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Your emotion moves into my motor system

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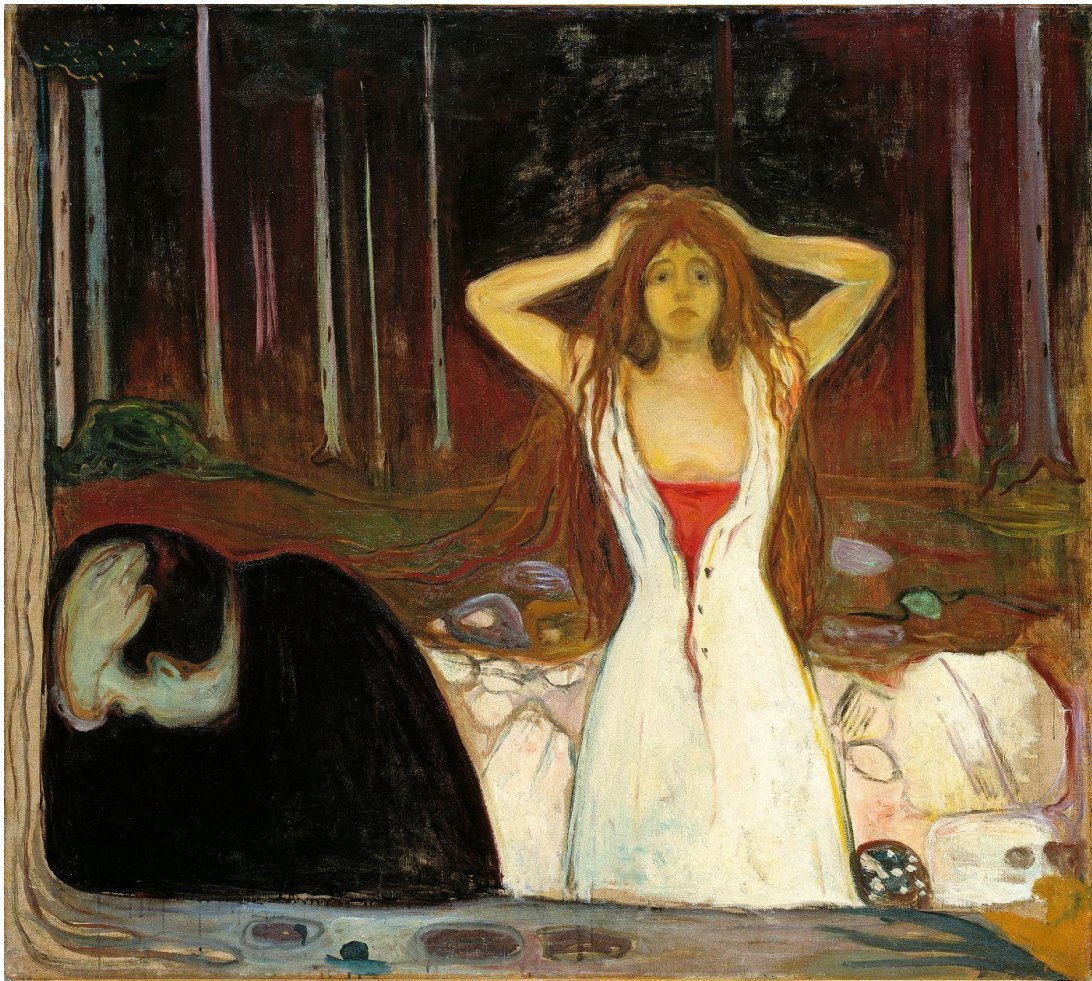
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*Your emotion
moves
into my motor system*



Sara Borgomaneri

Your emotion moves into my motor system

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. D.C. van den Boom

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op woensdag 27 mei 2015, te 10.00 uur

door Sara Borgomaneri

geboren te Gallarate, Italië

Promotiecommissie:

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Faculteit	Maatschappij- en Gedragwetenschappen	

Il faut toujours être ivre. Tout est là : c'est l'unique question. Pour ne pas sentir l'horrible fardeau du Temps qui brise vos épaules et vous penche vers la terre, il faut vous enivrer sans trêve.

Mais de quoi ? De vin, de poésie ou de vertu, à votre guise. Mais enivrez-vous.

Et si quelquefois, sur les marches d'un palais, sur l'herbe verte d'un fossé, dans la solitude morne de votre chambre, vous vous réveillez, l'ivresse déjà diminuée ou disparue, demandez au vent, à la vague, à l'étoile, à l'oiseau, à l'horloge, à tout ce qui fuit, à tout ce qui gémit, à tout ce qui roule, à tout ce qui chante, à tout ce qui parle, demandez quelle heure il est ; et le vent, la vague, l'étoile, l'oiseau, l'horloge, vous répondront : Il est l'heure de s'enivrer!

Pour n'être pas les esclaves martyrisés du Temps, enivrez-vous ; enivrez-vous sans cesse ! De vin, de poésie ou de vertu, à votre guise.

C. Baudelaire

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General Introduction

Human's lives are intertwined with those of other people and this makes them intensely social creatures. In this social environment, successful interactions require the ability to precisely understand emotions displayed by other individuals, which is critical to react in an adaptive way. Due to the extreme importance of emotion comprehension, over the past centuries, affective neurosciences have focused on the understanding of how our brain perceives and reacts to emotional stimuli. In our natural social environment, humans often have to encode emotional signals from facial expression, body postures, not only presented in isolation but also when these are included within more complex scenes. In the 1990s, functional magnetic imaging (fMRI) studies started to investigate the neural basis underlying emotional faces observation (Adolphs et al., 1995; Breiter et al., 1996; Kanwisher et al., 1997). The choice to start from faces is probably driven by the particularly present emphasis on the face in western culture and art, just think of the huge number of artistic portraits depicting only faces. Artists have focused on the face well before neuropsychologists had observed relative selective effects (as for example, prosopagnosia, the inability to selectively recognize faces) of bilateral or right-sided lesions of the occipital or temporal lobes (Sergent and Poncet, 1990) and neurophysiologists recording from single cells have provided evidence for specialized face processes (Perrett et al., 1988).



Fig 1. From left to right. Van Gogh: self-portrait (1889); Matisse: Study for the Virgin “head veiled” (1950); Manet: Berthe Morisot (1872); Matisse: woman with hat (1905).

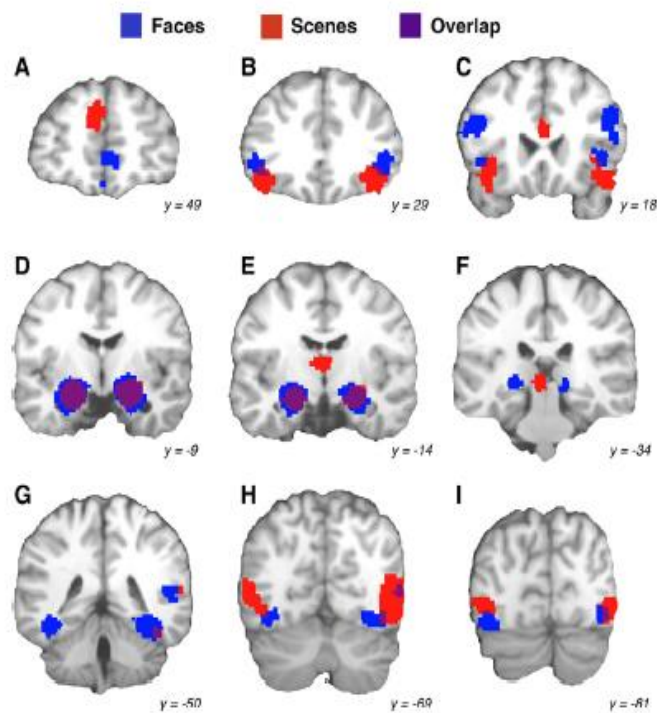
Traditionally the face is seen as our privileged access route to the thoughts and feelings of the people around us, presumably providing this information rapidly and automatically (de Gelder, 2009). To date, functional magnetic imaging (fMRI) studies on emotional faces observation have shown that emotionality increases activation in much of the basic circuit of face perception, which includes areas processing visual aspects (including fusiform gyrus, inferior and middle occipital gyri, lingual gyrus, middle/superior temporal gyrus) and areas involved in representing the emotional state of the observer (including the insula, amygdala, parahippocampal gyrus, posterior cingulate), parietal areas (inferior parietal lobule), prefrontal areas (medial frontal gyrus), subcortical areas (putamen) and the cerebellum (for reviews see Fusar-Poli et al., 2009; Sabatinelli et al., 2011). Although the exact functional interplay between these areas is not clear, the fact that faces activate many brain regions has led researchers to suggest that these stimuli are processed in a distributed network of connected areas (Haxby et al., 2000; Ishai, 2008; O'Neil et al., 2014).

Perceptual processing of faces is thought to take place in occipitotemporal lobes that might be involved in constructing detailed representations from the configuration of facial features while subcortical structures such as amygdala can be recruited, in fast and automatic emotional signal discrimination, in order to send feedback projections onto extrastriate cortex, which might enhance the explicit recognition processing of stimuli with emotional value (Vuilleumier, 2002; Furl et al., 2013).

Together with faces, our brain is also influenced by the perception of rather more complex emotional stimuli, such as complex scenes, which were also employed in electrophysiological and imaging studies in order to study brain activations when facing emotional stimuli in a more complex and naturalistic environment.

Commonly reported areas of the brain activated by emotional natural scene photographs, relative to not-emotional scenes, include the extrastriate occipital and inferotemporal cortex, superior parietal visual areas, the amygdala, insula, anterior cingulate, superior frontal gyrus, and medial prefrontal cortex (Hariri et al., 2002; Bradley et al., 2003; Northoff et al., 2004; Sabatinelli et al., 2005, 2007,

2009; Britton et al., 2006). In a recent ALE meta-analysis on emotional faces and scenes observation (Sabatinelli et al., 2011), authors found overlapping activations in the amygdala, the multimodal subcortical structure most consistently associated with emotional processing in the fMRI literature (van de Riet et al., 2009; Atkinson and Adolphs, 2011), which some (Adolphs, 2002; Vuilleumier, 2002; Adolphs and Spezio, 2006; Vuilleumier and Pourtois, 2007) interpret to have a role in directing the attention to salient stimuli and therefore invite the temporal lobe to do a deeper analysis of the situation. Both emotional stimuli types also prompted reliable BOLD signal in regions of the medial prefrontal, inferior frontal, inferior temporal and extrastriate occipital cortices (these results are reported also in Britton et al., 2006).



Sabatinelli et al., 2011; NeuroImage

Fig 2. Clusters resulting from an ALE analysis of 100 emotional face processing studies are shown in blue, clusters resulting from an ALE analysis of 57 emotional scene processing studies are shown in red, and the overlap of the two analyses is shown in purple, overlaid on a standardized structural volume. The neurological convention is used (right lateralized clusters are located on the right side of the slice).

Interestingly, emotional effects on fusiform activation have most commonly been reported for fearful faces (Breiter et al., 1996; Morris et al., 1996, 1998; Vuilleumier et al., 2001, 2003b, 2004; Pessoa et al., 2002), and much less frequently for happy faces (e.g., Breiter et al., 1996). In a recent fMRI study

directly comparing the effects of four different emotions (fear, disgust, happiness, and sadness) with two different intensities (mild and intense), fusiform cortex activity was enhanced by increasing intensities of all four types of facial emotions, but distinctively more so with fearful expressions than with any other expressions (Surguladze et al., 2003). Moreover, a boosting of activity in occipital and temporal cortices has similarly been observed for complex visual scenes with aversive contents relative to neutral scenes (e.g., Wik et al., 1993; Lane et al., 1998; Lang et al., 1998; Teasdale et al., 1999; Taylor et al., 2000), but much more rarely for pleasant pictures (Lane et al., 1998). These results suggest that such differential responses in visual areas might generally be more sensitive to negative or threatening than positive emotional signals (Surguladze et al., 2003; Vuilleumier et al., 2003a).

