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Your emotion moves into my motor system

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Chapter 2

Motor mapping of implied actions during perception of emotional body language*

* This chapter was adapted from:

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Abstract

Perceiving and understanding emotional cues is critical for survival. Using the International Affective Picture System (IAPS) previous TMS studies have found that watching humans in emotional pictures increases motor excitability relative to seeing landscapes or household objects, suggesting that emotional cues may prime the body for action. Here we tested whether motor facilitation to emotional pictures may reflect the simulation of the human motor behavior implied in the pictures occurring independently of its emotional valence. Motor-evoked potentials (MEPs) to single-pulse TMS of the left motor cortex were recorded from hand muscles during observation and categorization of emotional and neutral pictures. In experiment 1 participants watched neutral, positive and negative IAPS stimuli, while in experiment 2, they watched pictures depicting human emotional (joyful, fearful), neutral body movements and neutral static postures. Experiment 1 confirms the increase in excitability for emotional IAPS stimuli found in previous research and shows, however, that more implied motion is perceived in emotional relative to neutral scenes. Experiment 2 shows that motor excitability and implied motion scores for emotional and neutral body actions were comparable and greater than for static body postures. In keeping with embodied simulation theories, motor response to emotional pictures may reflect the simulation of the action implied in the emotional scenes. Action simulation may occur independently of whether the observed implied action carries emotional or neutral meanings. Our study suggests the need of controlling implied motion when exploring motor response to emotional pictures of humans.

Introduction

The ability to understand the emotions displayed by other individuals is critical to react adaptively in social environments. Nonverbal cues, such as body posture or hand gestures, convey important information about the emotional state of others (Darwin, 1872; James, 1890). Imaging studies suggest that reading the emotional language expressed in others' body postures and actions relies on the activity of a complex neural network which may include cortical-subcortical regions involved in emotional processing (e.g. the amygdala, anterior insula) and fronto-parietal sensorimotor regions involved in action planning and execution (Rizzolatti and Craighero, 2004; Keysers and Gazzola, 2009; de Gelder et al., 2010; Tamietto and de Gelder, 2010) (so called putative mirror neuron system, pMNS). Embodied simulation theories suggest that, since covert emotional states (e.g. happiness) are often associated with overt motor behaviors (e.g. smiling, joyful body postures and gestures), observers can simulate and understand the unobservable emotional state of others by embodying their observable motor behavior (Oberman et al., 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Niedenthal et al., 2010; Gallese and Sinigaglia, 2011). Thus, according to this view, emotion perception is inherently linked to action simulation.

Strong evidence for action simulation in humans comes from single-pulse transcranial magnetic stimulation (TMS) studies showing that action observation selectively increases the amplitude of motor-evoked potentials (MEPs) recorded from those muscles involved in the observed actions (Fadiga et al., 2005; Avenanti et al., 2007; Catmur et al., 2007; Avenanti and Urgesi, 2011). Similar motor facilitation is also found when seeing pictures of humans depicted in the middle of a motor act (implied action stimuli) (Avenanti et al., 2013; Candidi et al., 2010; Urgesi et al., 2010), indicating that the motor system can extract dynamic action information from static images that suggest an ongoing body movement.

These findings suggest that during observation of emotional expressions, even when displayed in a static snapshot, the motor system may be mainly involved in encoding motor features of the implied body movement, independently of its emotional meaning. Indeed, if the motor cortices are mainly involved in action simulation, as predicted by the embodied simulation account, then motor resonance should occur independently of the emotional content of the observed body movement.

However, another line of research has suggested that during emotional processing the motor system is mainly involved in reacting to emotional cues and preparing a potential motor act (Lang, 1993; Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009). In keeping with this notion, behavioral studies have found that unpleasant and pleasant emotional cues facilitate defensive and approaching movements, respectively (Chen and Bargh, 1999; Rotteveel and Phaf, 2004). Moreover, relevant to the present research, TMS studies have shown that perception of emotional pictures from the International Affective Picture System (IAPS, Lang et al., 1999) increases motor excitability (Oliveri et al., 2003; Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009; van Loon et al., 2010): in particular, seeing snapshots depicting humans in pleasant (e.g. sport, erotic scenes) or unpleasant situations (e.g. being assaulted, injured) increased the amplitude of MEPs from hand muscles relative to neutral control stimuli. However, in these studies, most of the neutral stimuli depicted landscapes or static objects but no humans. Therefore, it is unclear whether increased motor excitability for emotional IAPS stimuli reflected i) a reaction to emotionally arousing stimuli, possibly linked to the activation of approaching/avoidance motor programs; ii) the simulation of human actions implied in the emotional scenarios only.

Here we sought to clarify the involvement of the motor system in processing emotional and action cues embedded in scenes depicting human body movements. MEPs to single-pulse TMS of the left primary motor cortex (M1) were recorded during presentation of emotional and neutral stimuli during an active categorization task. In the experiment 1, we presented positive, neutral and emotionally negative complex scenes from the IAPS database (as in previous TMS research Hajcak et al., 2007;

van Loon et al., 2010) while in experiment 2, we compared positive and negative emotional body movements with neutral movements (i.e. with comparable perceived implied motion but no emotional meaning), and neutral static body postures (no implied motion). This way we tested whether motor facilitation for emotional pictures may reflect motor simulation independently of the emotional valence carried by the action implied in the observed scene.

