Primary somatosensory cortex necessary for the perception of weight from other people's action
Valchev, Nikola; Tidoni, Emmanuele; Hamilton, Antonia F. de C.; Gazzola, Valeria; Avenanti, Alessi

Published in:
Neuroimage

DOI:
10.1016/j.neuroimage.2017.02.075

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Primary somatosensory cortex necessary for the perception of weight from other people’s action: A continuous theta-burst TMS experiment

Nikola Valcheva,a,b,1, Emmanuele Tidoni,c,d,1, Antonia F. de C. Hamilton,e,f, Valeria Gazzola,a,g,h,⁎,1, Alessio Avenanti,c,d,e,⁎⁎

A B S T R A C T

The presence of a network of areas in the parietal and premotor cortices, which are active both during action execution and observation, suggests that we might understand the actions of other people by activating those motor programs for making similar actions. Although neurophysiological and imaging studies show an involvement of the somatosensory cortex (SI) during action observation and execution, it is unclear whether SI is essential for understanding the somatosensory aspects of observed actions. To address this issue, we used off-line transcranial magnetic continuous theta-burst stimulation (cTBS) just before a weight judgment task. Participants observed the right hand of an actor lifting a box and estimated its relative weight. In counterbalanced sessions, we delivered sham and active cTBS over the hand region of the left SI and, to test anatomical specificity, over the left motor cortex (M1) and the left superior parietal lobule (SPL). Active cTBS over SI, but not over M1 or SPL, impaired task performance relative to sham cTBS. Moreover, active cTBS delivered over SI just before participants were asked to evaluate the weight of a bouncing ball did not alter performance compared to sham cTBS. These findings indicate that SI is critical for extracting somatosensory features (heavy/light) from observed action kinematics and suggest a prominent role of SI in action understanding.

Introduction

When we observe somebody lifting a box, we can typically judge if the load is heavy or light. What are the brain mechanisms supporting this computation? Years of research on the mirror system, areas of the brain active both when we perform an action and when we observed a similar action performed by another individual, suggest the mirror mechanism as a possible basis for action understanding (Rizzolatti and Sinigaglia 2010, 2016). Motor mirroring engages a system of reciprocal anatomically connected cortical motor areas, including the inferior frontal cortex (IFC) and inferior parietal lobule (IPL) (di Pellegrino et al., 1992; Gallese et al., 1996; Keysers and Perrett, 2004; Fogassi et al., 2005; Gazzola and Keysers, 2009; Casile, 2013; Bonini, 2016). Lesion and transcranial magnetic stimulation (TMS) studies provide evidence for the functional relevance of IFC and IPL to action perception. For instance, as a consequence of a brain damage affecting the IFC, people become less accurate at detecting errors in action videos (Pazzaglia et al., 2008), ordering pictures of actions in temporal sequences (Fazio et al., 2009), and identifying what action was performed in point-light displays (Saygin, 2007). Also, lesions of the IPL impair detection of spatio–temporal errors in action sequences (Buxbaum et al., 2005; Weiss et al., 2008; Kalénine et al., 2010; see Urgesi et al. (2014) for a meta-analysis). Non-invasive transcranial stimulation of IFC and IPL transiently affects the discrimination of observed actions (Urgesi et al., 2009).
estimate the weight of a box by observing it being lifted. The task was Hamilton (2006) in four new TMS experiments. Participants had to functional specificity of a box from observed actions. In the first three experiments, we targeted SI to test its critical role in action understanding, and two neighboring sensorimotor regions, M1 and SPL, to test for anatomo-functional specificity (Chouinard et al., 2009; Eidenmüller et al., 2014). Both M1 and SPL possess functional and reciprocal connections with SI, and are found to respond to action execution (Vigneswaran et al., 2013; Bonini, 2016; Lloyd et al., 2002; Gazzola and Keysers, 2009; Keysers and Gazzola, 2009). In the fourth experiment, we applied cTBS over SI before participants judged the weight of a bouncing ball, to test for SI specificity to weight estimation when the action of a human agent is involved. Our results extend those of Bolognini et al. (2011, 2013, 2014) by showing that in addition to processing purely tactile information, SI also contributes to the processing of more proprioceptive information derived from action kinematics to infer weight; and those of Pobric and Hamilton (2006) by showing that weight judgment by observation requires SI in addition to IFC, supporting a behavioural relevance for the functional interplay between motor and somatosensory regions/representations in action perception suggested by our combined fMRI/TMS study (Valchev et al., 2016). Lastly, our results expand those of Jacquet and Avenanti (2015), Adolphs et al. (2000) and Paracampo et al. (2016) by showing that SI is critical not only for goal inference, but also for inferring proprioceptive qualities from observed kinematics.

Materials and methods

Participants

A total of 91 students from the University of Bologna took part in one of four TMS experiments (67 participants, 35 females, mean age ± S.D: 23.3 ± 1.9; see Table 1 for sample details) or in two psychophysical pilot studies (Pilot study 1: 12 participants, 8 females, mean age ± S.D: 23.8 ± 2; Pilot study 2: 12 participants, 6 females, mean age 26.6 ± 2.2). All participants provided written informed consent. All of them were right-handed (as assessed by verbal report of their manual preference) with normal or corrected to normal vision. 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. No discomfort or adverse effects during TMS were reported or noticed.

Weight estimation task

Participants watched 4.4 s movies showing either (i) a human hand lifting a box to place it on a shelf, or (ii) a ball falling from the top of the screen to then bounce at the bottom until it stops (no hand throwing the ball was visible) (Fig. 1A). The task consisted in judging, after each video, the weight of either the box or the ball by answering the question "How heavy is the box (or ball)?" on a 5 points scale, with 1 corresponding to the lightest and 5 to the heaviest weight estimation. Five different movies, representing 5 different box (or ball) weights were shown to the participants in a randomized order. Each movie was presented 12 times, 6 for each block, for a total of 30 movies per block. For both the box and ball task, each video was preceded by a 1 s fixation cross, and participants answered by pressing one of 5 keys with the left hand (ipsilateral to the stimulation site) to indicate a number from one to five. They were instructed to answer as quickly and accurately as possible. Participants wore headphones providing white noise to eliminate auditory information during task performance.