In addition to the spatial information offered by fMRI studies, electroencephalographic recordings (EEG) add important information about the time course in which emotions perception is able to influence electrophysiological brain responses (ERPs). Again, most of the EEG studies have focused on emotional faces or emotional scenes perception. Overall, emotional faces were found to increased EEG voltage fluctuation relative to neutral faces (for review see Eimer and Holmes, 2007; Vuilleumier and Pourtois, 2007) after 180 ms from the stimulus onset. However, in line with imaging findings, there are converging EEG results (Eimer and Holmes, 2002; Holmes et al., 2003; Pourtois et al., 2004a; Schupp et al., 2004) that support the existence of a negative bias in the way emotional faces are processed in the brain: perceiving fearful faces is able to modulate extremely early occipital components. For example, in a recent EEG study in which subjects were requested to perform a rapid serial visual presentation task, authors found that already at 100 ms (anterior N100 and the posterior P1 amplitudes) fearful faces elicited bigger waves than those elicited by happy and neutral faces (Luo et al., 2010). Remarkably, this negative bias take place even before the typical latency of occipito-temporal components of event-related potentials (ERPs), which are supposed to reflect the visual processing underlying the structural encoding of faces (i.e., the N170 component; Bentin et al., 1996), suggesting a rapid bias in visual attention allocation with greater resources devoted to negative stimuli (Vuilleumier, 2002; Carretié et al., 2009). Similar results were also found during observation of

complex scenes (Smith et al., 2003; Huang and Luo, 2006; Groen et al., 2013). In conclusion, from all these evidences (for a review see Olofsson et al., 2008), it seems that emotions are, overall, able to influence different structures in the brain and these modulations seem to affect very early occipitotemporal and frontal brain components. Moreover, there are convincing evidences on the existence of a negative bias in the way our brain process negative stimuli. After the aforementioned negative bias that affects occipitotemporal component at around 100-200 ms, electroencephalographic results suggest the existence of a subsequent step in the analysis of emotional signals: at around 300 ms from the stimulus onset, arousing stimuli observation (positive and negative) increased activity in more frontal regions of the brain, suggesting an increased resource allocation to motivationally relevant cues as suggested by ERPs studies (Cuthbert et al., 2000; Keil et al., 2002; Codispoti et al., 2007; see also Olofsson et al., 2008).

Another important aspect that has been addressed in the past years is whether emotion observation is able to influence our behavior (i.e., motor reaction) and to increase visual attention. In daily life, our brain encounters too many items in the visual environment to be able to fully analyze them at one time, so it is necessary to fast select the most important ones, in order to rapidly prepare a congruent reaction. One possible way to discriminate them is to evaluate the emotional significance (Compton, 2003) of the surrounding stimuli. Indeed, emotionally positive and negative faces are detected faster than neutral faces in visual search task (Calvo and Nummenmaa, 2008). However, although some evidences reported an happy face advantage (Byrne and Eysenck, 1995; Juth et al., 2005; Williams et al., 2005; Öhman et al., 2010; Becker et al., 2011), in line with evolutionary theory that fast perception and reactions to negative cues may best secure survival of the organism (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009), most of the visual search studies suggest faster detection of negative face expressions (Hansen and Hansen, 1988; Fox et al., 2000; Tipples et al., 2002). Fast perception of dangerous cues may provide faster reaction in order to protect the organism toward potential threat. Studies indicated that positive and negative stimuli could differently modulate motor behavior by favoring approach or avoidance movements, respectively (Cacioppo et

al., 1993; Chen and Bargh, 1999; Naugle et al., 2010). However, behavioral evidences reported faster movement times (Coombes et al., 2005, 2009) towards negative stimuli. De Oliveira and coworkers (2012) additionally found that negative stimuli elicited higher readiness potential (an electrophysiological marker of motor preparation) when they precede grasping relative to either positive or neutral stimuli presentation. All these evidences suggest that activation of defensive circuitry globally primes the human organism to move with greater haste.

In spite of the long-held view of the existence of a link between emotion perception and motor system (Lang, 1993; Ekman and Davidson, 1994; Frijda, 2009), only few studies used Transcranial Magnetic Stimulation (TMS) in order to directly asses the link between emotion observation corticospinal system modulations and its precise time-course (Coombes et al., 2005; Baumgartner et al., 2007; Hajcak et al., 2007; Schutter et al., 2008; Coelho et al., 2010; van Loon et al., 2010).

Transcranial Magnetic Stimulation (TMS) is a noninvasive technique that allows researchers to stimulate discrete brain areas. This technique involves delivering a brief magnetic pulse to the scalp through a coil; the stimulating effect depends on the geometry of the stimulating coil with respect to the head and of the waveform of the current pulse flowing through the coil. With the commonly used stimulation intensities and coils producing relatively focal types of stimuli, the cortex is activated within an area of a few square centimeters. After a single pulse stimulation (spTMS) of the primary motor cortex, a motor twitch named *motor-evoked potential* (MEP) can be recorded from the connected muscles: its amplitude (in millivolt) represent the main measure of the corticospinal excitability. Therefore, Transcranial Magnetic Stimulation is the ideal tool to directly address, with a high temporal resolution, possible fast corticospinal system modulations during emotional stimuli perception. Moreover, since TMS pulses disrupt neural activity in the targeted regions, it is useful to assess the causal role of the targeted brain area in a given process (such as emotion perception).

However, most of the existing studies employed single pulse TMS on emotion perception testing possible corticospinal modulations using emotional scenes (Coombes et al., 2005; Baumgartner et al., 2007; Hajcak et al., 2007; Coelho et al., 2010; van Loon et al., 2010) or emotional faces (Schutter et

al., 2008), without focusing on the causal role of the motor system in perceiving such stimuli. These studies have typically reported that the left motor cortex excitability was sensitive to emotional processing. In particular, they found larger motor evoked potentials (MEPs) when participants were asked to observe both pleasant and unpleasant stimuli (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009). In line with scholars that embrace an evolutionary perspective on emotion processing (Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009), which posit that the motor system of an observer is recruited in order to prepare adequate motor reactions, authors interpreted the aforementioned facilitatory modulations of the motor system as the sign of the preparation of a motor response towards relevant arousing stimuli.

Notably, however, in such studies, motor excitability was tested in a relatively late time window (i.e., at > 300ms after stimulus onset) when the amplitude of brain responses to emotional images is typically similar for positive and negative stimuli and likely reflects increased resource allocation to motivationally relevant cues as suggested by ERPs studies (Cuthbert et al., 2000; Keil et al., 2002; Codispoti et al., 2007; see also Olofsson et al., 2008). Differently to what already reported in behavioral studies (Chen and Bargh, 1999; Coombes et al., 2005, 2009) (i.e., faster reaction times to negative stimuli) and in electrophysiological findings (i.e., bigger components to negative stimuli; Eimer and Holmes, 2002; Holmes et al., 2003; Pourtois et al., 2004a; Schupp et al., 2004), these TMS studies fail to report a similar “negative bias” (i.e., higher motor cortex excitability for negative stimuli). One possible confound in these TMS experiments is that the motor system involvement was tested too late in time (i.e., >300 ms), while electrophysiological evidences suggested earlier brain modulation for emotional stimuli (i.e., greater visual cortex activations around 100-200 ms).

In Chapter 1, using Transcranial Magnetic Stimulation (TMS), we first aimed to replicate previous findings, namely the increased motor excitability during emotional complex scenes observation. We also aimed to investigate the existence of possible fast “negative bias” during emotional scenes perception in the motor system. We collected motor evoked potentials (MEPs) from the left motor

cortex (as most of the previous TMS study did) in a relatively late temporal window (300 ms after the stimulus onset). This time point was chosen based on electrophysiological results that found late emotion-related activations (300-400 ms) (Carretiè and Iglesias, 1995; Cuthbert et al., 2000; Krolak-Salmon et al., 2001; Campanella et al., 2002; Keil et al., 2002; Cano et al., 2009). Additionally, we tested possible negative-related modulations earlier in time, in a temporal window that is compatible with the “negative bias” reported in ERPs findings (150 ms, for a review see Olofsson et al., 2008). At 300 ms, we replicated previous findings supporting the idea that perceiving emotional cues in the environment has a facilitatory effect in the corticospinal system. Importantly, at 150 ms from the stimulus onset, we found that the early negative bias facing negative stimuli can be detected also in the motor system: negative images are able to clearly increase the amplitude of motor evoked potentials.

It is important to notice that the stimuli we used (the same employed in the other TMS studies) are complex scenes taken from the IAPS database (Lang et al., 1999). Positive complex scenes include erotic, family and sport related events; negative scenes depicted mutilations, threatening and disgusting scenarios; neutral stimuli are static landscapes, household objects and neutral faces. Most of the positive and negative stimuli depicted humans involved in dynamic complex situations while most of the neutral stimuli depicted static objects or landscapes with no humans. Previous TMS studies on action observation found that observing human actions compared to the relative static counterparts, 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) (Candidi et al., 2010; Urgesi et al., 2010; Avenanti et al., 2012a), indicating that the motor system can extract dynamic action information from static images suggesting an ongoing body movement. Embodied simulation theories suggest that when we observe another individual acting we strongly ‘resonate’ with his or her action (Gallese

et al., 2004; Goldman and Sripada, 2005; Gallese, 2007; Keysers and Gazzola, 2009). In other words, our motor system simulates “underthreshold” the observed action in a strictly congruent fashion. The involved muscles are the same as those used in the observed action and their activation is temporally strictly coupled with the dynamics of the observed action. In partial disagreement with scholars that embrace an evolutionary perspective on emotion processing (Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009), embodied theorists attribute to the motor system not only a role in basic motoric functions, like planning a motor reaction but they posit that it can have a role in more complex cognitive processing, like the comprehension of the observed motor acts, by internally simulate them.

Considering what embodied simulation theories propose, namely, the involvement of motor system when facing dynamic stimuli in order to better understand them, it is possible to speculate that previous TMS findings in emotional domain (more motor activation in facing emotional stimuli) reflect motor resonance processes. The motor system could be involved in encoding the motor features of the implied body movement depicted in the scenes, independently of their emotional meaning. Indeed, when facing with emotional stimuli, embodied simulation theorists posit that our motor system is involved in simulating and understanding the unobservable emotional states of others by embodying their observable motor behavior (Carr et al., 2003; Leslie et al., 2004; Gallese, 2007; Oberman et al., 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Niedenthal et al., 2010; Gallese and Sinigaglia, 2011).

In the previous experiment (Chapter 1) we found that, at 300 ms from the stimulus onset, the motor system of an observer is modulated by the observation of complex emotional scenes depicting humans involved in dynamic action relative to neutral control stimuli, like static objects or landscapes containing no implied motion and with no human presence. From these results it is not possible to assess whether this effect (greater MEPs amplitude for both positive and negative stimuli) was due to the emotional meaning conveyed by the stimuli, which therefore could activate the motor system in order to prepare a motor reaction, as suggested by evolutionary theorists or, as suggested by the

embodied simulation theories, the motor activation we found was simply related to the different quantity of implied motion conveyed by the emotional pictures relative to the neutral static ones. Subjects were asked to judge the quantity of implied movement conveyed by the different scenes and we observed that emotional (positive and negative) scenes contain significantly bigger quantity of implied motion relative to the static neutral ones. Emotional scenes depicted human beings in dynamic situation (erotic scenes, sportive events, people being assaulted, injured), while neutral scenes depicted static objects or landscapes.