Materials and methods

Participants

A total of 85 right-handed healthy participants took part in the study. Fourteen and fifteen subjects with no contraindication to TMS (Rossi et al., 2009) were tested in experiment 1 (6 men, mean age \pm S.D.: 24.1y \pm 1.4) and experiment 2 (6 men, age: 22.4y \pm 2.6), respectively. Moreover, 50 subjects (21 men) were tested in three pilot studies and 16 subjects (8 men) in two control behavioural experiments (see Supplementary Material). The study was approved by the University of Bologna, Department of Psychology ethics committee and carried out according to the Declaration of Helsinki. All subjects gave their written informed consent. No discomfort or adverse effects during TMS were reported or noticed.

Visual stimuli

Different types of pictures were presented on a 19-inch screen located 80 cm away from the participants. In experiment 1, 108 stimuli from the IAPS database (Lang et al., 1999) were selected (Figure 1A). All the stimuli (listed in the Supplemental Material) were already used in previous TMS experiments (Hajcak et al., 2007; van Loon et al., 2010) and included: 36 unpleasant (e.g. scenes of violence, threat, and injuries), 36 pleasant (e.g. sporting events, erotic scenes), and 36 neutral scenes

(e.g. household objects, landscapes). Several of these emotional IAPS stimuli depicted humans performing actions. In contrast, IAPS database did not include a sufficient number of emotionally neutral scenes depicting humans performing neutral actions. Hence, the influence of action-related information was further tested using a set of novel emotional and non-emotional stimuli. In experiment 2, 104 pictures depicting four different actors in emotional or neutral postures were used (Figure 1B). To focus specifically on body-related information, the faces were blanked out in all pictures. Images included 26 static neutral postures (static), 26 emotionally neutral actions (neutral), 26 emotionally negative (fear) and 26 positive body movements (joy). During the recording of neutral actions, actors' instructions specified the action to be performed. For emotional actions, instructions specified a familiar scenario (e.g. you have just won the lottery) or involved a potential threat (e.g. a tennis ball was thrown against the actor).

The 104 stimuli used in experiment 2 were selected from an initial sample of about 1000 stimuli based on two pilot studies (Supplementary Material) in which emotional ratings (pilot 1) and recognition performance (pilot 2) tasks were used to select 13 static, 13 neutral, 13 fearful and 13 joyful body expressions that were well recognized. For each category, the final set contained 13 original stimuli and 13 mirror-reflected copies of such stimuli. In a third pilot study ($N = 8$, 4 men, age: $26.9y \pm 4.8$), we assessed the perceived implied motion sensation evoked by actors' hands in each of the 104 pictures using an electronic 5-points Likert scale. Mean ratings for each category were analyzed by means of one-way repeated measure ANOVA. The analysis was significant ($F_{3,21} = 4.68$, $p < 0.05$): implied motion was lower for static stimuli (mean implied motion score \pm S.D.: 1.73 ± 0.90), than for neutral (3.19 ± 0.79), joyful (2.73 ± 1.00) and fearful stimuli (2.87 ± 0.62 ; all $p < 0.05$) which in turn did not differ from one another (all $p > 0.44$). Thus in the selected sample of body stimuli, emotional and neutral actions contained the same amount of implied hand motion.

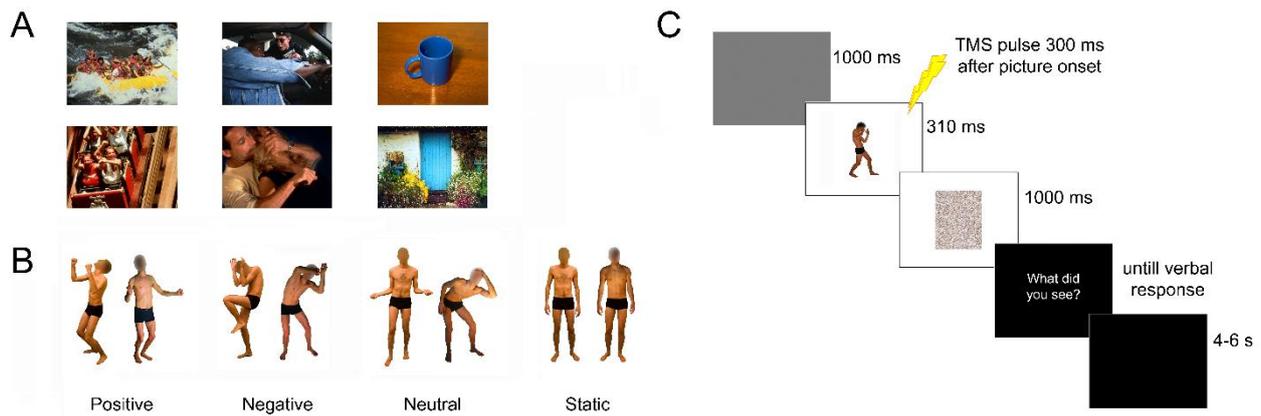


Figure 1. Examples of visual stimuli used in (A) experiment 1 (IAPS images) and (B) experiment 2 (Body images). For each visual condition two representative images are shown. (C) Trial sequence.

