substrates and effective connectivity of action simulation might be flexibly influenced by the sensorimotor features of the observed actions.

### Mapping Causative Corticospinal Functional Connectivity with TMS

The result that M1 inhibition (clearly supported in experiment 3 by the reduction of corticospinal excitability) does not influence a given MEP modulatory effect (in our study, the facilitation during action observation), along with the demonstration that the inhibition of other regions (in our study vPMc, S1) does, deserves a final methodological consideration. On one hand, these findings highlight the role of TMS as an important tool for studying the effective corticospinal connectivity; on the other hand, they challenge the assumption often made in TMS research that any modulating effect on the neural activity in premotor or motor areas is primarily originating from M1 or is causatively linked to M1 activity. Indeed, our data indicate that the motor cortex might be not crucially involved in the action-observation-related MEP modulation and suggest that a combination of rTMS and spTMS is necessary to investigate the neural origin of a given corticospinal modulatory effect.

### Supplemental Experimental Procedures

**Participants**

For experiments 1–3 there were 13, 13, and 13 subjects, respectively (4, 4, and 5 men), age 21–28, 21–29, and 22–32. Participants were right handed according to a standard handedness inventory [S23] and gave their written informed consent. They were paid 30 euros for their participation. None of them had neurological, psychiatric, or other medical problems or any contraindication to transcranial magnetic stimulation (TMS) [S24]. The protocol was approved by the ethics committee of the Fondazione Santa Lucia and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

**Visual Stimuli**

In each experiment, different types of video clips were presented on a 19 in screen located 80 cm from the subjects. The video clips showed the following: (1) the dorsal static view of a right hand (static), (2) a sequence of three biomechanically possible abduction-adduction movements of the right index finger (possible), and (3) a sequence of three biomechanically impossible abduction-adduction movements of the right index finger (impossible). Frequency of abduction-adduction was the same for the two types of movements (about 1 Hz). The duration of each video was 3000 ms. The biomechanically possible movements could be easily performed (0°–35° of angular displacement) and are likely to be seen in daily life. By contrast, the biomechanically impossible movements are never seen in naturalistic contexts. Indeed, these movements were performed in a workspace (60°–95°) clearly beyond the limits of the metacarpophalangeal joint (Movie S1). Thus, biomechanically possible and impossible index finger abduction-adduction movements had comparable ranges of angular displacement (35°) but were performed in independent workspaces.

**EMG, Single-Pulse TMS Recordings, and Study Design**

MEPs induced by single-pulse TMS (spTMS) were recorded simultaneously from first right dorsal interosseous (FDI, in the region of the index finger) and abductor digitii minimi (ADM, in the region of the little finger) by means of a Viking IV (Nicolet Biomedical, U.S.A.) electromyograph. EMG signals were band-pass filtered (20 Hz–2.5 kHz, sampling rate fixed at 10 kHz), digitized, and stored on a computer for offline analysis. Pairs of silver-silver chloride surface electrodes were placed over the muscle belly (active electrode) and over the associated joint or tendon of the muscle (reference electrode). A circular ground electrode with a diameter of 30 mm was placed on the dorsal surface of the right wrist.

A figure-of-8 coil connected to a Magstim Super Rapid Transcranial Magnetic Stimulator (Magstim, Whitland, Dyfed, U.K.) was placed over the left M1. The intersection of 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 [S25, S26].

With a slightly suprathreshold stimulus intensity, the coil was moved over the left hemisphere so that the scalp position from which maximal amplitude MEPs were elicited in the FDI muscle could be determined. With this TMS coil position, it was also possible to record a stable signal from ADM in all subjects. The optimal position of the coil was then marked on the scalp with a pen so that correct coil placement could be ensured throughout the experiment. The intensity of magnetic pulses eliciting MEPs was set at