Therefore, it was possible to speculate that, at 300 ms, our motor system was recruited in order to internally simulate the observed dynamic emotional scenes in order to understand them (Oberman et al., 2007). In Chapter 2, we aimed to deeper investigate this point. To do so, we decided to create a new set of stimuli in which the emotional body postures appeared in isolation (on a white background).

Over the last years, social neuroscience have focused on faces and relatively neglected bodies, in comparison. As suggested in the introduction, faces observation is seen as a more automatic and direct way to investigate others' feelings and intentions. However, faces and bodies are not separate but closely linked entities: the emotions conveyed by faces are closely related to the one expressed through body expressions, so that the face emotion recognition is impaired if the two sides express emotions that are incongruent rather than congruent (Meeren et al., 2005; Aviezer et al., 2008; Shields et al., 2011; Willis et al., 2011; Gu et al., 2013; Mondloch et al., 2013). This evidence supports the idea that, in everyday life, we use to collect information from both sides. Observing bodies for instance offers the possibility to capture signals and perceive emotions over longer distance than faces. This also shifts the attention away from the personal identity shown by the face, which may not always matter for rapid decoding of the expressions. Our aim is to investigate whether the motor system of an observer is merely involved in reacting to arousing relevant cues in the environment or whether corticospinal modulations in facing emotional stimuli reflect more cognitive processes, such as the visual recognition, as suggested by embodied theorists. In order to investigate this issue, we

need stimuli that are matched for implied movement either conveying or not an emotional meaning: human's bodies expressing happiness, fearful or mimic an action (dynamic by not emotional) represent the best stimuli to solve the question. Finally, investigation of bodies will extend the scope of face-based research and provide evidence that human emotion theories based on studies of facial expression may generalize to other affective signals.

Some of the few existing functional magnetic imaging studies on emotional body observation showed that observing emotional relative to neutral body expressions is able to modulate activity in "emotional areas" of the brain (amygdala and OFC), that partially overlapping with the ones observed for emotional faces and scenes (de Gelder et al., 2004; van de Riet et al., 2009; Van den Stock et al., 2012). Other studies, additionally found activation in motor (both cortical and subcortical) cortices, such as the inferior frontal gyrus, caudate nucleus and putamen (Hadjikhani and de Gelder, 2003; de Gelder et al., 2004; Grèzes et al., 2007; Peelen et al., 2007; van de Riet et al., 2009). Therefore, from these studies is possible to argue that the sensorimotor network seem to be modulated by the observation of emotional bodies. However, data from these imaging studies on the specific activation of primary motor cortices are conflicting: 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), another found the opposite effect, with M1 activations during perception of positive but not negative postures (van de Riet et al., 2009). Activations in the primary motor cortices are strictly related to modulations in the corticospinal system, which is involved in programming different motor reactions towards emotional stimuli. However, such motor reactions can be too fast to be detected using functional magnetic image, therefore, Transcranial magnetic stimulation appears to be the optimal tool to assess such modulations.

In Chapter 2 we aimed to assess whether emotional body postures, as well as complex scenes or emotional faces observation, was able to increase corticospinal activity of an observer. Moreover,

we aimed to answer to the unsolved question of whether the motor modulation we found for complex scenes was due to the emotional meaning or to the different implied motion conveyed by emotional stimuli. We created a database of human body postures in which actors were expressing both happiness and fear. Importantly, in order to balance the stimuli for the human presence as well as for implied movement, we decide to add a control neutral dynamic condition in which the body was actively mimicking an action but without expressing an emotion. Finally, the same actors were photographed in static postures (control for the implied movement).

We run two different experiments: MEPs were always recorded from the left motor cortex after 300 ms from the stimulus onset. In the first experiment, subjects were exposed to IAPS complex scenes (exactly as in Chapter 1); in a second experiment, subjects were asked to observe the set of body stimuli we created. Body stimuli expressed happiness, fear, dynamic actions or were in a static posture.

We replicated our previous findings (i.e., bigger MEPs amplitudes for emotional stimuli) but, importantly, in the second experiment, we found that such modulation was present also for the observation of neutral dynamic stimuli. These results clearly demonstrated that, as well as complex scenes, emotional bodies alone are able to affect motor cortices excitability but, more importantly, these data suggest that the modulations we observed in Chapter 1, were probably related to the amount of the implied movement depicted in the stimuli. In line with embodied simulation theory, it seem that, at this latency, the left motor cortex is involve in simulating the observed movement, independently of its emotional meaning.

In these two experiments we tested the excitability of the left motor cortex, in line with most of the previous TMS studies (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009). However, existing theories posit different roles of the two hemispheres in emotional processing.

The theory of the right hemisphere's supremacy in coding emotions (The Right Hemisphere Hypothesis) posits that the right half of the brain is specialized for processing all emotions, regardless

of the affective valence (Borod et al., 1998). This idea is supported by studies in patients with lesion to the right hemisphere who showed greater impairment in the perception of emotional faces, regardless of the valence of the expressed emotion, relative to patients with comparable lesions in the left hemisphere (Adolphs et al., 1996, 2000; Borod et al., 1998). Brain asymmetry in the perception of positive and negative facial expressions was found in an experiment in which, in each trial, a target and a distractor expression were presented simultaneously in a computer screen for 150 msec and participants had to determine the side (left or right) on which the target expression was presented. Results indicated that expressions of happiness and fear were identified faster when presented in the left visual field, suggesting an advantage of the right hemisphere in the perception of these expressions (Alves et al., 2009). Ishai and coworkers (2005) found that, in the right hemisphere, the response to famous and emotional faces was stronger than the response to unfamiliar faces. Keil and colleagues (2002) in an EEG study, found that P3 (a positive wave that arise 300 ms from the stimulus onset) and slow waves (elicited around 550-900 ms after the stimulus) displayed more reactivity to emotionally arousing (pleasant or unpleasant) pictures over the right hemisphere as has already been reported earlier, for comparable time ranges (Crites et al., 1995). Moreover, the middle sector of temporal cortex was found to be activated during facial emotion recognition, first, in the right hemisphere at about 200 ms (Streit et al., 1999).

*Due to this amount of evidences, in **Chapter 3** we decided to test to role of both hemispheres during emotional body's observation. Moreover, we directly tested the different predictions made by simulative and non-simulative theories regarding the time course of motor system responses to emotional bodies and the potential role of such motor responses for visual perception of emotional bodies. We tested the hypothesis that the motor system activity induced by viewing emotional body expressions may reflect both motor resonance and non-simulative motor reactivity, but at different time points. Additionally, since TMS pulses disrupt neural activity in the targeted regions, we also tested whether stimulation of M1 at 150 or 300 ms after stimulus onset affected visual perception of*

body expressions. This allowed us to combine correlational and causal approaches to test the role of the motor system in the perception of body expressions.

Motor excitability during emotional body perception was tested by stimulating the right M1 or the left M1 at two different times (as in Chapter 1), namely at 150 ms and 300 ms after stimulus onset. The early time point was chosen to explore possible short-latency motor response to emotional bodies and it was chosen based on the notion that visually presented complex scenes including facial and contextual emotional cues modulate visual event-related potentials (ERPs) in the 100-200 ms range (Vuilleumier and Pourtois, 2007; Olofsson et al., 2008) and motor excitability at 150 ms (Borgomaneri et al., 2013). If emotional body perception is associated to fast motor reactions to body emotional cues, we might expect, at early latency, differential excitability for the observation of emotional and non-emotional movements and stronger reactivity in the right hemisphere that seems to be dominant for emotional processing. The later time point was chosen based on action observation studies showing that neural activity reflecting action simulation is typically detected at about 250-350 ms after stimulus onset in the motor cortices (Nishitani et al., 2004; Catmur et al., 2011; Barchiesi and Cattaneo, 2013; Ubaldi et al., 2013). Thus, at this time point, we expect neural activity reflecting the encoding of the motor features of observed action, independently of their emotional meaning (as found in Chapter 2 for the left M1).

We found signs of motor resonance at 300 ms recording from both hemispheres (bigger MEPs for dynamic relative to static body postures observation) and, in keeping with results found in Chapter 1, at 150, a weak facilitatory effect in the left hemisphere for fearful body postures observation. Additionally, we found that seeing emotional body movements reduced MEP amplitude at 150 ms, only after stimulation of the right M1. This early inhibition of motor excitability was comparable for fearful and happy expressions and larger than for neutral movements. We interpreted this effect as an orienting reaction toward salient stimuli. Importantly we found that such orienting responses were

necessary for visual perception (TMS pulse interfered with perceptual accuracy only when delivered over the right M1 at 150 ms and not at 300 ms, neither over the left M1 or as sham).

In these three chapters, we found that:

Negative stimuli observation increases activity in the left motor cortex in an early time window (150 ms) to prepare the most opportune motor reaction.

Emotional bodies observation triggers a fast (150 ms) orienting reaction in the right motor cortex, which support visual recognition.

Dynamic bodies' observation (happy, fearful and neutral relative to static) subsequently (300 ms) increases activity in both motor cortices, reflecting motor resonance processes.

In these studies, we used single-pulse TMS, which allows routine evaluations of the excitability and conductivity of corticospinal motor pathways by measuring the amplitude of the motor evoked potentials (MEPs). However, it should be noted that MEP amplitude obtained with single-pulse TMS reflects the net effect of excitatory and inhibitory inputs to the corticospinal pathway, allowing to assess cortical but also spinal excitability (Di Lazzaro et al., 2001). Therefore, to date, it is not clear whether changes in MEPs reflect changes in the excitability of the spinal cord or in M1 or both (Di Lazzaro et al., 2001).

To overcome the limitations of single-pulse TMS, paired pulse techniques can be used to directly assess modulations of excitability of intracortical circuitry within M1. In paired pulse techniques, TMS stimulation can be delivered to a single cortical target using the same coil or to two different brain regions using two different coils. Paired pulse techniques can provide measures of intracortical facilitation (ICF) and inhibition (ICI) as well as study cortico-cortical interactions. In paired pulse protocols, two stimuli are released: the first (CS) or conditioning and the second or test stimulus (TS). Inhibition occurs by setting CS below the resting motor threshold and TS well above the resting motor threshold and by using extremely brief interstimulus intervals (ISIs) in the order of 1–3 ms, while

facilitation -with the same conditioning/test intensities- can be obtained by setting ISI in the order of 7–12 ms (Kujirai et al., 1993). This modulation of MEP size takes place at the cortical level and is thought to reflect the activation of separate populations of inhibitory and excitatory cortical interneurons without affecting spinal circuits (Kujirai et al., 1993). In particular it is held that SICI and ICF reflect the activation of low threshold GABAergic inhibitory interneurons (Fisher et al., 2002; Ilic et al., 2002) and glutamatergic interneurons within M1 (Nakamura et al., 1997; Ziemann, 2003) respectively. Therefore, paired-pulse TMS provides a reliable index of motor cortical activation and it appears to be a useful method to further investigate motor responses to emotional bodies.

In Chapter 4, taking advantage of the paired pulse paradigms, we aimed to further investigate whether the excitatory or inhibitory intracortical neural circuits within the right and left primary motor cortex (M1) are modulated during observation of emotional body expressions. In particular, we tested the possible existence of an earlier additional stage of the further involvement of the motor cortex during perception of emotional bodies. Our previous studies suggest comparable motor reactivity for positive and negative body expressions when motor excitability is tested in the 150- 300 ms temporal window (Borgomaneri et al., 2012, 2014). Here, based on the evolutionary contention that threat-related stimuli should evoke extremely rapid motor reactions (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009; Costa et al., 2013), we tested the hypothesis that a “negative bias” in motor reactivity can be detected for fearful bodies if motor excitability is assessed very early in timing. To this aim, we investigated motor excitability in the right and left M1 within the same temporal window in which fearful faces and bodies are known to induce the earliest modulation of occipito-temporal cortices (i.e., at 100-125 ms corresponding to the timing of the P1 component (Smith et al., 2003; Williams et al., 2004; Pourtois et al., 2005; van Heijnsbergen et al., 2007; Jessen and Kotz, 2011).