Transcranial magnetic stimulation and electromyography recording

In both experiments, MEPs induced by TMS were recorded from the right first dorsal interosseus (FDI) and abductor pollicis brevis (APB) by means of a Biopac MP-150 (Biopac, U.S.A.) electromyograph. We selected these two muscles based on previous TMS studies that explored motor excitability during observation of emotional pictures (Oliveri et al., 2003; Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009; van Loon et al., 2010). EMG signals were band-pass filtered (30-500 Hz), sampled at 5 kHz, digitized and stored on a computer for off-line analysis. Pairs of silver-chloride surface electrodes were placed in a belly-tendon montage with ground electrodes on the wrist. A figure-of-8 coil connected to a Magstim Rapid2 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. By using a slightly suprathreshold stimulus intensity, the coil was moved to determine the optimal position from which maximal amplitude MEPs were elicited in the contralateral FDI muscle. The optimal position was then marked on the scalp to ensure correct coil placement throughout the experiment. The intensity of magnetic pulses was set at 120% of the resting motor threshold (rMT), defined as the

minimal intensity of the stimulator output that produces MEPs with amplitude of at least 50 μ V in the higher threshold muscle with 50% of probability (Rossini et al., 1994). This way a stable signal could be recorded from both muscles. The absence of voluntary contraction was continuously verified visually throughout the experiment. When muscle tension was detected the experiment was briefly interrupted and subjects were invited to relax.

Procedure and experimental design

The experiments were programmed using Matlab software to control picture presentation and to trigger TMS pulses. In both experiments MEPs were collected in four blocks. The first and the last blocks served as baseline: subjects held their eyes closed with the instruction to imagine watching a sunset at the beach (Fourkas et al., 2008) while receiving TMS over M1 (inter-pulse interval ~10s). Ten trials were recorded for each of the baseline blocks. In the other two blocks subjects performed an emotion evaluation task, in which they were presented with a picture and were asked to categorize it as positive, negative or neutral picture (experiment 1) or as a joyful, fearful, neutral or static posture (experiment 2). Two control behavioral experiments ruled out that baseline procedures biases emotion categorization in experiment 1 and 2 (Supplementary Material).

Emotional evaluation blocks included 54 (experiment 1) or 52 trials (experiment 2) each (108 and 104 trials in total). In the emotion evaluation blocks, trial sequence was the following: a grey screen (1s duration) indicated the beginning of the trial and it was followed by the test picture (310ms) projected at the centre of the screen (Figure 1C). The TMS pulse was delivered 300ms after the onset of the stimulus. The picture was followed by a random-dot mask (obtained by scrambling the corresponding sample stimulus by means of a custom-made image segmentation software) lasting 1s and then, subject had to verbally answer to the question “What did you see?” that appeared on the screen (forced choice). An experimenter collected the answer by pressing a corresponding computer

key. To avoid changes in excitability due to verbal response (Tokimura et al., 1996; Meister et al., 2003), participants were invited to answer only during the question screen, few seconds after the TMS pulse. After response, the screen appeared black for 4-6 sec. This way the inter-pulse interval was >10 sec, thereby avoiding changes in motor excitability due to TMS *per se* (Chen et al., 1997). This was directly confirmed by the lack of changes in FDI or APB MEP amplitudes between the first and the last baseline blocks in both experiments (all $p > 0.40$). To reduce the initial transient-state increase in motor excitability, before each block two magnetic pulses were delivered over M1 (inter-pulse interval ~10s). Each baseline and experimental block lasted about 2 and 10 min respectively.

After TMS, subjects were presented with all the stimuli (shown in a randomized order) and asked to judge arousal, valence and perceived movement using an electronic 5-points Likert scale. To avoid building up artificial correlations between the different judgments, each rating was collected separately during successive presentation of the whole set of stimuli.

Data analysis

Neurophysiological data were processed off-line. Mean MEP amplitude values in each condition were measured peak-to-peak (in mV). Since background EMG is known to modulate the MEP amplitudes (Devanne et al., 1997) pre-TMS EMG was assessed by calculating the mean rectified signal across a 100ms interval prior to TMS. MEPs with preceding background EMG deviating from the mean by more than 2 S.D., were removed from further analysis (less than 5%). Moreover, MEPs associated to incorrect answers were discarded from the analysis. Mean accuracy was high (experiment 1 mean accuracy \pm S.D.: 91% \pm 8%; experiment 2: 92% \pm 4%) and comparable across the experiments ($t_{27} = 0.42$; $p = 0.68$).

Mean MEP amplitude in each condition was normalized by using the average of two baseline blocks (condition-baseline)/(condition+baseline). This MEP ratio index proved adept to normalize data

distribution (as revealed by Shapiro-Wilk test) and was analyzed by means of two repeated measures two-way ANOVA (one for each experiment) with factors: Muscle (2 levels: FDI, APB) and Stimulus (3 levels for experiment 1: positive, negative and neutral; 4 levels for experiment 2: positive, negative, neutral and static). In all ANOVAs, post-hoc comparisons were carried out by means of the Newman-Keuls test.

In the TMS experiments, mean ratings for arousal, valence and perceived movement induced by the different images were not normally distributed and thus were analyzed by means of nonparametric Friedman ANOVAs and Bonferroni-corrected planned nonparametric comparisons. Specifically, in experiment 1 we tested whether emotional IAPS stimuli had not only greater arousal but also greater implied motion than neutral IAPS stimuli. Moreover, in experiment 2 we tested whether emotional (positive, negative) body stimuli had greater arousal than non-emotional neutral action stimuli, but comparable implied motion. We also assured that emotional and neutral action stimuli had greater implied motion than static stimuli. Furthermore, in both experiment we tested whether valence of positive stimuli was greater than neutral and negative stimuli and valence of negative stimuli was lower than that of neutral stimuli.

Results

Experiment 1

The Muscle x Stimulus ANOVA on normalized MEP amplitudes recorded during observation of IAPS stimuli showed a main effect of the Stimulus ($F_{2,26} = 6.50$, $p < 0.01$; Figure 2) accounted for by the greater MEP amplitude during observation of emotionally positive ($p < 0.01$) and negative ($p < 0.05$) relative to neutral scenes. No difference between positive and negative scenes was found ($p = 0.37$). No main effect or interaction with factor Muscle was found indicating that similar changes in

excitability were detected in the FDI and APB muscles (all $F < 2.19$, $p > 0.13$; raw MEP amplitudes in Table 1).