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### Table S2. Visual Analog Scale Ratings in the TMS Experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Static</th>
<th>Possible</th>
<th>Impossible</th>
<th>Static</th>
<th>Possible</th>
<th>Impossible</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08 (0.14)</td>
<td>0.48 (0.95)</td>
<td>0.70 (0.51)</td>
<td>0.26 (0.37)</td>
<td>0.53 (0.83)</td>
<td>4.45 (3.34)</td>
</tr>
<tr>
<td>2</td>
<td>0.03 (0.04)</td>
<td>0.46 (0.95)</td>
<td>0.95 (0.88)</td>
<td>0.47 (1.20)</td>
<td>0.52 (0.85)</td>
<td>5.09 (2.68)</td>
</tr>
<tr>
<td>3</td>
<td>0.04 (0.05)</td>
<td>1.23 (2.80)</td>
<td>0.94 (0.87)</td>
<td>0.56 (1.19)</td>
<td>0.61 (0.84)</td>
<td>4.83 (2.97)</td>
</tr>
</tbody>
</table>

Subjective ratings along the visual analog scale (VAS) of the movie clips in the three TMS experiments. Each cell reports mean (SD). After each TMS experiment, participants were asked to rate any aversive somatic feelings they experienced during observation of the different video clips on a 10 cm VAS and to judge the biomechanical plausibility of the stimuli. For both types of judgments, impossible received higher ratings than did possible and static stimuli (p < 0.0001). In keeping with the psychophysical pilot experiment (Table S1), participants judged the finger abduction-adduction impossible movements as strongly violating the biomechanical constraints of the metacarpophalangeal joint. Moreover, all subjects reported higher aversive somatic feelings in the impossible condition than in the static and the possible conditions.
130% of the 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 [S27]. Mean values (standard deviations [SDs]) of rMT were 40.85 (6.90) in experiment 1, 42.08 (6.32) in experiment 2, and 41.34 (6.32) in experiment 3. The absence of voluntary contractions was continuously verified visually and prior to the recording session by the auditory monitoring of the EMG signal.

Each experiment included two spTMS sessions (each lasting 5.25 min) in which MEPs were recorded during the observation of the different video clips. One session (in-win) was performed within the inhibition window created by repetitive TMS (rTMS) and the other outcome outside the influence of rTMS. The two sessions were separated by 1.5 hr, and their order was counterbalanced in each experiment, with seven participants starting with the out-win session and the remaining six subjects beginning with the in-win session. Figure S1 shows a schematic representation of the experimental design.

Each spTMS session (out-win, in-win) included 15 trials for each video clip (45 trials in total per session) presented in a randomized order. In all of the experiments, a central cross (1000 ms) indicated the beginning of a trial and initiated EMG recording. On each trial, a magnetic pulse was randomly delivered between 1500 and 2000 ms after the end of the movie (lasting 3000 ms) so that any priming effects that could affect MEP size could be avoided. A blank screen was shown for 3000 ms in the intertrial intervals. The experiments were programmed with Psychophysics Toolbox (www.psychotoolbox.org/) and Matlab (www.mathworks.com) software so that sequence and duration of video clips could be controlled and TMS and EMG recording could be triggered.

rTMS and Neuroavigation
The in-win spTMS session was preceded by 15 min of low-frequency 1 Hz rTMS (100% rMT) over the target area (left PVMc in experiment 1, left S1 in experiment 2, and left M1 in experiment 3). Subjects were asked to keep their muscles as relaxed as possible during the rTMS because contraction could reduce the effect of rTMS on MEP size [S28]. In the in-win session, MEP collection started 45 s after the cessation of the rTMS. This short time interval allowed the changing of the stimulating coil and the TMS intensity. All the MEPs were recorded within 6 min after the end of rTMS. Therefore, the entire in-win session was performed during the reduced excitability temporal window induced by 1 Hz rTMS. This allowed us to assess whether rTMS over specific cortical regions can disrupt the MEP modulation induced by observation of different categories of action movies.