We found that, regardless of the stimulated hemisphere, perceiving an emotional bodies induced a strong modulation of the observers' M1. In particular, seeing fearful bodies strongly reduced the magnitude of ICF relative to watching happy or neutral bodies. Moreover, happy bodies reduced ICF relative to neutral bodies. No modulations were found for SICI or corticospinal excitability in the 100-125 ms range. ERP studies suggested the existence of a negative bias during fearful bodies observation; however, these ERP studies did not include positive emotional expressions and thus, to date, it was unclear whether P1 modulation merely reflected an arousal response or some threat-related reactivity i.e., a negative bias. Therefore, our finding provides the first direct evidence of an early negative bias in the human motor cortices during perception of emotional bodies. This bias consisted in a modulation of ICF but not of corticospinal excitability or SICI.

Chapter 1

Temporal dynamics of motor cortex excitability during perception of natural emotional scenes*

* This chapter was adapted from:

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Abstract

Although it is widely assumed that emotions prime the body for action, the effects of visual perception of natural emotional scenes on the temporal dynamics of the human motor system have scarcely been investigated. Here, we used single-pulse transcranial magnetic stimulation (TMS) to assess motor excitability during observation and categorization of positive, neutral and negative pictures from the International Affective Picture System (IAPS) database. Motor-evoked potentials (MEPs) to TMS of the left motor cortex were recorded from hand muscles, at 150 and 300 ms after picture onset. In the early temporal condition we found an increase in hand motor excitability that was specific for the perception of negative pictures. This early negative bias was predicted by interindividual differences in the disposition to experience aversive feelings (personal distress) in interpersonal emotional contexts. In the later temporal condition, we found that MEPs were similarly increased for both positive and negative pictures, suggesting an increased reactivity to emotionally arousing scenes. By highlighting the temporal course of motor excitability during perception of emotional pictures, our study provides direct neurophysiological support for the evolutionary notions that emotion perception is closely linked to action systems and that emotionally negative events require motor reactions to be more urgently mobilized.

Introduction

Perceiving and immediately reacting to potential threats are critical for survival (Darwin, 1872). Negative stimuli require perceptual processing and action resources to be more intensely and urgently mobilized to minimize negative consequences associated with unpleasant cues. In support of this view, behavioral and electrophysiological studies have found that unpleasant stimuli are detected more quickly (Hansen and Hansen, 1988; Fox et al., 2000; Öhman et al., 2001) and are associated with larger early (100-200 ms) occipito-temporal components of event-related potentials (ERPs) relative to pleasant or neutral stimuli (Schupp et al., 2003; Smith et al., 2003; Pourtois et al., 2004 for a review see Olofsson et al., 2008) suggesting a rapid bias in visual attention allocation with greater resources devoted to negative stimuli (Vuilleumier, 2002; Carretié et al., 2009). This “negative” bias may reflect interactions between cortical and subcortical structures as suggested for example by the rapid amygdala activation during processing of aversive stimuli (Oya et al., 2002; Tamietto and de Gelder, 2010) and the reduced amplitudes of occipito-temporal ERPs components for negative stimuli when the amygdala is lesioned (Rotshtein et al., 2010).

The impact of negative bias has also been explored at the motor level. For example, studies indicate that while both positive and negative stimuli can modulate motor behavior (e.g. by favoring approach and avoidance movements, respectively), negative stimuli are particularly adept at evoking rapid reactions in the observer (Cacioppo et al., 1993; Bradley et al., 2001; Coombes et al., 2005, 2009; Freina et al., 2009; Naugle et al., 2010) providing support to the evolutionary contention that increased motor readiness in the face of threat may best secure survival of the organism (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009). These findings would also suggest that seeing emotionally negative stimuli while at rest should automatically trigger short-latency activation of the motor representation of the dominant hand.

Single-pulse TMS is a valuable method to non-invasively stimulate the human motor cortex in resting conditions and thus instantaneously assess changes in the excitability of distinct corticospinal

representations of the body (Hallett, 2007; Serino et al., 2009; Avenanti et al., 2012b). This approach has been classically used to probe motor excitability when imaging (Fadiga et al., 1999; Fourkas et al., 2006), observing (Fadiga et al., 2005; Urgesi et al., 2010; Avenanti et al., 2013a, 2013b), planning or performing an action (Hoshiyama et al., 1997; Hasbroucq et al., 1999; Michelet et al., 2010). The high temporal resolution of the technique and the possibility to distinguish between excitatory and inhibitory activity allows effectively exploration action-related dynamics and can provide direct information about how neural processing in the motor cortex unfolds over time (Prabhu et al., 2007; Michelet et al., 2010; Avenanti et al., 2012a; Barchiesi and Cattaneo, 2013).

Recently, studies have used TMS to investigate the excitability of the hand representation in the left motor cortex during observation of natural emotional scenes. These studies have typically reported comparable increase in motor excitability for both emotionally positive and negative stimuli (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009; Borgomaneri et al., 2012). Notably, however, in such studies, motor excitability was tested in a relatively late time window, i.e. at >300ms after stimulus onset, when the amplitude of brain responses to emotional images is typically similar for positive and negative stimuli and likely reflects increased resource allocation to motivationally relevant cues as suggested by ERPs studies (Cuthbert et al., 2000; Keil et al., 2002; Codispoti et al., 2007; Olofsson et al., 2008).

Here we tested the hypothesis that a greater increase in excitability of the left motor cortex occurs for negative stimuli before increased motor reactivity for positive stimuli can be detected. We capitalized on ERPs studies showing that responses in the visual system are: i) larger for negative stimuli in an early temporal window (~100-200ms); and ii) similar for positive and negative stimuli at a later time (~300-600ms) (Vuilleumier, 2002; Olofsson et al., 2008). We reasoned that to ensure survival, action reactivity should be closely coupled with visual processing and thus we predicted greater facilitation of the hand motor representation for negative stimuli in an early time window (at 150 ms from stimulus onset) followed by a comparable motor facilitation for both negative and positive stimuli (at 300 ms as in Borgomaneri et al., 2012). To test these hypotheses we administered single-pulse TMS

over the hand region of the left motor cortex to induce motor-evoked potentials (MEPs) in right hand muscles while participants observed and actively categorized positive, neutral and emotionally negative visual scenes from the International Affective Picture System (IAPS) database. Emotional pictures in the IAPS typically depict humans in emotionally arousing scenarios (e.g. smiling or distressed individuals, or people in sexual or threatening contexts etc.) that may also induce empathy-related processing or personal distress (Lang et al., 1999, 2000; Lamm et al., 2007a, 2008; Morelli et al., 2012); notably, studies have shown that stable empathy or personal distress dispositions predict motor reactivity during social perception (Gazzola et al., 2006; Avenanti et al., 2009a, 2010; Minio-Paluello et al., 2009; Ferri et al., 2010; Lepage et al., 2010). Thus we investigated whether empathy and personal distress dispositions predicted the magnitude of motor reactivity to the different classes of pictures.

Materials and methods

Participants

Fourteen healthy participants (6 men, mean age \pm S.D.: 24.1y \pm 1.4) took part in the study. All the subjects were right-handed according to a standard handedness inventory (Briggs and Nebes, 1975), had normal or corrected-to-normal visual acuity in both eyes, and were naïve as to the purposes of the experiment. None of the participants had neurological, psychiatric, or other medical problems or any contraindication to TMS (Rossi et al., 2009). Participants provided written informed consent, and the procedures were approved by the ethics committee at the Psychology Department of Bologna University and were in accordance with the ethical standards of the 1964 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

Visual stimuli

Color pictures from the IAPS database (Lang et al., 1999; Figure 1A) were presented on a 19-inch screen located 80 cm away from the participants. A total of 32 pleasant, 32 unpleasant and 32 neutral pictures (subtending a 21.2° by 16.2° region) were shown. The stimuli were already included in previous TMS studies (Hajcak et al., 2007; van Loon et al., 2010; Borgomaneri et al., 2012) and are listed in Supplementary Table 1.

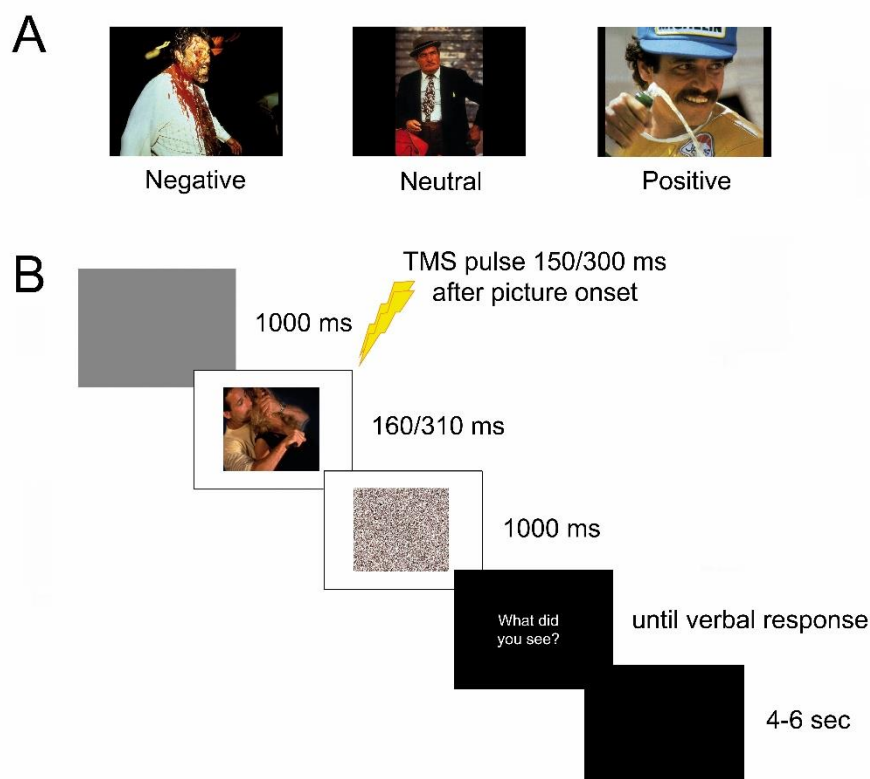


Figure 1. Examples of visual stimuli and trial sequence.

Transcranial magnetic stimulation and electromyography recording

MEPs induced by TMS were recorded from the right first dorsal interosseus (FDI) and abductor pollicis brevis (APB) with a Biopac MP-35 (Biopac, U.S.A.) electromyograph. We selected these two muscles to compare our results with previous studies that explored the excitability of the FDI (Oliveri

et al., 2003), the APB (Hajcak et al., 2007; van Loon et al., 2010) or both FDI and APB motor representations (Borgomaneri et al., 2012) during the observation of emotional pictures. Electromyographic (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. 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 50µV in the higher threshold muscle with 50% of probability (Rossini et al., 1994). This way a stable signal could be recorded from both muscles (Avenanti et al., 2007). The absence of voluntary contractions was continuously visually verified throughout the experiments. When muscles tension was detected the experiment was briefly interrupted and subjects were invited to relax.