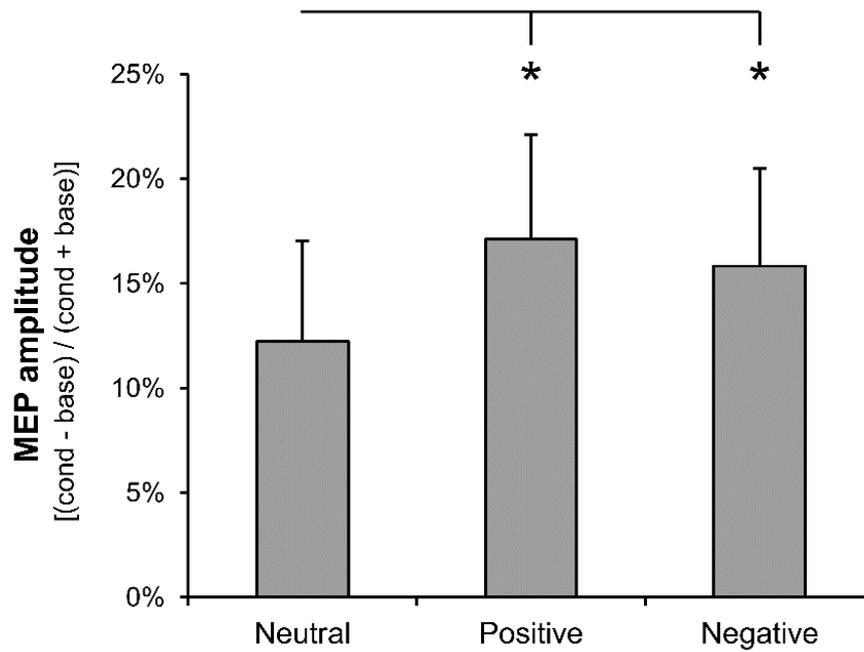


Figure 2. MEP amplitude [(condition - baseline)/(condition + baseline)] during the observational conditions of experiment 1 (IAPS images), average of the two muscles (FDI and APB). Error bars indicate s.e.m. Asterisks (*) denote significant post-hoc comparison ($p < 0.05$).

	Baseline	Neutral	Positive	Negative
FDI	1.08 ± 0.85	1.40 ± 0.92	1.55 ± 0.96	1.47 ± 0.89
APB	0.78 ± 0.44	0.96 ± 0.46	1.04 ± 0.51	1.01 ± 0.47

	Baseline	Static	Neutral	Positive	Negative
FDI	1.00 ± 0.40	1.22 ± 0.47	1.31 ± 0.48	1.32 ± 0.51	1.35 ± 0.50
APB	0.86 ± 0.59	1.21 ± 0.69	1.29 ± 0.78	1.33 ± 0.8	1.27 ± 0.71

Table 1. Raw MEP amplitudes ± standard deviation recorded in the two muscles (FDI and APB) during the conditions of experiment 1 (top) and experiment 2 (bottom).

Table 2 illustrates subjective evaluations of IAPS stimuli. Friedman ANOVAs carried out on arousal, valence and implied motion scores of IAPS stimuli were all significant (all $\chi^2_2 > 26.14$, $p < 0.001$). Follow-up comparisons confirmed that valence was lower for negative relative to positive and neutral IAPS stimuli (all $p < 0.001$); moreover, positive IAPS stimuli obtained higher valence scores than neutral IAPS stimuli ($p < 0.001$). Critically, not only arousal but also implied motion scores were higher for positive and negative IAPS stimuli relative to neutral IAPS stimuli (all $p < 0.001$). Thus, greater motor excitability for emotional scenes may be due to arousal (as hypothesized in previous studies (Hajcak et al., 2007; van Loon et al., 2010), but also to the greater (implied) motion perceived in emotional relative to neutral scenes of IAPS database. Experiment 2 was designed to directly test whether implied motion per se may explain changes in motor excitability during observation of emotional and neutral body movements.

	Neutral	Positive	Negative
Arousal	1.24 ± 0.23	2.98 ± 0.60	3.98 ± 0.70
Valence	0.01 ± 0.06	1.10 ± 0.20	-1.31 ± 0.31
Perceived motion	1.28 ± 0.50	3.60 ± 0.50	2.40 ± 0.41

	Static	Neutral	Positive	Negative
Arousal	1.13 ± 0.20	2.25 ± 0.55	3.05 ± 0.84	3.53 ± 0.76
Valence	0.01 ± 0.03	0.16 ± 0.18	1.21 ± 0.31	-1.25 ± 0.38
Perceived motion	1.06 ± 0.12	2.94 ± 0.49	3.28 ± 0.58	2.94 ± 0.51

Table 2. Mean ± standard deviation of subjective evaluations (arousal, valence and perceived implied motion) of stimuli used in experiment 1 (top) and experiment 2 (bottom).

Experiment 2

The Muscle x Stimulus ANOVA performed on normalized MEPs recorded during observation of body stimuli showed a main effect of the Stimulus ($F_{3,42} = 4.24$, $p < 0.01$; Figure 3) accounted for by the greater amplitude for positive, negative and neutral body movement relative to static body postures (all $p < 0.05$). MEP amplitude during observation of positive, negative and neutral movements did not differ from one another (all $p > 0.46$). No main effect or interaction with factor

Muscle was found indicating that similar changes in excitability were detected in the FDI and APB muscles (all $F < 0.86$, $p > 0.37$; see also Table 1).