Visual stimuli

All the video stimuli came from previous experiments (Pobric and Hamilton, 2006; Hamilton et al., 2007). Briefly, the five different videos of the hand lifting a box (Experiment 1–3) were generated by down sampling a single high-speed movie of a lifting hand to create the perception of 5 different box weights, ranging from approximately 50 g to 850 g. Since they all derived from the same video, they are very well controlled for visual differences not relevant for the task. The videos of the bouncing balls were generated using Matlab (www.mathworks.
Table 1

<table>
<thead>
<tr>
<th>Task</th>
<th>anatomical description</th>
<th>Mean Talairach and Task coordinates in mm (± SD)</th>
<th>No of participants</th>
<th>Mean participant’s age in y (± SD)</th>
<th>Stimulation intensity as % of max stim. output (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1 SI</td>
<td>MNI coordinates</td>
<td>−41.3 (± 5.8) x −34.7 (± 3.8) z</td>
<td>17 (9)</td>
<td>23.1 (± 1.6) x 43.5 (± 5.1) y</td>
<td>43.5 (± 6.2) z</td>
</tr>
<tr>
<td>Exp. 2 M1</td>
<td></td>
<td>−41.9 (± 5.9) x −38.8 (± 3.7) z</td>
<td>16 (9)</td>
<td>23.5 (± 1.8) x 43.5 (± 5.1) y</td>
<td>43.4 (± 7.3) z</td>
</tr>
<tr>
<td>Exp. 3 SFL</td>
<td></td>
<td>−41.0 (± 2.9) x −38.2 (± 4.7) z</td>
<td>17 (9)</td>
<td>24.1 (± 2.1) x 43.7 (± 5.1) y</td>
<td>40.4 (± 6.2) z</td>
</tr>
<tr>
<td>Exp. 4 SI</td>
<td></td>
<td>−41.3 (± 5.2) x −38.2 (± 3.6) z</td>
<td>17 (9)</td>
<td>24.1 (± 2.0) x 43.7 (± 5.8) y</td>
<td>40.7 (± 5.8) z</td>
</tr>
</tbody>
</table>

The study included two pilot and four TMS experiments, in which participants had to estimate the weight of the box or the ball (Fig. 1A).

The first pilot experiment was conducted on 12 volunteers (8 females, mean age 22.8 y ± 2.0) not participating in the TMS experiments to check if the accuracy in judging the weight of the ball was comparable to that of the box. Participants were asked to complete the box and ball weight estimation tasks in two separate sessions whose order was counterbalanced across subjects. Each session started with 60 practice trials, and continued with two blocks of 30 experimental trials each.

The box and ball task might not only differ in difficulty, but also have a different susceptibility to TMS interference. The second pilot experiment, on 12 additional volunteers (6 females, mean age 26.6 y ± 2.2), was therefore performed to test whether the box and ball weight estimation tasks presented comparable sensitivity to external interference. To this aim, we experimentally manipulated stimulus visibility in both tasks by visually masking the phases critical to the estimation of the box’s or ball’s weight. The informative time window (IFT) for the box weight estimation’s videos started from the frame in which the hand touched the cube before lifting it, and lasted until the frame in which the hand released the cube. The IFT for the ball weight estimation’s videos started from the frame in which the ball touched the ground for the first time and ended with the frame in which the ball stopped. For each video, we applied two static masks of different duration (15% and 30% of the IFT), starting at 6 different onsets (at 0-10-20-30-40-50% of IFT duration), which were obtained by freezing the very last frame before mask onset. At the end of the mask, the video resumed showing the remaining frames. In this way, we obtained 60 new movies (2 masks×6 onsets×5 weights) for each task. Participants in the pilot study performed the box and ball weight estimation tasks in two separate sessions whose order was counterbalanced across subjects. For each task, the original version of the videos (0% of the IFT) and the two masking conditions (15–30% of the IFT) were presented in separate blocks. Each session started with 60 practice trials, and continued with the three blocks of 60 experimental trials (180 experimental trials in total). By using the masking manipulation, we provide an independent measure of task sensitivity that can be used to better interpret the results of the TMS experiments. A similar sensitivity in the two tasks will make it less likely that the changes in performance induced by TMS in one of the tasks could be simply explained by differences in sensitivity to external manipulation.

All four TMS experiments were composed of three parts: a preparatory, an active and a sham cTBS session (Fig. 1B). The order of the active and sham cTBS sessions was counterbalanced across participants. Additionally, active and sham cTBS sessions were separated by 90 min to ensure that no cTBS effects were carried over from one session to the other. During these 90 min participants were asked to remain relaxed and seated on a comfortable chair. Participants were randomly assigned to the different experiments.

During the preparatory session the optimal scalp position and the resting motor threshold were evaluated by means of motor-evoked potentials (MEPs) recording (see Transcranial magnetic stimulation...
paragraph for more details). Once the target site was individuated (Fig. 1C), it was marked on the scalp, and Talairach coordinates were estimated using the neuro-navigation system (see Target sites and neuro-navigation paragraph for more details; Table 1). Participants were then familiarized with the experimental task by performing a practice block of 60 trials. At the end of the practice, there was a 10 min rest period in which participants were required to remain in front of the computer.