Procedure

The experiment was programmed using Matlab software to control picture presentation and to trigger TMS. MEPs were collected in four blocks. The first and the last blocks (10 trials each) served as baseline: subjects kept their eyes closed with the instruction to imagine watching a sunset at the beach (Fourkas et al., 2008; Tidoni et al., 2013) while receiving TMS over M1 (inter-pulse interval ~10 s). In the other two blocks (48 trials each) 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. It is held that active categorization maximizes the chance of detecting emotion specific modulations in

different brain regions, including the motor system as suggested by previous imaging (Gur et al., 2002; Hariri et al., 2003; Habel et al., 2007), TMS (Oliveri et al., 2003) and ERPs studies (Mikhailova and Bogomolova, 2000; Hajcak et al., 2006). A grey screen (1000 ms duration) indicated the beginning of the trial. This was followed by a test picture lasting 160 or 310 ms projected at the centre of the screen (Figure 1B) and by a TMS pulse delivered at 150 or 300 ms after picture onset, respectively. 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 1000 ms. Then, the question “What did you see?” appeared on the screen, and participants answered verbally (forced choice: positive, neutral or negative). An experimenter recorded the answer by pressing a 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, a few seconds after the TMS pulse (Tidoni et al., 2013). After response, the screen appeared black for 4-6 sec. This way the inter-pulse interval was >10 s, thereby avoiding changes in motor excitability due to TMS *per se* (Chen et al., 1997). To reduce the initial transient-state increase in motor excitability, before each block two magnetic pulses were delivered over M1 (inter-pulse interval >10 s). Each baseline and experimental block lasted about 2 and 10 minutes, respectively. At the end of the TMS sessions, all stimuli (shown in a randomized order for 160 and 310 ms as in the TMS experiment) were presented to subjects, who were asked to rate arousal and valence of each picture using an electronic 5-points Likert scale. Arousal and valence ratings were collected in two separate blocks whose order was counterbalanced across subjects. Afterwards, to assess empathy and personal distress dispositions, subjects were asked to complete the Interpersonal Reactivity Index (IRI) (Davis, 1996), a 28-item self-report survey that consists of four subscales, namely Perspective Taking (PT, that assess the tendency to spontaneously imagine and assume the cognitive perspective of another person), Fantasy scale (FS, that assess the tendency to project oneself into the place of fictional characters in books and movies), Empathic Concern (EC, that assess the tendency to feel sympathy and compassion for others in need) and

Personal Distress (PD, that assess the extent to which an individual feels distress in emotionally distressing interpersonal contexts). PT and FS assess cognitive components of empathy, while EC and PD correspond to the notions of other-oriented empathy reaction and self-oriented emotional distress, respectively (Davis, 1996). The PD subscale reflects an anxiety-related interpersonal reactivity that may interfere with mature forms of empathy; thus it tends to drop as the other scales rise and is negatively related to measures of overall social functioning.

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 amplitude, 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% and similarly distributed across conditions). It should be noted that pictures were presented for relatively short time and online TMS may potentially distract participants. To assure that motor excitability reflected effective perception of IAPS stimuli, MEPs associated to incorrect picture classification were discarded from the analysis (see Supplemental data for a discussion of the relation between classification accuracy and motor excitability). Mean accuracy in the emotion evaluation task was high (mean accuracy \pm S.D: 92% \pm 4). Accuracy and MEP data across subjects were normally distributed (as revealed by Shapiro-Wilk test) and were analyzed by means of repeated measure analysis of variance (ANOVA). Significant effects in the ANOVAs were followed by post-hoc analyses using Duncan tests corrected for multiple comparisons. To investigate the relationship between early motor reactivity, dispositional empathy and personal distress, MEP contrasts (negative minus mean of positive and neutral pictures; mean of the two muscles) recorded at 150 ms and the four subscales of the IRI were entered into a correlation analysis. Partial correlations were computed between MEP contrasts and each IRI subscale while controlling for the remaining subscales. A further partial correlation analysis was computed on MEP contrasts

(mean of positive and negative minus neutral pictures; mean of the two muscles) computed at 300 ms and the four IRI subscales. The two different MEP contrasts were chosen based on the results of the main ANOVA that showed a selective increase of motor excitability for negative pictures at 150 ms and a comparable increase of motor excitability for positive and negative pictures at 300 ms. MEP contrasts and IRI subscales were normally distributed as shown by Shapiro Wilk test. The significance level for the two correlation analyses was set at $p = 0.025$.

To confirm emotional features of the IAPS stimuli, we assessed mean scores of arousal and valence. These data were not normally distributed and thus were analyzed by means of nonparametric Friedman ANOVAs. Preliminary Wilcoxon test comparisons revealed that subjective ratings were statistically comparable for pictures presented for 150 ms and 300 ms (all $p > 0.27$) and thus data were collapsed across temporal conditions. Bonferroni-corrected planned Wilcoxon tests were used to analyze significant Friedman ANOVAs effects.

Results

Subjective judgements and classification accuracy of IAPS pictures

Subjective judgements confirmed emotional features of the IAPS stimuli (Table 1). Friedman ANOVAs carried out on mean valence and arousal scores were significant (all $\chi^2 > 26.14$ $p < 0.001$). Bonferroni-corrected planned Wilcoxon test 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 ($p < 0.001$). Arousal scores were higher for positive and negative IAPS stimuli relative to neutral IAPS stimuli (all $p < 0.001$).

	Negative	Neutral	Positive	Negative	Neutral	Positive
	150	150	150	300	300	300
Arousal	4.0 ± 0.8	1.3 ± 0.3	3.0 ± 0.6	4.0 ± 0.7	1.3 ± 0.2	3.0 ± 0.6
Valence	-1.4 ± 0.3	0.0 ± 0.05	1.1 ± 0.2	-1.3 ± 0.3	0.0 ± 0.1	1.1 ± 0.2

Table 1. Mean ± S.D. of arousal and valence ratings of stimuli in the different conditions.

Overall, classification accuracy during the TMS session was high. A Time (2 levels: 150 ms and 300ms) x Emotional scene (3 levels: positive, neutral and negative) repeated measure ANOVA on percentage of correct responses indicated a main effect of Emotional scene ($F_{2,26} = 4.24$, $p < 0.05$). Duncan tests indicated greater accuracy for negative (mean accuracy ± standard deviation; $97\% \pm 7$) relative to positive images ($87\% \pm 14$, $p < 0.01$). Accuracy for neutral images ($91\% \pm 11$) was statistically comparable to negative and positive images (all $p > 0.10$). No main effect of or interaction with the factor Time (all $F < 1.98$, $p > 0.16$).

Temporal dynamics of motor excitability during perception of emotional scenes

Preliminary neurophysiological analyses assured that no change in excitability due to TMS *per se* was detected throughout the experiment and showed that motor excitability during the emotion evaluation task was greater relative to baseline levels (Figure 1; Supplementary Data).

To specifically investigate the effect of time and visual conditions on motor excitability a Muscle (2 levels: FDI and APB) x Time (2 levels: 150 ms and 300ms) x Emotional scene (3 levels: negative, neutral and positive) repeated measure ANOVA on MEP amplitudes recorded during observation of IAPS stimuli was carried out. The analysis showed a main effect of Emotional scene ($F_{2,26} = 6.77$, $p < 0.01$) and, most importantly, a significant Time x Emotional scene interaction ($F_{2,26} = 4.76$, $p < 0.05$; Figure 2).

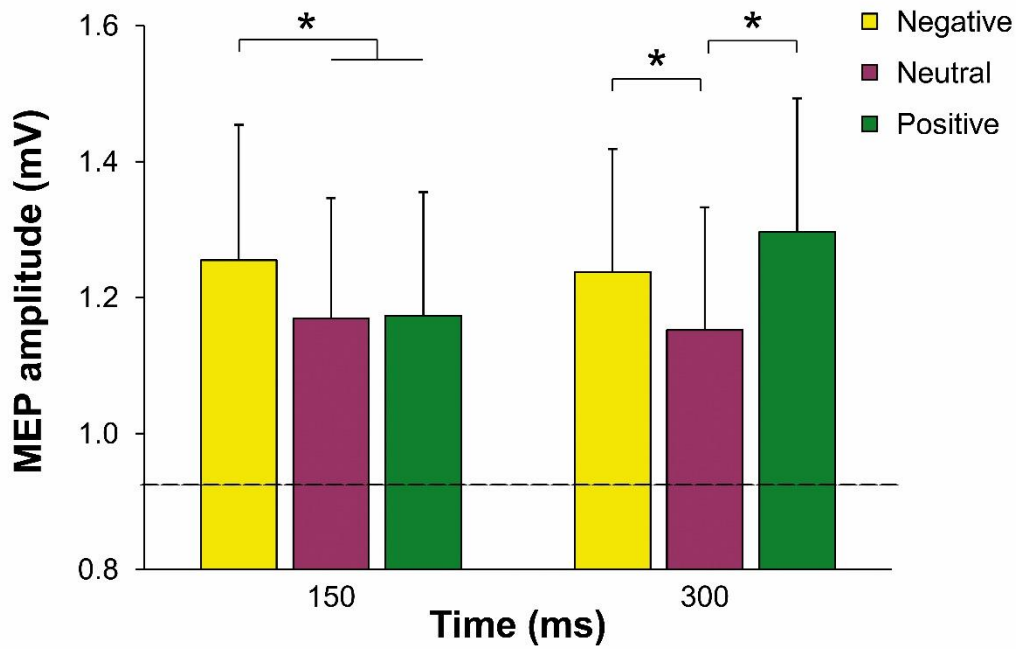


Figure 2. MEP amplitude (in mV) recorded at 150 ms and 300 ms after presentation of negative, neutral and positive pictures (average of the two muscles, FDI and APB). The dashed line represents mean MEP amplitude during baseline blocks. Error bars indicate s.e.m. Asterisks (*) denote significant post-hoc comparisons ($p < 0.05$).

	Negative 150	Neutral 150	Positive 150	Negative 300	Neutral 300	Positive 300
FDI	1.50 ± 0.99	1.38 ± 0.81	1.38 ± 0.90	1.46 ± 0.89	1.37 ± 0.89	1.55 ± 0.95
APB	1.01 ± 0.50	0.96 ± 0.51	0.97 ± 0.47	1.01 ± 0.46	0.94 ± 0.46	1.04 ± 0.52

Table 2. Raw MEP amplitude ± S.D. (in mV) from the two target muscles during the experimental conditions.

Post-hoc analysis (performed with the Duncan test to account for multiple comparisons) indicates that at 150 ms MEP amplitude was greater during the observation of emotionally negative relative to

both positive ($p < 0.05$) and neutral scenes ($p < 0.05$), which in turn did not differ from one another ($p = 0.93$). At 300 ms, MEP amplitude was greater during observation of both emotionally positive ($p < 0.01$) and negative ($p < 0.05$) relative to neutral scenes. No difference between positive and negative scenes was found ($p = 0.15$). No other main effects or interactions were significant, including those with the factor Muscle, indicating that similar changes in excitability were detected in the FDI and APB muscles (all $F < 3.04$, $p > 0.10$; Table 2).

Relation between personality and motor reactivity

The comparable motor facilitation for positive and negative IAPS images that we observed in the 300 ms condition, confirms previous TMS studies that tested motor excitability within the same temporal frame (Borgomaneri et al., 2012) or at a later time (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009). Notably, our study reveals that at an earlier stage of processing (150 ms) a selective increase of excitability for negative images occurs within the motor system. We explored whether inter-individual differences in dispositional empathy and personal distress predicted the magnitude of these two neurophysiological effects.

An index of early motor reactivity for negative pictures (MEP contrast computed at 150) entered in a partial correlation analysis with the four IRI subscales. Partial correlations showed that the PD was marginally significantly related to MEP contrast at 150 ms ($r = 0.66$, $p = 0.027$; Table 3). This relation strongly increased after the removal of one outlier with standard residuals $< -2\sigma$ ($r = 0.90$, $p < 0.001$; Figure 3).