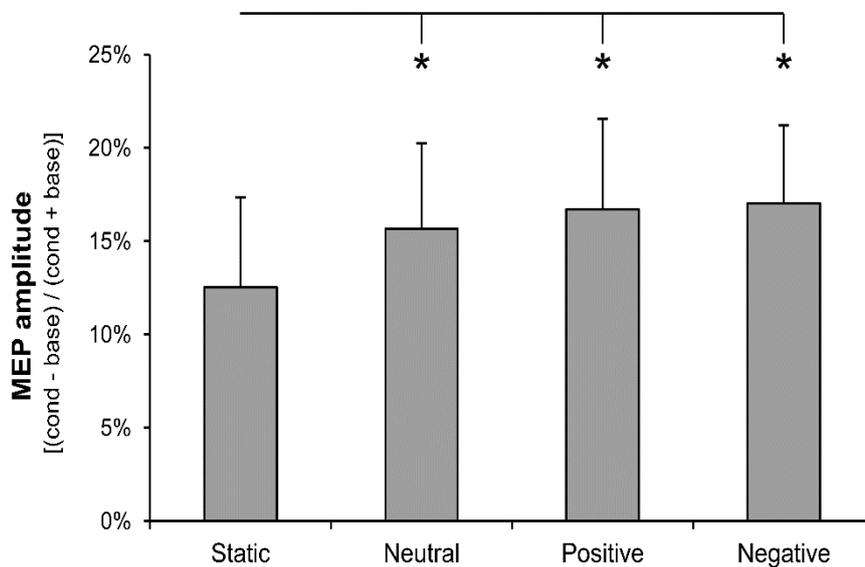


Figure 3. MEP amplitude [(condition - baseline)/(condition + baseline)] during the observational condition of experiment 2 (Body images), average of the two muscles (FDI and APB). Error bars indicate s.e.m. Asterisks (*) denote significant post-hoc comparison ($p < 0.05$).

Table 2 illustrates subjective evaluations of body stimuli. Friedman ANOVAs carried out on arousal, valence and implied motion scores of body stimuli of experiment 2 were all significant (all $\chi^2_2 > 42.79$, $p < 0.001$). Follow-up comparisons confirmed that valence was lower for negative relative to positive, neutral and static body stimuli (all $p < 0.0001$); moreover, positive body stimuli obtained higher valence scores than neutral and static body stimuli (all $p < 0.0001$); and neutral action were considered more positive than static postures ($p < 0.008$).

On average, greater arousal was associated to emotional relative to neutral and static body stimuli ($p < 0.001$); moreover, greater arousal was found for neutral actions relative to static postures ($p < 0.001$). Critically, implied motion was greater for positive and negative relative to static body stimuli

(all $p < 0.0001$) and for neutral actions relative to static body stimuli ($p < 0.0001$); moreover, implied motion was comparable for emotional relative to neutral action stimuli ($p > 0.07$). These data indicate that emotional and neutral body movement differed for arousal and valence, but contained the same amount of implied motion. Since MEPs were greater when seeing emotional and neutral movement than for static postures, implied motion but not arousal or valence can explain the pattern of changes in motor excitability.

Discussion

In the first experiment, we used single-pulse TMS to stimulate the left M1 and thus explore motor excitability during perception and active categorization of complex scenes from the IAPS database (Lang et al., 1999). We found greater MEP amplitudes from two hand muscles (FDI and APB) when seeing emotionally positive and negative scenes relative to emotionally neutral scenes. These findings confirm the results obtained in a previous study (Hajcak et al., 2007) during passive observation of the same IAPS stimuli and suggest that perception of emotional scenes is associated to an increase of hand motor excitability, in keeping with the view that emotions may prime the body for action (Lang, 1993; Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009). In experiment 1 (and in the study using the same stimuli Hajcak et al., 2007), positive and negative stimuli elicited comparable motor facilitation, suggesting that arousal but not valence may in principle account for by the pattern of changes in motor excitability (see also Baumgartner et al., 2007; Coombes et al., 2009). However, pleasant and unpleasant IAPS pictures mostly showed humans involved in dynamic situations - that is, engaged in some motor activity or operating an object such as a gun or a knife – while most of the neutral scenes depicted still objects and landscapes. Notably, our data also indicate that participants perceived more implied motion in the emotional than in the neutral scenes. Thus, it is unclear whether the pattern of motor excitability found in experiment 1 (and likely in previous TMS studies using the

same IAPS stimuli) may be due to the arousing content of the emotional scenes or the human actions implied in such scenes.

In the second experiment, we directly addressed the issue of action-related changes in motor excitability during perception of a novel set of emotional body stimuli. Similar to experiment 1, MEPs to left M1 stimulation were recorded from the APB and FDI muscles during presentation of pictures that had to be actively categorized. To eliminate any possible confounding present in experiment 1, only scenes depicting humans were presented. Moreover, to directly test the influence of action-related information on motor excitability, snapshots of humans in dynamic situations (i.e. during emotionally positive, negative or neutral movements involving the two target hand muscles) and static postures were shown, and faces and contextual information were blanked out. This way, we specifically tested how perception of emotional body language would affect motor excitability.

A major point of novelty of our study is the demonstration that seeing positive (happy) and emotionally negative (fearful) body movements increased motor excitability relative to observation of static neutral postures. Critically, motor facilitation was also detected during observation of neutral body movements, and did not significantly differ from that found with emotional stimuli. Notably, pictures of neutral actions received lower emotional but comparable implied motion ratings relative to pictures of emotional body movement. Thus, after controlling for the amount of the perceived motion in static snapshots of body expressions, we found no evidence of emotional specific motor facilitation.