In experiment 1, rTMS was performed over the left PVMc. Coil position was identified on each participant’s scalp with the SofTaxic Navigator system (Electro Medical Systems, Bologna, Italy), as in our previous TMS research [S29, S30]. Skull landmarks (nasion, inion, and two preauricular points) and about 60 points providing a uniform representation of the scalp were digitized by means of a Polhemus digitizer (Polhemus, Colchester, VT). Coordinates in Talairach space [S31] were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template. The scalp location that corresponded best to the vPMc coordinates was identified by means of the SofTaxic Navigator system and marked with a pen. On the basis of our previous fMRI study, in which the same set of visual stimuli was used [S7], we targeted vPMc in the pars opercularis of the inferior frontal gyrus. To do so, we also checked that small EMG phasic responses in FDI or ADM could be detected during voluntary hand-muscle contraction (about 20% of maximal contraction) by delivering TMS pulses at 110% rMT over the marked scalp position [S3]. In keeping with a previous study, no MEPs were elicited by spTMS over vPMc in resting hand muscles [S33].

In experiment 2, rTMS was delivered over the left S1. TMS studies that successfully targeted the somatosensory hand area positioned the coil 1–4 cm posterior to the motor hotspot [S32–S37]. In light of this, we assumed that positioning the coil 3 cm from the previously marked optimal scalp position (OSP) for activation of the right FDI muscle would reduce the activity of S1 with minimum effects on M1. To test this assumption directly, we checked that TMS pulses delivered with the coil in the above position did not elicit any detectable MEPs. Moreover, this position was identified on each observer’s scalp with the SoftTaxic Navigator system. In all subjects, the chosen site was localized in the postcentral gyrus, suggesting that this procedure allowed us to target the hand region representation in S1.

In experiment 3, we performed rTMS on left M1 by placing the coil over the optimal scalp position corresponding to the scalp projection of the primary motor cortex [S24]. The SoftTaxic Navigator system was used for the measurement of the Talairach coordinates of this position.

Psychophysical Testing
In experiments 1–3, after TMS sessions, subjects were asked to judge the biomechanical implausibility of each visual stimulus by marking a vertical, 10 cm visual analog scale (VAS) with 0 cm indicating “it is not biomechanically impossible, everyone can perform it” and 10 cm “it is biomechanically impossible, nobody can perform it.” We also asked participants to report aversive somatic feelings induced by the three types of stimuli by means of 10 cm VAS with 0 cm indicating “no effect” and 10 cm indicating “maximum effect imaginable” (Table S2).

In the psychophysical pilot study aimed at selecting the finger-movement video clip with the highest ratings of biomechanical implausibility, participants (M = 23, nine men, aged between 19 and 30) were asked to observe different types of possible and impossible index- and little-finger-movement (adduction-abduction, flexo-extension) video clips and to judge the biomechanical implausibility and the unpleasantness of the visual stimuli on a VAS. Participants were also requested to report any somatic feelings (joint stretch, touch, and pain sensation) evoked by the observation of the two types of movies on a VAS. Table S1 reports the VAS ratings.

Data Analysis
Neurophysiological data were processed offline. Trials with EMG activity prior to TMS were discarded from the analysis. In experiments 1–3, mean MEP amplitude values in each condition were measured peak to peak (in mV). Outliers (±2.0 SD of the mean) were identified for each muscle in each condition, and the data were removed. MEPs were analyzed by means of a three-way repeated-measures ANOVA with muscle (FDI, ADM), session (out-win, in-win) and condition (static, possible, impossible) as within-subjects factors. When a significant triple interaction was found, two separate two-way repeated-measure ANOVAs, one for each muscle, with session (out-win, in-win) and condition (static, possible, impossible) as within-subjects factors, were performed. Mirror MEP facilitations to action observation (movement/static ratio) in the FDI muscle were also compared in the two sessions by paired t tests.

In experiments 1–3, subjective data were analyzed by means of one-way repeated-measure ANOVA with condition (static, possible, impossible) as within-subjects effect. Post-hoc comparisons were made by means of the Newman-Keuls test. In the psychophysical pilot study, subjective data were analyzed with paired t tests.

Supplemental References