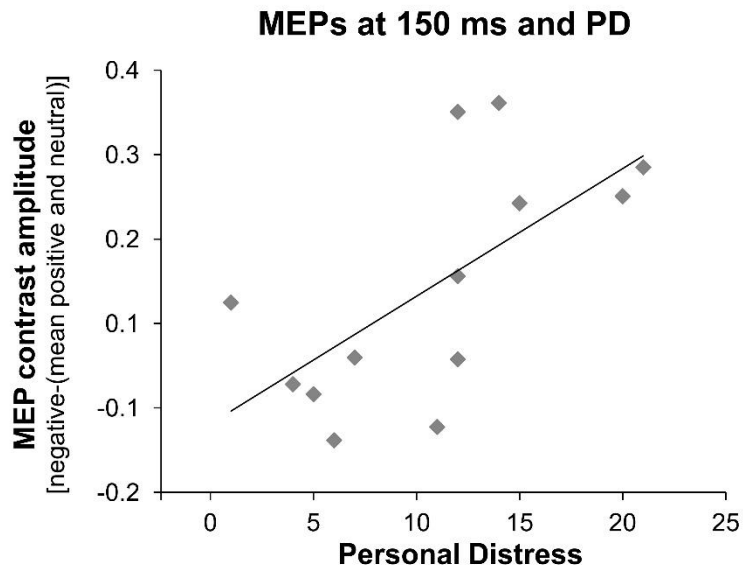


Figure 3. Simple correlation between MEPs contrasts at 150 ms (amplitude during negative pictures minus mean amplitude in the positive and neutral conditions) and Personal Distress subscale of the Interpersonal Reactivity Index.

IRI subscale	MEP contrast 150 ms		MEP contrast 300 ms	
	correlation coefficient	associated p-level	correlation coefficient	associated p-level
PD	0.66	0.03	0.57	0.06
EC	-0.25	0.46	-0.23	0.50
PT	-0.02	0.95	0.37	0.26
FS	-0.42	0.20	-0.17	0.61

Table 3. Partial correlations between MEP contrasts and IRI subscales.

An index of late motor reactivity for positive and negative pictures (MEP contrast computed at 300 ms) was entered in a further partial correlation analysis showing no significant relation with the IRI subscales (Table 3).

Discussion

Although it is widely assumed that emotions prime the body for action, the effects of visual perception of natural emotional scenes on the dynamics of the human motor system have rarely been investigated. In the present study we directly tested the hypothesis that perception of emotionally negative scenes triggers fast reactivity in the corticospinal system. We used single-pulse TMS to monitor changes in excitability of the dominant hand cortical motor representation while participants observed and actively categorized pictures from the IAPS database. We found that seeing negative pictures increased the amplitude of MEPs from the right FDI and APB muscles at 150 ms from stimulus onset. The magnitude of this early increase in hand motor reactivity was predicted by inter-individual differences in the disposition to experience personal distress. Moreover, the early motor excitability increase was followed by a comparable motor facilitation for both positive and negative pictures detected at 300 ms. These findings suggest that perception of natural emotional scenes dynamically modulates the functional state of the human corticospinal system with faster reactivity for potentially threatening scenarios. Moreover, they highlight a functional link between interpersonal anxiety-related personality traits and motor reactivity to complex negative emotional scenes.

The neural network involved in the perception of emotional scenes has been highlighted in a series of functional imaging studies (see Sabatinelli et al., 2011, for a recent meta-analysis of 57 studies). This network includes not only occipitotemporal visual regions but also medial frontal cortices (orbitofrontal and cingulate cortex) that are densely connected to the motor cortex either directly or via premotor and supplementary motor areas (Morecraft and van Hoesen, 1998; Cavada et al., 2000; Oliveri et al., 2003); and may thus provide a cortical pathway for emotional cues to modulate motor

excitability (Pessoa and Adolphs, 2010). Remarkably, motor reactions to aversive visual stimuli may also be implemented through predominantly subcortical routes as suggested by studies on brain damage patients with permanent cortical blindness (Tamietto et al., 2009). Moreover, imaging evidence indicates that subcortical structures (i.e. pulvinar, thalamus and amygdala) are active during perception of complex emotional scenes (Sabatinelli et al., 2011) and in principle these structures may provide additional signals to different segments of the motor pathway during processing of emotional stimuli (Tamietto and de Gelder, 2010; de Gelder et al., 2011).

ERPs studies have highlighted the cortical dynamics of emotional scene processing and, particularly relevant to the present findings, they have reported an initial greater reactivity for negative scenes in occipitotemporal regions (within 100-200 ms from stimulus onset; Olofsson et al., 2008) and, in some cases, also in frontal regions. For example, Carretié and colleagues (2001, 2006) have reported that seeing negative scenes increases the amplitude of frontal positive ERP components with latency in the 160-200 ms range which are thought to reflect the activation of medial prefrontal cortices (see also Northoff et al., 2000 and Kawasaki et al., 2001). Our data significantly expand these findings by demonstrating that this early “negative bias” is not limited to neural regions involved in sensory processing and affective evaluation but extends to the cortical motor representation of the dominant hand, supporting the notion that emotional cues drive action preparation in the brain and, that potential threats require particularly quick motor reactions to secure the survival of the organism (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009).

The early motor facilitation for negative pictures appears strictly related to inter-individual differences in personal distress but not empathy dispositions. Personal distress is an aversive, self-focused emotional reaction to the negative state of another and the PD subscale of the IRI assesses this anxiety-related interpersonal disposition (Davis, 1996). While personal distress may counteract mature forms of empathy (Avenanti et al., 2009; Batson et al., 1997; Lamm et al., 2007), studies have reported that participants who score high on the PD scale show enhanced reactivity of the insula when seeing negative facial expressions (i.e. pain or disgust, Jabbi et al., 2007; Saarela et al., 2007). Seeing

stimuli depicting painful stimulation of the body of another reduces motor excitability, an effect that may be due to the ‘resonant’ activation of pain representations in the observer (Minio-Paluello et al., 2006; Avenanti et al., 2009b). Interestingly, participants with high PD scores tend to show increased motor excitability when seeing the pain of others (Avenanti et al., 2009), in line with the notion that anxiety-related traits are associated with greater motor excitability (Wassermann et al., 2001). Notably greater PD scores are also associated with heightened action readiness but weaker motor control when facing negative images, suggesting that increased motor reactivity may be non-functional (Ferri et al., 2010). Our study is consistent with such evidence and supports the view that anxiety-related traits influence the way in which social and emotional signals are processed in the brain (Azevedo et al., 2005; Lawrence et al., 2006; Moriguchi et al., 2006; Kret et al., 2011a).

The early motor facilitation for negative pictures may seem at odds with previous TMS studies testing the excitability of the left motor cortex during perception of emotional scenes. Most of these studies reported comparable motor facilitation of right hand muscles for positive and negative pictures, either during passive observation (Hajcak et al., 2007) or active categorization of the images (Borgomaneri et al., 2012), or when planning a movement during observation of task-irrelevant pictures (Coombes et al., 2009). Similar findings were also reported by Baumgartner and colleagues (2007) during passive presentation of emotional scenes and emotionally congruent auditory stimuli. Notably in all such studies, MEPs were collected at 300 ms from picture onset (Borgomaneri et al., 2012) or at a later time (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009) and ERP studies suggest that electrophysiological responses to positive and negative pictures are similar in the 300-600 ms range (Olofsson et al., 2008). Thus, our study confirms these temporal dynamics and, on the other hand, indicates the presence of an earlier modulation of the motor cortex when facing emotionally negative scenes.

While MEPs were collected at two discrete time points, it is likely that during perception of emotional scenes the excitability of the observer’s motor cortex gradually changes over time, in line with traditional models of continuous information processing (Miller et al., 1992; Massaro and Cohen,

1995). Future studies testing motor excitability at earlier time windows and with more intervals have the potential to disclose how neural responses to emotional scenes gradually build up (see Michelet et al., 2010; Barchiesi and Cattaneo, 2013) for recent examples in the domain of action execution and observation).

It is worth noting that the reported modulations of motor excitability occurred in the left hemisphere. Traditionally, two theories have linked emotion perception to the issue of hemispheric laterality. According to one view, the right hemisphere is specialized to process all emotions, whereas another view suggests that the right and the left hemispheres are relatively specialized in processing negative and positive emotions, respectively (Silberman and Weingartner, 1986; Davidson and Hugdahl, 1995; Borod, 2000). Since our results showed that negative scenes are able to modulate the left motor cortex, none of these laterality effects can be considered as confounds for the reported changes in motor excitability. Further studies are needed to directly investigate how perception of negative and positive emotional scenes may differentially influence neural motor dynamics in the two hemispheres.

That negative scenes could be particularly adept at facilitating action mechanisms was already suggested by behavioral studies exploring motor performance and action readiness (Coombes et al., 2005, 2009; Naugle et al., 2010). This notion has also been supported by studies reporting enhanced motor cortex plastic reactivity to repetitive TMS during presentation of negative pictures (Koganemaru et al., 2012) as well as increased behavioral imitation (Grecucci et al., 2011) and motor and premotor cortex response to the observation of other's actions (Enticott et al., 2012; Ferri et al., 2013) when action stimuli were primed by unpleasant cues. Moreover, increased EEG motor readiness potentials were found when planning to grasp unpleasant relative to pleasant or neutral objects (de Oliveira et al., 2012) or when moving a finger after negative relative to neutral pictures (Grecucci et al., 2009). Relevant to the present findings are TMS studies in which MEPs were recorded during action execution. In a first study participants pressed a button when seeing emotionally negative IAPS pictures (Oliveri et al., 2003). In such condition, hand motor excitability was greater than in neutral control conditions. However, only negative and neutral conditions were

tested and thus it was not clear whether motor facilitation was driven by unpleasant valence or emotional arousal. More recently, van Loon et al. (2010) asked participants to respond to symbolic targets during presentation of task-irrelevant IAPS pictures. MEPs were recorded at various delays from target onset. Overall, MEP amplitude was larger during positive and negative than during neutral pictures, similarly to previous studies in which MEPs were recorded at rest (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009; Borgomaneri et al., 2012). However, when the TMS pulse was delivered during actual finger movement, MEPs were larger for negative relative to positive and neutral pictures.

In sum, despite the long-held view that negative stimuli require fast processing and reactions, to date, previous TMS studies on the perception of natural emotional scenes tested the excitability of the left motor cortex only in a relatively late temporal window. In these conditions a negative bias was detected only when the motor system was pre-activated (van Loon et al., 2010). Our study provides direct evidence that a negative bias can be detected also at rest when motor excitability is tested early. This negative bias is then followed by a comparable increase in motor excitability for both negative and positive stimuli. In conclusion, our study highlights the temporal dynamics of the human corticospinal system during perception of natural emotional scenes.

Supplementary Data

Supplementary Table 1 - List of IAPS stimuli

Positive pictures: 1601, 2000, 2070, 2080, 2091, 2092, 2165, 2311, 2340, 4002, 4220, 4290, 4572, 4608, 4658, 4659, 4660, 4664, 4800, 4810, 7325, 8032, 8080, 8200, 8280, 8320, 8330, 8370, 8400, 8465, 8490, 8540.
Neutral pictures: 2480, 2570, 2840, 2880, 5390, 5500, 5510, 5532, 5731, 5740, 5800, 5900, 7000, 7002, 7004, 7006, 7009, 7025, 7034, 7035, 7040, 7060, 7090, 7100, 7140, 7175, 7190, 7205, 7217, 7233, 7235, 7491.
Negative pictures: 2800, 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, 9810, 9920, 9921.