Previous research has investigated whether activation in the motor system is influenced by the emotional meaning of body movements or postures (de Gelder et al., 2010; Tamietto and de Gelder, 2010). One of the first imaging studies on the topic found increased M1 activation during the perception of negative (fearful) but not positive (joyful) body postures compared to neutral ones (de Gelder et al., 2004); however another study found the opposite effect, with M1 activations during perception of positive but not negative postures (van de Riet et al., 2009). Moreover, a number of

additional imaging studies have found very little or no evidence of M1 activation when contrasting emotional expressions versus neutral actions with comparable real or implied motion (Grèzes et al., 2007; van der Gaag et al., 2007; de Gelder et al., 2010; Tamietto and de Gelder, 2010; Kret et al., 2011b). To date no direct neurophysiological assessment of motor system excitability during processing of emotional and neutral actions was provided. Here, we found motor facilitation to depend more on the perceived motion implied in the observed scene/action than on the evoked arousal or emotional valence. Jointly, these results seem more compatible with activity in the motor system reflecting a "cold" motor simulation of the observed actions than a specific response to emotional cues (Fadiga et al., 2005; Avenanti et al., 2007; Oberman et al., 2007; Avenanti et al., 2013a; Catmur et al., 2007; Oberman and Ramachandran, 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Urgesi et al., 2010; Candidi et al., 2010; Niedenthal et al., 2010; Avenanti and Urgesi, 2011).

The idea that motor system activation may reflect the internal simulation of others' motor behavior comes from TMS studies showing that seeing or imaging others' actions modulate the excitability of the motor representations of those muscles involved in the observed/imagined body movements (Strafella and Paus, 2000; Fadiga et al., 2005; Fecteau et al., 2005, 2010; Fourkas et al., 2006; Avenanti et al., 2007, 2013a; Catmur et al., 2007; Schütz-Bosbach et al., 2009; Cattaneo et al., 2009; Urgesi et al., 2010; Candidi et al., 2010; Avenanti and Urgesi, 2011; Cavallo et al., 2012). These motor modulations are temporarily coupled with the observed action phase (Borrioni et al., 2005; Urgesi et al., 2010) and are mediated by neural activity in the premotor cortex (Avenanti et al., 2007, 2013a; Catmur et al., 2011) which may suggest that motor modulations reflect action simulation activity of the anterior node of the human pMNS (Rizzolatti and Craighero, 2004; Gazzola et al., 2007; Keysers and Gazzola, 2009, 2010).

Evidence for similar embodied simulation mechanisms comes from studies exploring motor excitability during observation of somatosensory stimulations in others: watching painful (Minio-Paluello et al., 2006; Fecteau et al., 2008; Avenanti et al., 2009b) or touching (Wood et al., 2010)

stimuli shown on the body of other people induces modulations of motor excitability that are specific to the muscles involved in the observed stimulation. These motor modulations are inhibitory as those observed during first-hand experience of touch or pain and correlate with sensorimotor (e.g. intensity, localization) but not emotional (e.g. unpleasantness) features of the somatic experience (Avenanti et al., 2006, 2009a). Thus, seeing others' actions or somatic feelings activates fine-grained sensorimotor representation of the observed state in the observer (Preston and de Waal, 2002; Rizzolatti and Craighero, 2004; Fadiga et al., 2005; Avenanti and Aglioti, 2006; Oberman and Ramachandran, 2007; Oberman et al., 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Keysers and Gazzola, 2009; Keysers et al., 2010; Niedenthal et al., 2010; Gallese and Sinigaglia, 2011), and this notion is supported by imaging and neurophysiological evidence that motor and somatosensory networks involved in action execution, touch and pain perception are also activated during observation of similar actions (Caetano et al., 2007; Raos et al., 2007; Etzel et al., 2008; Kilner et al., 2009; Arnstein et al., 2011; Turella et al., 2012) and similar somatic feelings in others (Keysers et al., 2004; Bufalari et al., 2007; Lamm et al., 2007b, 2011; Valeriani et al., 2008; Bolognini et al., 2011; Morrison et al., 2011; Aziz-Zadeh et al., 2012). Our study supports this view and suggests that the motor system encodes motor features of the observed movement, independently of its emotional content.

It should be noted that in both experiment 1 and 2, MEPs were recorded during active categorization of emotional pictures. We used an emotional categorization task because previous imaging (Gur et al., 2002; Hariri et al., 2003; Winston et al., 2003; Habel et al., 2007), TMS (Oliveri et al., 2003) and event-related potentials (Mikhailova and Bogomolova, 2000) (ERPs) studies have shown that active categorization maximizes the chance of detecting emotion specific modulations in different brain regions, including the motor system. Nevertheless, our study fails to reveal any emotion specific modulation of motor excitability.

It is unlikely that the pattern of results may be accounted for by the semantic meaning of the verbal response, as the very same response (“neutral”) was associated to reduced excitability in experiment 1 (neutral IAPS stimuli) and motor facilitation in experiment 2 (neutral actions with implied motion).