During the active cTBS session the experimenter administered 40 s of off-line continuous theta-burst stimulation over the target site, by placing the intersection of the coil tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline (Balslev et al., 2004; Azañón and Haggard, 2009; Jacquet and Avenanti, 2015). Two blocks of 30 trials (~5 min duration each) were performed at five and twelve minutes after the stimulation (Fig. 1B). Between blocks and trials, participants were asked to rest. Active cTBS is known to disrupt functions related to the target area for about 30–60 min (Huang et al., 2005, 2011; Franca et al., 2006; Bertini et al., 2010; Wischnewski and Schutter, 2015). Since the task was completed within 20 min after active cTBS administration, performance should reflect the influence of active cTBS over the stimulated site. The sham cTBS session was exactly the same as the active cTBS session except that the coil was positioned, over the target site, perpendicular to the scalp, so that no current was induced in the brain.

In Experiments 1–3, participants performed the weight lifting task on the box videos after receiving stimulation over the left SI, left M1 and left SPL respectively (Fig. 1C). In Experiment 4, stimulation was delivered over the left SI and participants performed the weight estimation task on the falling ball videos. The number of trials was the kept the same throughout Experiments 1 to 4.

**Transcranial magnetic stimulation protocol**

The cTBS protocol lasted 40 s and consisted of bursts of 3 TMS pulses delivered at 50 Hz, with each train burst repeated every 200 ms (5 Hz) for a total of 600 pulses (Huang et al., 2005). Stimulation was administered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK).

Previous studies have suggested that motor experience before or after the administration of cTBS may alter its effect on cortical excitability (Iezzi et al., 2008, 2011; Todd et al., 2009), possibly leading to large inter-individual differences in the cTBS effect. To minimize the influence of motor activity before task execution, participants rested for at least 10 min before active cTBS in all the TMS experiments. After active cTBS, they rested for 5 min before performing the task to allow the active cTBS’ effect to reach its maximum level (Huang et al., 2005). To minimize the influence of motor activity after cTBS, participants performed the weight estimation task using their left hand, ipsilateral to the stimulated sites. To be consistent, the same rest periods were included in the sham cTBS sessions.

Pulse intensity was set at 80% of the resting motor threshold (rMT) and was comparable in the four experiments ($F_{(3,63)}=0.003$, $P>0.99$; Table 1). In those participants with rMT > 64% of maximum stimulator output (2, 3, 3 and 3 participants in Experiment 1, 2, 3 and 4 respectively) the intensity was set at the maximum allowed by the stimulator (51%; on average this intensity corresponded to 76% ± 3 of rMT; Bertini et al., 2010). The rMT evaluation was performed by recording motor-evoked potentials (MEPs) induced by single-pulse TMS of the left motor cortex. MEPs were recorded from the right first dorsal intersosseus (FDI) by means of a Biopac MP-150 electromyo-
graph (Biopac Corp, Goleta, CA). EMG signals were band-pass filtered (20 Hz to 1.0 kHz, sampled at 5 kHz), digitized and displayed on a computer screen. Pairs of silver/silver chloride surface electrodes were placed over the muscle belly (active electrode) and over the associated joint of the FDI muscle (reference electrode). A ground electrode was placed on the ventral surface of the right wrist. The optimum scalp position (OSP) was chosen so as to produce maximum amplitude MEPs in the FDI muscle. The rMT was defined as the lowest level of stimulation able to induce MEPs of at least 50 µV with 50% probability (Rossini et al., 1994, 2015).

Target sites and neuronavigation

In Experiment 1 and 4 scalp locations corresponding to the left SI were targeted by moving the coil 2.5 cm back with respect to the OSP (corresponding to the M1 hand area). TMS studies that successfully targeted the somatosensory hand area positioned the coil 1–4 cm posterior to the motor hotspot (Avenanti et al., 2007; Harris et al., 2002; Balslev et al., 2004; Merabet et al., 2004; Fiorio and Haggard, 2005; Azañón and Haggard, 2009; Jacquet and Avenanti, 2015). We therefore assumed that positioning the coil 2.5 cm from the previously marked optimal scalp position (OSP) for activation of the right FDI muscle would interfere with the activity of the hand representation area of SI with minimum effects on M1. To test this assumption directly, we checked that TMS pulses at 105% rMT with the coil in the above position did not elicit any detectable MEPs.

To directly test the anatomical and functional specificity of the effect of tCBS on SI activity, in Experiments 2 and 3, we applied tCBS over M1 and SPL, two brain areas recruited during action observation (Keyser and Gazzola, 2009; Casile, 2013; Bonini, 2016), respectively. In Experiment 2, left M1 was stimulated by placing the coil over the OSP, corresponding to the scalp projection of motor cortex hand area (Rossini et al., 1994, 2015). In Experiment 3, left SPL was stimulated by moving the coil 5 cm back with respect to the OSP (Balslev et al., 2004). Thus, stimulation of M1 and SPL occurred 2.5 cm forward and backward to SI, respectively.

In all the TMS experiments, we only stimulated brain regions (SI, M1 or SPL) in the left hemisphere. This choice was dictated by several factors including: (i) being able to directly compare our results with those of previous studies, which also stimulated the left hemisphere (Pobric and Hamilton, 2006; Cattaneo, 2010; Cattaneo et al., 2010, 2011; Tidoni et al., 2013; Jacquet and Avenanti, 2015; Avenanti et al., 2017; Valchev et al., 2016); (ii) the use of right-hand actions in the box videos; (iii) the inclusion of right-handed participants in our sample. Indeed, although responses to action observation is bilaterally distributed (van Overwalle and Baetsens, 2009; Grosbras et al., 2012; Borgomaneri et al., 2015), studies have shown a gradient of lateralization which depends on the laterality of the body part involved in the observed action, as well as the observers’ hand preference. In particular, during observation of right hand actions, activations of right-handers tend to be stronger (Aziz-Zadeh et al., 2002; van Schie et al., 2004; Shmuelof and Zohary, 2005; Gazzola and Keysers, 2009; Cabино et al., 2010; Caspers et al., 2010) in the left, relative to the right, hemisphere, and some studies showed that stimulation of the left hemisphere is more effective than the right hemisphere to modulate perception of observed right hand actions (Avenanti et al., 2017; but see Urgesi et al., 2007). Thus, by targeting the left hemisphere, we could test whether performance in the box weight estimation task was affected by interference with observer’s sensorimotor regions representing the very same hand shown in the action movies.