Preliminary neurophysiological analyses

Before analyzing motor responses to emotional pictures we first tested whether TMS during the experimental blocks altered motor excitability *per se*, by comparing MEP amplitudes recorded in the first and the last baseline blocks using a Muscle (2 levels: FDI, APB) x Block (2 levels: first, last) repeated measure ANOVA. The analysis yielded no significant main effects or interaction (all $F < 1.63$, $p > 0.22$). Thus, motor excitability of the two muscles was comparable in the baseline and did not change from the first (mean MEP amplitude \pm SD: FDI = 1.06 mV \pm 0.87; APB = 0.81 mV \pm 0.46) to the last block (FDI = 1.09 mV \pm 0.84; APB = 0.75 mV \pm 0.46), ruling out changes in motor excitability due to the prolonged magnetic stimulations (Chen et al., 1997). We then checked whether motor excitability during the emotion evaluation task differed from baseline levels. We compared MEPs collected during the six experimental conditions with those recorded during baseline (average of the two blocks) using a Muscle (2 levels: FDI, APB) x Condition (7 levels: baseline, negative-150, neutral-150, positive-150, negative-300, neutral-300 and positive-300) repeated measure ANOVA. The analysis revealed a significant main effect of Condition ($F_{6,78} = 8.95$, $p < 0.0001$). Dunnett tests showed that MEPs recorded in all the experimental conditions were greater than those recorded in the baseline control condition (all $p < 0.001$). No main effect of or interaction with the factor Muscle resulted significant (all $F < 2.90$, $p > 0.11$). Thus, during the emotion evaluation task there was an increase of motor excitability relative to baseline levels and this increase was similar in the FDI and APB muscles (151% \pm 58 and 143% \pm 84, respectively; Borgomaneri et al., 2012; Tidoni et al., 2013).

Relation between classification accuracy and neurophysiological data

The analysis of the emotion evaluation task accuracy indicates that overall negative pictures were recognized more frequently than positive pictures (~31 and ~28 correct classifications out of 32 pictures, respectively).

Thus, one may ask to what extent this difference may explain the greater MEP amplitude for negative relative to positive emotional scenes in the early time window.

We believe MEP changes reflect adaptive motor reactions to negative and positive emotional cues and do not reflect unspecific factors linked to the different recognizability of the two classes of pictures. Contrary to MEP data, the difference between negative and positive pictures in classification accuracy occurred independently of the factor time. Thus, were picture recognizability the key factor affecting motor excitability, we should have found greater response for negative relative to positive also in the late time window. Moreover, it should be noted that classification accuracy was comparable for negative and neutral pictures whereas motor excitability was greater for negative than for neutral pictures in both time windows. Thus, overall classification accuracy and MEPs data exhibited different patterns of results.

We nevertheless carried out a further analysis to provide direct evidence that motor excitability for positive and negative pictures remained the same after balancing classification accuracy. We ordered pictures based on participants' accuracy in the emotion evaluation task (independently of time of presentation, since this factor did not influence accuracy) and started removing positive pictures with the lowest accuracy values and negative pictures with the highest values until a match was reached in the remaining sample. This procedure yielded to a sample of 19 negative and 19 positive pictures with comparable classification accuracy ($94.4\% \pm 10$ and $94.3\% \pm 9$, respectively; $t_{13} = 0.04$, $p = 0.97$). The analysis of neurophysiological data associated to this subsample of stimuli confirmed that at 150 ms MEPs were larger for negative ($1.28 \text{ mV} \pm 0.65$; mean of the two muscles) than for positive pictures ($1.15 \text{ mV} \pm 0.54$; $p < 0.05$) while they remained similar in the 300 ms condition ($1.25 \text{ mV} \pm 0.52$ and $1.30 \text{ mV} \pm 0.56$; $p = 0.37$). These data speak against the possibility that a different recognizability of the emotional scenes may have played a role in determining the observed pattern of motor excitability.

Supplementary References:

Borgomaneri, S., Gazzola, V., & Avenanti, A. (2012) Motor mapping of implied actions during perception of emotional body language. *Brain Stimulation*, 5, 70-6.

Chen, R., Classen, J., Gerloff, C., et al. (1997) Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, 48, 1398-1403.

Tidoni, E., Borgomaneri, S., di Pellegrino, G., & Avenanti, A. (2013) Action simulation plays a critical role in deceptive action recognition. *Journal of Neuroscience*, 33, 611-23.

Chapter 2

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

* This chapter was adapted from:

Borgomaneri S, Gazzola V, Avenanti A (2012) Motor mapping of implied actions during perception of emotional body language. *Brain Stimul* 5:70–76. doi: 10.1016/j.brs.2012.03.011.

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.

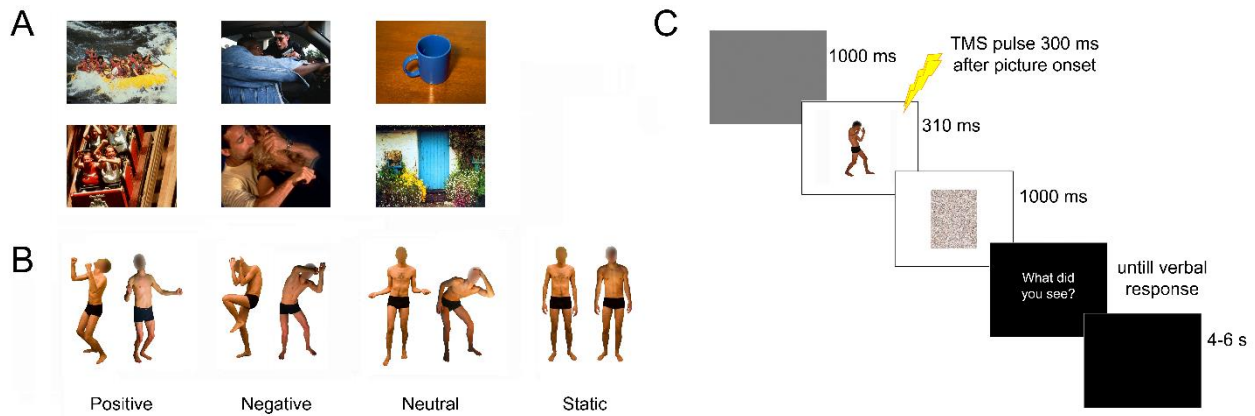


Figure 1. Examples of visual stimuli used in (A) experiment 1 (IAPS images) and (B) experiment 2 (Body images). For each visual condition two representative images are shown. (C) Trial sequence.

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).

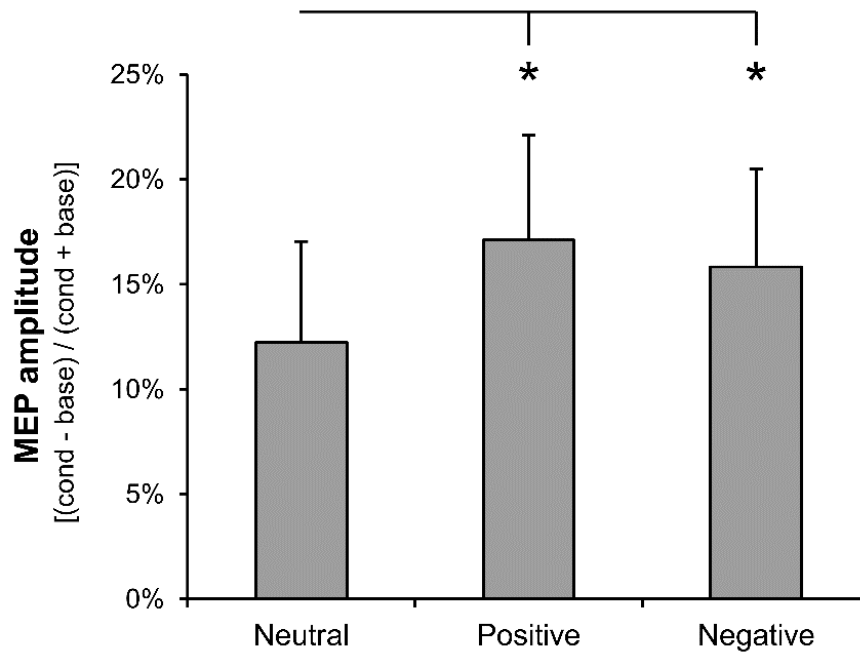


Figure 2. MEP amplitude [(condition - baseline)/(condition + baseline)] during the observational conditions of experiment 1 (IAPS images), average of the two muscles (FDI and APB). Error bars indicate s.e.m. Asterisks (*) denote significant post-hoc comparison ($p < 0.05$).

	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).

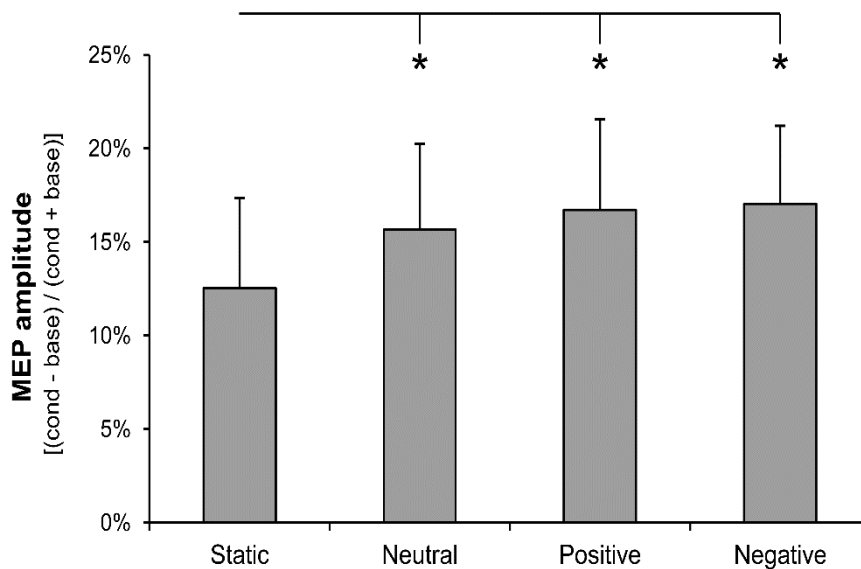


Figure 3. MEP amplitude [(condition - baseline)/(condition + baseline)] during the observational condition of experiment 2 (Body images), average of the two muscles (FDI and APB). Error bars indicate s.e.m. Asterisks (*) denote significant post-hoc comparison ($p < 0.05$).

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.

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Chapter 3

Transcranial magnetic stimulation reveals two functionally distinct stages of motor cortex involvement during perception of emotional body language*

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Abstract

Studies indicate that perceiving emotional body language recruits fronto-parietal regions involved in action execution. However, the nature of such motor activation is unclear. Using transcranial magnetic stimulation (TMS) we provide correlational and causative evidence of two distinct stages of motor cortex engagement during emotion perception. Participants observed pictures of body expressions and categorized them as happy, fearful or neutral while receiving TMS over the left or right motor cortex at 150 and 300 ms after picture onset. In the early phase (150 ms), we observed a reduction of excitability for happy and fearful emotional bodies that was specific to the right hemisphere and correlated with participants' disposition to feel personal distress. This 'orienting' inhibitory response to emotional bodies was also paralleled by a general drop in categorization accuracy when stimulating the right but not the left motor cortex. Conversely, at 300 ms, greater excitability for negative, positive and neutral movements was found in both hemispheres. This later motor facilitation marginally correlated with participants' tendency to assume the psychological perspectives of others and reflected simulation of the movement implied in the neutral and emotional body expressions. These findings highlight the motor system's involvement during perception of emotional bodies. They suggest that fast orienting reactions to emotional cues – reflecting neural processing necessary for visual perception – occurs before motor features of the observed emotional expression are simulated in the motor system and that distinct empathic dispositions influence these two neural motor phenomena. Implications for theories of embodied simulation are discussed.