Our findings suggest that motor response to emotional stimuli found in previous TMS research (Oliveri et al., 2003; Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009; van Loon et al., 2010) may reflect, at least in part, action simulation activity triggered by the processing of the action cues depicted in the observed emotional scene. However, the lack of emotion specific motor facilitation in experiment 2 requires two final considerations. First, similarly to previous TMS research we focused on the excitability of the hand motor representations in the left hemisphere. Thus, we do not exclude that a different reactivity (i.e. emotion specific modulation) may be found in the right hemisphere which is specialized for emotional processing (Borod et al., 1998) or outside the hand region. Future studies will assess whether whole-body emotional actions may induce emotion specific facilitation in other sector of the motor system. Second, it should be noted that in the present study we specifically explored motor cortex reactivity at 300 ms after stimulus onset. Thus we do not exclude that emotion specific signals may specifically modulate motor excitability at different time points. Moreover, since observed (neutral) actions are known to modulate the motor system at 200-400ms (Nishitani and Hari, 2002; Nishitani et al., 2004; Proverbio et al., 2009), one may even wonder whether the present paradigm was optimized to assess the activity of the pMNS rather than the tendency of the motor system to react to emotional cues. However, ERPs studies suggest that emotionally arousing stimuli induce activity in the same temporal window at various electrodes, including fronto-central electrodes (Olofsson et al., 2008). Moreover, a previous TMS study found increased motor excitability for facial emotional expressions (i.e. expressions with implied motion) relative to neutral (static) expressions at 300 ms after stimulus onset (Schutter et al., 2008). Such evidence indicates that the explored temporal window is potentially adept to reveal motor reactivity to emotional body language.

In conclusion, our study suggests that seeing others' emotional expressions and neutral actions in static pictures induces comparable action simulation activity in the observer's motor system. In keeping with embodied simulation accounts (Fadiga et al., 2005; Avenanti et al., 2007; Oberman and Ramachandran, 2007; Oberman et al., 2007; Avenanti et al., 2013a; Catmur et al., 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Urgesi et al., 2010; Candidi et al., 2010; Niedenthal et al., 2010; Avenanti and Urgesi, 2011; Gallese and Sinigaglia, 2011), we found that motor resonance occurred independently of the emotional meaning of the observed behavior. Our data also suggest that caution should be paid when selecting emotional stimuli (e.g. in the IAPS or in other databases) and the motion implied in such stimuli should be controlled when investigating neural activity within the motor system.

Supplementary Material Online

Motor mapping of implied actions during perception of emotional body language

Supplemental methods:

List of IAPS images used in experiment 1

Positive images: 1601, 2000, 2070, 2080, 2091, 2092, 2165, 2311, 2340, 4002, 4220, 4290, 4572, 4608, 4658, 4659, 4660, 4664, 4800, 4810, 5470, 5621, 5626, 5628, 7325, 8032, 8080, 8200, 8280, 8320, 8330, 8370, 8400, 8465, 8490, 8540. Neutral images: 2480, 2570, 2840, 2880, 5390, 5500, 5510, 5532, 5534, 5731, 5740, 5800, 5900, 7000, 7002, 7004, 7006, 7009, 7010, 7025, 7030, 7034, 7035, 7040, 7060, 7090, 7100, 7140, 7150, 7175, 7190, 7205, 7217, 7233, 7235, 7491. Negative images: 2800, 2900, 3051, 3102, 3110, 3261, 3530, 3550, 6230, 6242, 6250, 6260, 6313, 6370, 6540, 6570, 6571, 6821, 9040, 9050, 9253, 9300, 9400, 9405, 9410, 9433, 9490, 9520, 9530, 9570, 9800, 9810, 9910, 9911, 9920, 9921.

Stimuli validation for experiment 2: Pilot studies 1-3

Since the aim of experiment 2 was to test whether perception and categorization of positive and negative emotional body stimuli modulate motor excitability, we decided to test MEPs modulation during perception of happy and fearful body expressions. Fear and joy are among the most studied emotions and few previous imaging studies have suggested that seeing fearful [S1] or happy [S2] bodies may increase activity in M1.

Thus, in a preliminary phase of the study three raters selected a sample of 214 images from an initial pool of >1000 stimuli depicting emotional and neutral body expressions. Only stimuli that were categorized by at least 2 raters as realistic representations of joy, fear or neutral expressions were included in the set. In all the emotional expression and neutral action stimuli, the selected pictures represented a whole-body movement with a clear involvement of upper-limbs (implied motion stimuli). In none of the stimuli the model interacted with objects or other individuals. To then validate the set of body expressions and to select the most representative stimuli, we performed two additional pilot studies. Lastly, a third pilot study was performed on the final set of stimuli to assess the amount of the perceived implied motion.

Pilot study 1: emotional intensity ratings

A first pilot study was conducted to assure that joy and fear were recognized more than other basic emotions such as sadness, disgust, angry and surprise. To this aim, 15 participants (8 men; mean age \pm S.D.: 25.3y \pm 2.7) were presented with the selected set of 214 emotional and neutral dynamic body expressions and were asked to rate the intensity of each body expression on the following six labels: happy, sad, disgusted, angry, afraid, surprised. Participants used an electronic 9-points Likert scale ranging from 1 (no emotion) to 9 (maximal intensity of the emotion). To avoid building up artificial correlations between the different

judgments, each rating was collected separately during successive presentation of the whole set of stimuli. The order of the different judgments was randomized across subjects. Based on mean intensity ratings we selected a pool of 167 stimuli which included 53 joyful, 56 fearful and 58 neutral expressions. For joyful images inclusion criteria were: i) high ratings in the “happy” scale (mean intensity > 6); ii) low ratings (< 4) in the other emotion scales (afraid, sad, disgusted, angry, surprised); iii) a spread >3 in the “happy” relative to the other emotion scales. For fearful images we used a similar procedure. This procedure ensured that the selected joy or fear stimuli conveyed appropriate and unambiguous basic emotional information. For neutral expressions the inclusion criterion was a low rating (< 3) in all the emotional scales.