Brain surface Talairach coordinates corresponding to projection of the stimulated sites in SI (Experiments 1 and 4), M1 (Experiment 2) or SPL (Experiment 3) were identified on each participant’s scalp with the SoftTaxis Navigator system (Electro Medical Systems, Bologna, Italy) as in previous research (Avenanti et al., 2007, 2013a, 2017; Bertini et al., 2010; Serino et al., 2011; Tidoni et al., 2013; Jacquet and Avenanti, 2015; Paracampo et al., 2016). Skull landmarks (nasion, inion, and two preauricular points) and about 100 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northern Digital Inc., Ontario, Canada). An individual estimated magnetic resonance image (MRI) was obtained for each subject through a 3D warping procedure fitting a high-resolution MRI template with the participant’s scalp model and cranioemetric points. This procedure has been proven to ensure a global localization accuracy of roughly 5 mm, a level of precision closer to that obtained using individual MRIs than can be achieved using other localization methods (Carducci and Brusco, 2012). Coordinates of target regions in Talairach space (corresponding scalp projections on brain surface) were automatically estimated by the SoftTaxis Navigator from the MRI-constructed stereotaxic template and later transformed to the MNI space for better visualisation (Table 1). For illustrative purpose, spherical rois of diameter 4 mm around the mean target point from each TMS experiment were created using Marsbar (Brett et al., 2002) running in MATLAB 7.5 (Mathworks Inc., Sherborn, MA, USA) and then overlaid on the MNI brain template from MRcron (http://www.cabiatl.com/mricro/mricron/index.html; Fig. 1C).

Data analysis

In all experiments, separately for each session, we calculated participants’ performance as the sum of squared errors (SSE), which was calculated on the difference between the subjects’ reported weight, and the weight associated with the stimulus based on Pobric and Hamilton (2006) and Hamilton et al. (2007). SSE was preferred to the R² measure used in previous studies (e.g. Pobric and Hamilton, 2006) for two reasons. First, by subtracting averages, R² ignores possible differences in the mean values between reported and stimulus weights, and thus fails to capture systematic shifts in reports. Second, while R² is good at estimating linear relationships between reported and stimulus weights, it does not necessarily reflect performance. For instance, a participant reporting values of 1 1 1 1 5 to stimuli with assigned values of 1 2 3 4 5, would receive R²=0.5; one reporting 1 2 1 1 5, R²=0.4. Hence, the latter reporting 3 weights correctly would have a performance lower than the former, reporting only 2 weights correctly. The SSE does not suffer from these limitations, as it directly sums the deviations from the stimulus weights (for the two examples above SSE_{11115}=14, and SSE_{12115}=13). Importantly, all our participants performed the exact same number of trials, which is why sums can be directly compared.

A paired t-test was used in the first pilot study to compare the performance (SSE) in estimating the box weight to that of the bouncing ball. In the second pilot study a Task (hand, ball)×Masks (0%, 15%, 30%) ANOVA was performed with repeated measures on the two factors. Results are reported in Table 2.
The effect of cTBS on our regions of interest, SI, was directly tested by using a paired t-test on the SSE. Site- and task-specificity of the observed effects were then tested by examining the interaction term of a general linear model implementation of mixed models ANOVAs with factor Conditions (SI box, M1 box, SPL box, SI ball) and cTBS (sham, active), i.e. by examining if the cTBS effect on performance was different depending on condition or site. To further understand the interaction, we used two series of Duncan post-hoc tests. The first series, fully exploratory, compared performance after sham vs active cTBS across the four conditions. The second series compared the sham-active contrast across the 4 conditions to test whether the cTBS effect, as measured using the sham-active difference, varied across conditions. This directly follows up on the significant interaction.

To confirm that a parametric test was suited for analyzing our SSE values, we checked for normality. A Lilliefors tests on our SSE differences did not reveal a deviation from normality for any of our condition (all $p > 0.2$). To maximize the power to detect deviation from normality we also pooled all but the SI box conditions (as their means did not differ), but even in this case the Lilliefors test found no evidence to reject normality ($p > 0.5$). We also repeated the normality analysis using the ChiSquare goodness of fit, which confirmed the Lilliefors test results.

The same analyses (paired t-tests and ANOVAs) used for our main outcome measure were performed on other indices of task performance, namely the Pearson $R^2$ (to keep the comparability of current results with that of Pobric and Hamilton (2006)), mean weight estimation values and response times (RTs). Only one participants in Exp. 1 had values above 2 SD for both SSE (SSE $p_{\text{17}}=125$; SSE group $=67.2 \pm 25.38$ SD) and RTs (RT $p_{\text{17}}=2.18$; RT group $=0.63 \pm 0.06$ SD) during the sham cTBS condition, indicating the possibility of being an outlier. Because the relatively contained sample size for each group, which limits the confidence of identifying outliers, the significance of the ANOVAs are reported first excluding that participant, then including the participant.

**Results**

**Behavioral pilot experiments**

In the first pilot experiment a t-test confirmed that performance was indeed comparable in the box (mean SSE ± s.e.m.$\cong 86.92 \pm 8.5$) and ball (93.58 ± 14.77) weight estimation tasks ($t_{11} < 1$, $p=0.48$)

The two conditions (hand, ball)×three masks (0%, 15%, 30%) one-way ANOVA applied to the data from the second pilot experiment (Table 2) showed a main effect of masks, indicating that the task became more difficult as the percentage of mask increased ($F_{2,22}=14.55$, $p < 0.0001$), independently of whether participants estimated the weight of a box being lifted or a bouncing ball. No other significant effects were observed. In particular, there was no interaction between conditions and masks ($F_{2,22}=0.09$, $p > 0.9$), suggesting that the box and the ball weight estimation tasks were comparably sensitive to this manipulation.

One may note that performance in the no mask condition of the second pilot study appears greater than performance in the first pilot study. Importantly, this between-experiment difference occurred for both tasks (which in turn did not differ from each other, all $p > 0.48$), and is likely due to the difference in total number of experimental trials (180 experimental trials in Pilot 2, and 60 in Pilot 1) enabling different levels of learning. Such an effect of trial number is compatible with SSE during the four TMS experiments, in which participants performed 120 experimental trials, falling between the first and second pilot. Despite different practice, the two pilot studies confirmed that the box and ball weight estimation tasks were matched for difficulty and were similarly

30%) repeated measures ANOVA was used on the SSE (Table 2) to compare the effect the different masks had on the performance in the different conditions.
sensitive to visual interference.

**TMS experiments: is SI functionally relevant to weight estimation?**

With our experiment, we wanted to test two main questions. First, we tested if SI carries information relevant to deducing the weight of a box from the observation of human actions, which we tested by investigating whether cTBS had an effect in the SI-box condition - i.e. by directly comparing the SSE in the box weight estimation task after sham and active cTBS over SI (Experiment 1). A paired t-test revealed greater SSE values after active relative to sham cTBS and this difference could be detected regardless of whether the outlier participant was included in the analysis or not (N16: t_{15}=−3.35, p=0.004; N17: t_{16}=−2.77, p=0.013; Fig. 2A).

Second, we wanted to know if the observed effect was site- and task-specific. To this aim we performed a 4 condition (SI box, M1 box, SPL box, SI ball)×2 cTBS (Sham vs Active) ANOVA. The ANOVA revealed a significant condition×cTBS interaction (N16: F_{3,62}=3.46, p=0.02; N17: F_{3,63}=2.88, p=0.04; Fig. 2A). Duncan post-hoc tests indicated that the SI box condition (Experiment 1) showed only the significant SSE difference between sham and active cTBS (N16: p=0.02; N17: p=0.04), whereas no difference between sham and active cTBS were found in the other three conditions (Experiment 2–4, all paired wise comparisons p > 0.34). Comparable SSE was found across the sham condition of the four experiments (all p > 0.88).

Directly comparing sham and active cTBS differences across all conditions confirmed that this difference was significantly larger in the SI box condition (Experiment 1) than in the other three conditions (Experiment 2–4; N16: all p < 0.014; N17: all p < 0.028) which in turn did not differ from one another (all p > 0.84 for N16 and N17). The data indicate a selective reduction of performance in the SI box condition after active cTBS. Fig. 2B shows the distribution of the SSE differences (sham – active cTBS) for the four experiments. While a clear disruption could be observed at the group level, the cTBS effect was variable across participants in the SI box condition (Experiment 1): 11 out of the 17 participants showed larger SSE during active relative to sham cTBS whereas the remaining 6 participants showed an opposite trend although smaller in size (average SSE sham-active cTBS SI box ± s.e.m.=4.94 ± 3.76). In sum, there was a relative decrease after active cTBS in the remaining participants (average SSE sham-active cTBS PPC box ± s.e.m.=4.94 ± 3.76). In summary, the SSE performance decreased after active cTBS only for the SI box condition (Experiment 1), indicating a specific causal role of SI when the weight of an object is deduced from biological motions.

**TMS experiments: control analyses**

These findings on SSE were confirmed by the condition x cTBS ANOVA performed on another index of task performance, namely the Pearson R² (as used in Pobric and Hamilton (2006)). This ANOVA showed a significant condition×cTBS interaction (N16: F_{3,62}=3.03, p=0.036; N17, F_{3,63}=2.6, p=0.054). Duncan post-hoc tests indicated SI box condition as the only condition with a significant R² difference between sham and active cTBS (N16, p=0.03). No difference was found between sham and active cTBS in the other conditions (all p > 0.22). Directly comparing sham and active cTBS differences across all conditions using the same Duncan post-hoc procedure confirmed that this difference was significantly larger in the SI box condition than in the other three conditions (N16: p < 0.031) which in turn did not differ from one another (all p > 0.6). As expected, the SSE and R² measures were highly and inversely correlated across the four conditions and 2 types of cTBS (all r > .86, all p < .001). Moreover, in Experiment 1, changes in SSE (sham – active) significantly correlated with changes in R² (r > −.74, p < .001), suggesting that disruption of performance could be similarly detected with the two indices of performance.

To further explore whether the increase of errors in the SI box condition (Experiment 1) was due to a systematic change in perceived weight, or a reduction in the reliability of estimation, we also compared the mean estimated box weight after sham cTBS (mean weight ± s.e.m: 3.13 ± 0.07 for both N16 and N17) and active cTBS (3.24 ± 0.08 for N16; and 3.22 ± 0.08 for N17) in the SI box condition (paired t-test, for N16: p=0.027, and for N17: p=0.074), suggesting a tendency for perceiving greater weight after active cTBS over SI (Fig. 2C). No sham-active cTBS differences in box weight estimation was found in the other conditions (all p >.40). However, the condition×cTBS ANOVA did not show significant main effects or interaction (N16: all F < 2.05, all p > 0.11; N17: all F < 1.89, all p > 0.14), suggesting that changes in the mean estimated weight were less consistent than changes in SSE. Further t-tests suggested that in all conditions participants tended to overestimate the box weight (Supplementary Table 1). Moreover, in Experiment 1, changes in SSE (sham-active) correlated with changes in the estimated mean box weight (r=0.53, p=0.028). Thus, in Experiment 1, the disruption of performance induced by active cTBS over SI was at least in part due to an increased bias toward weight overestimation (a bias that was present in all groups but increased only after SI perturbation).