Pilot study 2: emotion recognition task

Since the aim of the TMS experiments was to investigate neural correlates of emotional categorization, we conducted a further pilot study to select stimuli that could be well interpreted as positive, negative and neutral body expressions. To this aim, 27 participants (9 men, age: $26.5 \text{ y} \pm 3.5$) were presented with the 167 stimuli selected in pilot study 1 and were asked to recognize, for each image, the depicted body expression as static, neutral, fearful or joyful. For each category we selected 13 stimuli with accuracy >75%.

Pilot study 3: implied motion ratings

In the TMS experiment 2 we planned to explore the excitability of the hand motor representation during the observation of stimuli selected in pilot study 1 and 2. Thus, we conducted a third pilot study to ensure that more implied motion was perceived in actors’ hands in emotional (joy, fear) and neutral actions stimuli relative to static stimuli. Details are reported in the main text.

Control for baseline manipulation: behavioral control experiment 1 and 2

During baseline subjects held their eyes closed with the instruction to imagine watching a sunset at the beach [S3] while receiving TMS over M1. It was stressed to imagine a static scene, with no humans, animals or moving entities, and not to imagine self body movements (as this may increase motor excitability [S7,S8]). After each baseline block, subjects confirmed to have followed the imagery instructions. This procedure was used to minimize “task unrelated thoughts” [S6,S7] that may greatly vary across subjects and may also include motor imagery or arousing thoughts.

While the content of the imagery task was not arousing (and typically, imaging landscapes is used as emotionally neutral conditions in mood-induction studies [S8,S9]), nonetheless imaging a sunset may be a mild positive experience for some participants. Thus we checked whether the content of the imagery task may have induced some bias in the categorization of positive IAPS or body stimuli. To address the issue, we performed two additional behavioral experiments in which two groups of participants categorized IAPS (8 subjects, 4 men, mean age \pm S.D.: $27.5 \text{ y} \pm 2.9$) or emotional body stimuli (8 subjects, 4 men, age: $25.5 \text{ y} \pm 1.4$) with no preceding imagery task. As shown in Supplementary Table 1, very similar accuracy for positive/joy stimuli (hit) and total number of positive/joy answers (hit + false alarms) were obtained in the

TMS and control experiments (all comparisons using non-parametric Mann-Whitney U tests, $p > 0.25$). These findings speak against the presence of a categorization bias induced by the baseline procedure in experiments 1 and 2.

Supplementary Table 1

	TMS experiment 1			Control behavioral experiment 1		
	Neutral	Positive	Negative	Neutral	Positive	Negative
Accuracy	92% ± 11	85% ± 15	96% ± 5	94% ± 12	82% ± 12	95% ± 6
N of responses	39.4 ± 7.5	32.6 ± 6.8	36.1 ± 1.3	42.4 ± 6.6	31.0 ± 6.6	34.6 ± 2.1

	TMS experiment 2				Control behavioral experiment 2			
	Static	Neutral	Joy	Fear	Static	Neutral	Joy	Fear
Accuracy	97% ± 4	92% ± 7	87% ± 12	92% ± 9	100% ± 1	98% ± 3	91% ± 9	96% ± 5
N of responses	25.7 ± 1.0	28.7 ± 5.7	24.5 ± 4.2	25.1 ± 2.7	25.9 ± 0.4	28.8 ± 3.4	24.4 ± 2.4	25.0 ± 1.4

Supplementary Table 1. Mean ± S.D. of accuracy (hit) and total number of answers (hit and false alarms) in each category of stimuli used in experiment 1 (top, left) and experiment 2 (bottom, left). The right side of the table shows data from the two control behavioral experiments.

Supplemental References

- [S1] de Gelder B, Snyder J, Greve D, Gerard G, Hadjikhani N. Fear fosters flight: a mechanism for fear contagion when perceiving emotion expressed by a whole body. *Proc Natl Acad Sci USA* 2004;101:16701-6.
- [S2] van de Riet WA, Grezes J, de Gelder B. Specific and common brain regions involved in the perception of faces and bodies and the representation of their emotional expressions. *Soc Neurosci* 2009;4:101-20.
- [S3] Fourkas AD, Bonavolontà V, Avenanti A, Aglioti SM. Kinesthetic imagery and tool-specific modulation of corticospinal representations in expert tennis players. *Cereb Cortex* 2008;18:2382-90.
- [S4] Fadiga L, Buccino G, Craighero L, Fogassi L, Gallese V, Pavesi G. Corticospinal excitability is specifically modulated by motor imagery: a magnetic stimulation study. *Neuropsychologia* 1999;37:147-58.

- [S5] Mazoyer B, Zago L, Mellet E, Bricogne S, Etard O, Houde´ O, et al. Cortical networks for working memory and executive functions sustain the conscious resting state in man. *Brain Res Bull* 2001;54:287-298.
- [S6] Binder JR, Frost JA, Hammeke TA, Bellgowan PSF, Rao SM, Cox RW. Conceptual processing during the conscious resting state: a functional MRI study. *J Cogn Neurosci* 1999;11:80-93.
- [S7] Munzert J, Lorey B, Zentgraf K. Cognitive motor processes: the role of motor imagery in the study of motor representations. *Brain Res Rev* 2009;60:306-26.
- [S8] Westermann R, Spies K, Stahl G, Hesse FW. Relative effectiveness and validity of mood induction procedures: a meta-analysis. *Eur J Soc Psychol* 1996;26:526-80.
- [S9] Gilet AL. Mood induction procedures: a critical review. *Encephale* 2008;34:233-9.