The ANOVA on RTs during sham and active cTBS (Fig. 2D) did not show a significant condition×cTBS interaction (N16: F_{3,62}=1.11, p=0.35; N17: F_{3,63}=1.3, p=0.28) suggesting that active cTBS over SI impaired the accuracy in the weight estimation of observed lifted box and not the speed of the response (as measured by RT). The direct comparison between the mean RTs for the sham (mean RTs ± s.e.m.: N16: 514 ms ± 60; N17: 632 ms ± 112) and active cTBS (N16: 601 ms ± 81; N17: 714 ms ± 125) conditions showed a non-significant trend towards an increase of RTs after active cTBS in the SI box condition (paired t-test, N16: p=0.09; N17: p=0.06). No similar trend was observed in the other conditions (all p > 0.64). Importantly, that cTBS in the SI box condition reduced accuracy (significantly) and increased RTs (marginally), speaks against the possibility of a speed-accuracy trade-off. Indeed, there was also a significant correlation between changes (sham-active) in SSE and RTs (r=0.59, p=0.012), suggesting that in Experiment 1 greater impairments in accuracy induced by SI cTBS were associated with slower response.

**Discussion**

Our results show that, compared to sham stimulation, active cTBS perturbation of SI selectively worsened participant’s ability to estimate the weight of a box when seen lifted (Experiment 1). In contrast, participants’ performance after active cTBS remained comparable to sham stimulation when (i) participants judged the weight of a bouncing ball (Experiment 4), and (ii) stimulation was applied over the adjacent M1 (Experiment 2) or (iii) SPL (Experiment 3). Notably, disruption of performance after active cTBS over SI consisted of a clear reduction in the accuracy of estimations in the box weight, with an increased tendency for weight overestimation and slower responses. This suggests SI is a critical part of a system of brain regions sub-serving weight estimation when a human agent is involved, and supports the idea that SI may enrich action understanding by providing vicarious representa-
tions of the proprioceptive consequences of the observed actions (Gazzola et al., 2007a, 2007b; Avenanti et al., 2007; Raos et al., 2007; Caspers et al., 2010; Keysers et al., 2010; McGregor et al., 2016; Valchev et al., 2016).

This extends the network of brain regions necessary for optimal action perception, as evidence for necessity was so far mostly restricted to the IFC and IPL, as shown by TMS (Pobric and Hamilton, 2006; Urgesi et al., 2007; Cattaneo, 2010; Cattaneo et al., 2010, 2011; Tidoni et al., 2013; Avenanti et al., 2013b; Jacquet and Avenanti, 2015), transcranial direct current stimulation (Avenanti et al., 2017), and neurological lesion studies (Tranel et al., 2003; Battelli et al., 2003; Saygin et al., 2004; Buxbaum et al., 2005; Saygin, 2007; Pazzaglia et al., 2008; Weiss et al., 2008; Fazio et al., 2009; Kalènine et al., 2010; Urgesi et al., 2014).

If the effect of cTBS over SI were not the result of a perturbation of SI but of a spread of the magnetic impulse onto nearby motor or parietal regions, moving the coil forward or backwards should increase rather than decrease its detrimental effects on perception. This was not the case, supporting the conclusion that the effect was indeed mediated by SI. However, imaging and neurophysiological studies show SI does not work in isolation during action observation, but is rather part of an entire network composed of ventral and dorsal premotor, anterior and posterior parietal cortices activated in both action observation and execution (Avikainen et al., 2002; Rossi et al., 2002; Hasson et al., 2004; Caetano et al., 2007; Gazzola et al., 2007a, 2007b; Raos et al., 2007; Kilner et al., 2009; Pierno et al., 2009; Caspers et al., 2010; Arnstein et al., 2011; Turella et al., 2012). Indeed, we found that cTBS over SI alters brain activity in the premotor cortices (Valchev et al., 2015a, 2016), known also to contribute to weight estimation (Hamilton et al., 2006; Pobric and Hamilton, 2006). Accordingly, the current results should not be interpreted to suggest that the impairment of performance reflects cTBS-induced changes of activity in SI alone, but rather that disrupting SI activity using cTBS is likely to have disrupted the functioning of a somatosensory-motor, parieto-frontal network of which SI is an active element.

Given the importance of both IFC (Pobric and Hamilton, 2006) and SI (this paper) to weight estimation by observation, as well as the exchange of information between these regions during action observation (Kokal and Keysers, 2010; Schippers and Keysers, 2011, Valchev et al., 2016, McGregor et al., 2016), it is relevant to consider what the roles of each region may be. TMS studies show that seeing biomechanically possible and extremely overstretching movements facilitates the corticospinal representation of the muscles involved in the observed movements (Romani et al., 2005). Notably, rTMS over IFC disrupted motor facilitation during the observation of possible actions, while rTMS over SI disrupted the facilitation during observation of overstretching movements (Avenanti et al., 2007, 2013a). The IFC could therefore provide primarily vicarious motor representations derived from the kinematics that would enable the observer to produce a similar action, if the movement is biomechanically possible. SI, on the other side, could primarily contribute vicarious somatosensory (tactile and/or proprioceptive) action components, that emerge for instance during observation of overstretching finger movements. The contribution of SI in mapping somatosensory consequences of observed actions is supported by the findings that SI activity is increased when seeing other people grasping or manipulating objects (Keysers et al., 2010) or when seeing extreme joint stretching movements (Costantini et al., 2005). Evidence that somatosensory cortices are recruited both when sensing the body and during perception of others being touched or painfully stimulated (Bufalari et al., 2007; Valeriani et al., 2008; Keysers et al., 2010; Lamm et al., 2011), and that rTMS over SI impairs the ability to detect touch in others (Bolognini et al., 2011, 2014) further supports this interpretation.

While manipulation of biomechanical plausibility may dissociate somatosensory and motor components during action observation, typically these two components are tightly interlinked. This is particu-}

larly evident when observing somebody else lifting objects. Recently, Alaerts et al. (2010) found that when participants observe an actor lift objects of different weights, motor-evoked potentials are facilitated mainly by two factors: the kinematics of the movement and the degree of contraction of the hand (see also Tidoni et al. (2013) and Valchev et al. (2015b)). This facilitation could be the result of the integration in M1 of motor plans inferred via IFC and proprioceptive/tactile information inferred via SI. In our experiments, as the stimuli were generated by modifying kinematics alone, the source of somatosensory information has to be kinematic. Importantly, we propose that SI main contribution to action perception relates to the extraction of proprioceptive/tactile information derived from observed action kinematics, rather than to encoding action kinematics per se. On the other hand, observed kinematics is likely processed in other (visual and/or motor) brain regions including the IFC. This proposal fits with previous studies showing impaired recognition of action kinematics following lesion or interference with the IFC (Avenanti et al., 2013b; Urgesi et al., 2014), and with the recent study of Jacquet and Avenanti (2015) showing that visual discrimination of observed hand kinematics (i.e., grip aperture) was disrupted by IFC but not SI online TMS interference.

Our study supports the notion that SI provides a vicarious somatosensory representation of seen actions and this representation is necessary for accurate performance in the box weight estimation task. However, we argue that the contribution of SI to action understanding may be more general. Indeed, studies using causal methods have shown that SI is critical for recognizing the facial or vocal emotional expressions of others (Adolphs et al., 2000; Pitcher et al., 2008; Banissy et al., 2010; Paracampo et al., 2016). While the TMS adaptation study of Jacquet and Avenanti (2015) suggested no critical role of SI for accurate perception of action kinematics, the same study also highlighted a state-dependent effect of SI (and IFC) stimulation in a task requiring to discriminate between action goals. TMS pulses over SI and IFC hand representations (but not over control regions) facilitated the recognition of test pictures showing a repeated (adapted) action goal, regardless of the kinematics used to perform the action (Jacquet and Avenanti, 2015). These state-dependent effects suggested a causal role of SI and IFC in the encoding of action goal. Remarkably, this role is not in contrast with the proposal that SI and IFC may mirror somatosensory and motor components of observed actions, respectively (Gazzola et al., 2007; Avenanti et al., 2007; Keysers et al., 2010; McGregor et al., 2016). Indeed, goal processing may involve the prediction of both motor and somatic afferent action components which could be processed in partially separated networks (Christensen et al., 2007; Etzel et al., 2008; Gazzola and Keysers, 2009).

Our study significantly expands this previous evidence by showing that disruption of SI with active cTBS impairs performance in the box weight estimation task (Experiment 1), and this provides strong evidence that SI is causally essential for accurate action perception. On the other hand, active cTBS over SI did not affect the ability to judge the weight of a bouncing ball (Experiment 4). The selectivity of the cTBS disruption cannot be accounted by a difference in the difficulty between the box and ball weight estimation tasks. Indeed, the two tasks were matched for difficulty (as shown in pilot study 1 and 2) and presented similar sensitivity to external (visual masking) interference (as shown in pilot study 2). Thus, the selective interference with box weight estimation by SI perturbation cannot be accounted by confounding effects like a different difficulty of the two tasks and rather suggests that SI is critically involved in inferring somatosensory qualities (i.e., light/heavy) from human actions and not from non-human motion.

In all the conditions, participants tended to slightly overestimate the ‘correct’ weight in the box weight estimation task. Interestingly, active cTBS over SI tended to further increase such overestimation relative to sham cTBS, suggesting that the disruption in accuracy was in part due to a tendency to perceive the weight of the box as heavier. Thus, altered signals in SI may make people less accurate in judging the
weight of lifted objects, by biasing their weight judgment. Again, these effects were specific for human action as no weight bias was induced in the ball estimation task. Notably, a contribution of SI, IFC and other sensorimotor regions to perceiving the weight of objects seen to be lifted was suggested by previous studies showing that: i) lifting a box influences participant’s perceptual judgments of the weight of a box lifted by others (Hamilton et al., 2004); and, ii) the strength of this perceptual bias correlated with neural activity in a network of cortical regions including SI, IFC, M1 and SPL (Hamilton et al., 2006). However, these methods could not establish whether activity in SI was necessary for accurate action perception. While previous evidence showed that IFC is necessary for correct performance in the box weight estimation task (Pobric and Hamilton, 2006), the present study provides the first causative evidence that also SI, but not M1 or SPL, is critical for the social perception of weight.

The lack of a significant effect with M1 stimulation (Experiment 2) is not surprising. First, although SI and M1 are anatomically close and highly interconnected, there is physiological and behavioral evidence that the aftereffect of SI and M1 cTBS can dissociate (Ishikawa et al., 2007; Mochizuki et al., 2007; Ragert et al., 2008; Schabrun et al., 2008). Second, although neural activity in M1 may be modulated by action observation (Nishitani and Hari, 2000; Fadiga et al., 2005; Caetano et al., 2007; Schütz-Bosbach et al., 2009; Gazzola and Keysers, 2009; Vigneswaran et al., 2013), it is debated whether such activity might play a major role in action perception (Lepage et al., 2008; Pineda, 2008; Bonini, 2016). On the one hand, the activity may be a simple downstream consequence of the strong reciprocal cortico-cortical connections, for example with IFC and/or SI (Geyer et al., 2000; Rizzolatti and Luppino, 2001). Similarly, previous TMS studies reported that offline inhibition of M1 excitability did not influence mirror-like motor facilitation (Avenanti et al., 2007), and online M1 interference did not affect perceptual judgments of seen actions (Cattaneo et al., 2011), whereas these processes were affected by stimulation of IFG or SI (Pobric and Hamilton, 2006; Avenanti et al., 2007; Cattaneo et al., 2011; Jacquet and Avenanti, 2015; and present study). On the other hand, a few recent studies have documented a disruption of effector recognition (Naish et al., 2016) or body posture recognition (Borgomaneri et al., 2015) after online TMS of the hand representation in M1. However, online M1 stimulation may cause peripheral motor responses and the lack of control area eliciting similar responses in a different body part or the lack of control task for assessing nonspecific, distracting effects of online TMS makes the interpretation of such studies not conclusive. Recently, Palmer et al. (2016) found no net change in action perception following offline cTBS over M1, a null result that is in keeping with the present data. However, Palmer et al. (2016) also showed that the physiological effect of cTBS was variable across participants, leading to inhibitions of M1 excitability in some and increases in other participants. Interestingly, participants showing M1 inhibition after cTBS also showed a clear disruptive effect on action perception. It should be noted that also in our data, the cTBS effect was variable across participants. Variability was observed in all the experiments, including Experiment 2 (M1 box condition) where approximately half of the participants showed improved performance and the remaining participants showed decreased performance, thus resembling the proportion observed by Palmer et al. (2016). Thus, although current and previous data suggest no major action perception impairment when M1 is stimulated, further studies combining cTBS with physiological and behavioral assessment might test whether effective inhibition of M1 affects performance in the box weight estimation task. Nerveless, the inconsistency in the effect of M1 stimulation is at variance with the disruption we found after SI cTBS (Experiment 1). Although also in this case cTBS effects were variable, a clear reduction of performance was observed at the group level, suggesting that SI may be more important than M1 for estimating weight from seen actions.

The absence of effects after rTMS over SPL (Experiment 3) may be less expected. The SPL is a high-order multisensory region integrating visual and somatosensory information about limb position (Johannes et al., 2002). Direct stimulation of SPL (area 7) in awake neurosurgery patients produces sensations on the body but not motor output (Desmurget et al., 2009). Moreover, rTMS over this region impairs performance in proproceptive tasks, although less than rTMS over SI (Balslev et al., 2004). Also, studies show activation in SPL both during action execution and observation (Raos et al., 2007; Keysers and Gazzola, 2009) and that the effect of cTBS over SI spreads to SPL (Valchev et al., 2016), suggesting a possible role of SPL in action perception. However, during action observation this region is less consistently activated relative to other sectors of the parietal cortex (Van Overwalle and Baetens, 2009). It may thus be that SPL (and in particular area 7, the target of Experiment 3), plays a role in action perception that is relatively minor relative to nearby parietal regions, including SI and IPL that appears more critical for action perception (Cattaneo et al., 2010; Urgesi et al., 2014; Jacquet and Avenanti, 2015).
interindividual variability, in the present research we limited motor activity of participants’ right hand (i.e., the hand contralateral to the target sites and corresponding to the actor’s hand depicted in the movies) before and after cTBS, possibly leading to a consistent suppression of neural activity in the stimulated sites (Huang et al., 2005; Ishikawa et al., 2007; Poreisz et al., 2008). However, even if we observed a disruptive effect at the group level, the effect of SI cTBS was variable across participants of Experiment 1 and not all showed a disruption (see Jones et al., 2016). Additionally, we do not rule out that either increases or decreases of neural activity could move brain activity away from its optimal state, and thereby could reduce behavioural accuracy. Future studies combining brain stimulation and brain imaging techniques will directly address these issues and clarify the relationship between physiological and behavioural effects induced by SI cTBS.

In conclusion, earlier evidence supported the claim that somatosensory cortices are activated not only during action execution, but also during perception of others’ actions, but whether such activation of SI is necessary to efficiently judge the somatosensory aspects of the actions of others remained unclear (Gazzola et al., 2007b; F内的, 2012; Vannuscorps and Caramazza, 2015). Indirect evidence came from sensory neuropathy patients that lack a sense of touch on their own body. These patients showed impaired performance in a task requiring weight estimation from lifting actions (Miall et al., 2000) and inference of another’s expectation of a weight when seeing him lifting a box (Bosbach et al., 2005). Our findings, that cTBS over SI negatively influences the capacity to judge the weight of a box by observing the action (lifting) of other people, now provides direct evidence that SI is a crucial part of a system of brain areas necessary for the optimal perception of at least certain aspects of other people’s hand actions. Together with evidence that SI is also critical for recognizing the facial expression of others (Adolphs et al., 2000; Pitcher et al., 2008; Banissy et al., 2010; Paracampo et al., 2016) and encoding action goals (Jacquet and Avenanti, 2015), this suggests that SI seems to play a more important role in action understanding than previously thought.

**Funding**

The work was supported by a grant from the Portuguese Foundation for Science and Technology SFRH/BD/47576/2008 to N.V.; a grant from the European Research Council of the European Commission (ERC consolidator grant INTERACT 313398) to A.H.; grants from the Netherlands Organisation for Scientific Research (VENI 451-09-006, VIDI 452-14-015) to V.G.; and grants from the Ministero della Salute (GR-2010-231935), Cogito Foundation (R-117/13; and 14-139-R), Ministerio Istruzione, Università e Ricerca (RBFR12F0BD) and BIAL Foundation (298/16) awarded to A.A.

**Conflict of interest**

The authors declare that they have no conflicts of interest for this study.

**Appendix A. Supplementary material**

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.neuroimage.2017.02.075.

**References**


Aziz-Zadeh, L., Maeda, F., Zaidel, E., Mazziotta, J., Iacoboni, M., 2002. Lateralization in the role of the right primary somatosensory cortex in encoding the a


Pineda, J.A. 2008. Sensorimotor cortex as a cortical component of an “extended” mirror neuron system: does it solve the development, correspondence, and control problems of empathy? Behav. Brain Funct. 4, 47.