Introduction

Perceiving and reacting to the emotional states of other individuals are critical for survival. Facial and bodily expressions convey important information about another person's feelings and intentions. Nevertheless, to date most investigations of emotion perception have focused on brain activity generated by the perception of facial expressions (see Fusar-Poli et al., 2009 and Sabatinelli et al., 2011 for meta-analysis) and neglected the body by comparison. Imaging studies have suggested that processing emotional body expressions recruits a complex neural network which includes not only visual areas, but also cortical and subcortical regions involved in emotional processing (e.g., the amygdala, anterior insula, and orbitofrontal cortex) and fronto-parietal sensorimotor regions involved in action planning and execution (de Gelder et al., 2010; Tamietto and de Gelder, 2010). However, the nature of such motor activation is unclear. According to embodied simulation theories, since covert emotional states (e.g., happiness) are often associated with overt motor behaviors (e.g., smiling, joyful body postures and gestures), observers can understand the unobservable emotional states of others by embodying their observable motor behavior through motor (or somato-motor) resonance mechanisms that tap onto the motor (somato-motor) response associated with generating the perceived expression (Adolphs, 2002; Gallese et al., 2004; Goldman and Sripada, 2005; Keysers and Gazzola, 2006, 2009; Gallese, 2007; Oberman et al., 2007; Bastiaansen et al., 2009; Niedenthal et al., 2010; Gallese and Sinigaglia, 2011). Most radical "motoric-centric" versions of these theories contend that motor resonance occurs prior to the activity in emotion-related regions (thus very early in time) and is necessary for assigning emotional meaning to visual signals (thus they would play a causal role in visual perception) (e.g., Carr et al., 2003; Iacoboni, 2009). However, to date, these hypotheses were mainly based on the indirect imaging evidence of a co-activation of motor and emotional regions during observation of emotional expressions. Although studies suggest that portions of the motor system indeed transmit information to emotion-related regions during emotion perception (Jabbi and Keysers, 2008) and that manipulation of posture and motor activity affects

perception of emotions in others (Oberman et al., 2007; Niedenthal et al., 2010), whether the cortical motor system is engaged early and whether this engagement reflects resonance mechanisms necessary for visual perception remain speculative. Support for a casual role of somatosensory (rather than motor) regions comes from brain damage and transcranial magnetic stimulation (TMS) studies showing that stable lesion or transient disruption of the right somatosensory cortex impairs the recognition of facial expression of emotions (Adolphs et al., 2000; Pourtois et al., 2004b; see also Banissy et al., 2011). In particular, Pitcher and colleagues (2008) showed this effect by administering pairs of TMS pulses early during visual perception (at 100-140 ms and 130-170 ms from stimulus onset), suggesting that the right somatosensory cortex is promptly engaged during the perception of facial expression. However, it is unclear whether similar engagement would be critical for the recognition of body rather than facial expressions. Moreover, it is unclear whether early somatosensory (or motor) activity reflects resonance mechanism or some other neural processing. For the motor system, the picture is complicated by the fact that emotional cues may trigger fast motor reactions (Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009) rather than motor resonance. Indeed, other scholars embracing an evolutionary perspective on emotion processing have proposed that attribution of emotional value to visual stimuli occurs, at least initially, in subcortical circuits (e.g., amygdala, pulvinar, superior colliculus etc; Morris et al., 1999; Luo et al., 2007; Tamietto et al., 2009; de Gelder et al., 2010; LeDoux, 2012). In this vein, early motor reactivity during perception of emotional bodies would reflect emotionally appropriate (non-simulative) motor reactions serving adaptive purposes (e.g., fight/flight reactions), rather than motor resonance processing necessary for visual perception (Tamietto et al., 2009; de Gelder et al., 2010; LeDoux, 2012). In the present study, we directly tested the different predictions made by simulative and non-simulative theories regarding the time course of motor system responses to emotional bodies and the potential role of such motor responses for visual perception of emotional bodies. To this aim, we used single-pulse transcranial magnetic stimulation (TMS) during an emotion recognition task in which participants observed and actively categorized pictures of happy, fearful and neutral body movements and static postures. TMS

was administered over the right M1 (Exp1M1right) or left M1 (Exp2M1left) at two critical time points, i.e., at 150 and 300 ms from picture onset. This paradigm allowed us to record TMS-induced motor-evoked potentials (MEPs) during perception of emotional body expressions. This way, we non-invasively monitored changes in motor excitability reflecting neural responses hypothesized by embodied simulation theories (i.e., embodiment of the observed motor behavior, that is, motor resonance) or other types of responses subserving perception (e.g., orienting responses) or body survival (e.g., freezing or fight/flight reactions). Indeed, assessment of MEPs is a well-established approach to explore motor resonance in humans (Fadiga et al., 1995, 2005; Avenanti et al., 2007, 2013a; Aglioti et al., 2008; Candidi et al., 2010; Urgesi et al., 2010; Catmur et al., 2011) and a number of studies have also shown that perceptually salient and emotional stimuli affect motor excitability (Farina et al., 2001; Oliveri et al., 2003; Makin et al., 2009; Serino et al., 2009). TMS seems, therefore, to be a valuable tool for assessing the interplay between action and emotion processing within the motor system. Importantly, since TMS pulses disrupt neural activity in the targeted regions, we also tested whether stimulation of M1 at 150 or 300 ms after stimulus onset affected visual perception of body expressions. This allowed us to combine correlational and causal approaches to test the role of the motor system in the perception of body expressions. The early time point was chosen to explore possible short-latency motor responses to emotional bodies and was based on the notion that visually presented complex scenes including facial and contextual emotional cues modulate visual event-related potentials (ERPs) in the 100-200 ms range (Vuilleumier and Pourtois, 2007; Olofsson et al., 2008) and motor excitability at 150 ms (Borgomaneri et al., 2013). Moreover, this timing corresponds to the study of Pitcher and colleagues (2008). If emotional body perception is associated with fast motor reactions to emotional body cues, we might expect differential excitability for emotional and non-emotional movements at this latency and stronger reactivity in the right hemisphere, which may be dominant for emotional processing (Borod, 2000). The later time point was chosen based on action observation studies showing that neural activity reflecting motor resonance is typically detected at about 250-350 ms after stimulus onset in the motor cortices (Nishitani et al., 2004; Catmur et al.,

2011; Barchiesi and Cattaneo, 2013) and on the finding that observation of emotional and non-emotional movements induces motor resonance in the observer's left M1 at 300 ms after stimulus onset (Borgomaneri et al., 2012). Thus, at this time point we expected neural activity reflecting the encoding of the motor features of observed actions, independent of their emotional meaning (as found in Borgomaneri et al. 2012 for the left M1). Since studies suggest that participants with a greater tendency to take the psychological perspective of another may show stronger resonant activations (Gazzola et al., 2006; Cheng et al., 2008; Avenanti et al., 2009a; Minio-Paluello et al., 2009; Martínez-Jauand et al., 2012; Schaefer et al., 2012) and different empathy traits may modulate neural activity during social perception (Singer et al., 2004; Lamm et al., 2007b, 2010; Borgomaneri et al., 2013; Bufalari and Ionta, 2013; Melloni et al., 2013) we explored the relation between changes in motor excitability and individual scores of dispositional empathy using the Interpersonal Reactivity Index (IRI) (Davis, 1996). If early M1 reactivity reflects pure motor reactions to emotional cues that are epiphenomenal for visual recognition (as suggested by non-simulative theories), whereas later motor resonance plays an active role in perception (as suggested by embodied simulation theories), we might expect that M1 stimulation at 300 ms but not at 150 ms from stimulus onset would disrupt participants' performance in the emotion recognition task. Conversely, if early motor activity reflects neural processing necessary for perceiving body expressions, whereas motor resonance at 300 ms reflects an embodiment of the observed expression occurring *after* its visual recognition, we might expect that M1 stimulation at 150 ms but not at 300 ms would impair task performance.

Material and methods

Participants

Fifty-six healthy subjects took part in the study. Twenty participants (10 men, mean age \pm S.D.: 23.7 y \pm 2.4) were randomly assigned to Experiment 1 in which the right M1 was stimulated

(Exp1M1right) and other 20 (9 men, $23.7 \text{ y} \pm 1.6$) to Experiment 2 in which the left M1 was stimulated (Exp2M1left). A further group of 16 participants (7 men, $25.5 \text{ y} \pm 3.1$) took part in a third control experiment in which sham stimulation was performed (Exp3Sham). The experiments were carried out at the Centro studi e ricerche in Neuroscienze Cognitive, Department of Psychology, University of Bologna. All participants were right-handed according to a standard handedness inventory (Oldfield 1971) and free from any contraindication to TMS (Rossi et al. 2009). They gave their written informed consent to take part in the study, which was approved by the local ethics committee and carried out according to the Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

Visual stimuli

In all the experiments, different types of pictures were presented on a 19-inch screen located 80 cm away from the participants. Sixty pictures were selected from a validated database (Borgomaneri et al. 2012). Pictures depicted four different actors in emotional and neutral postures (Fig. 1a). To focus specifically on body-related information, the face was blanked out in all the pictures. Stimuli included pictures of emotionally positive (happy) and negative (fearful) movements, neutral movements (i.e., actions with implied movement comparable to emotional body expressions but with no emotional meaning) and static neutral postures (baseline).

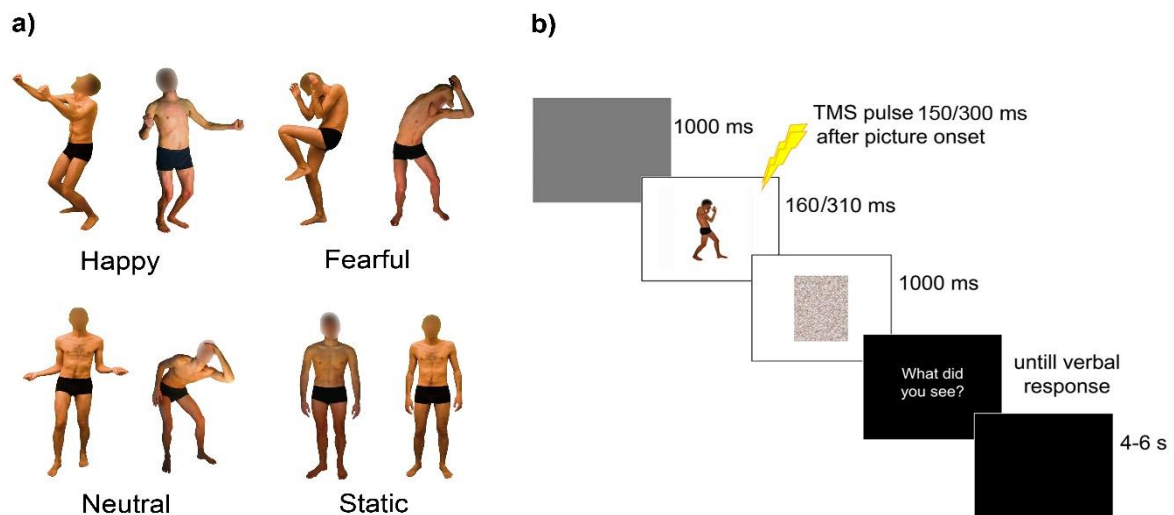


Fig. 1 a Examples of visual body stimuli. **b** Trial sequence

During the recording of neutral movements, instructions to the actors specified the action to be performed (e.g., jump rope). For emotional expressions, 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 at the actor). Stimuli were selected from an initial sample of about 1000 images based on two pilot studies in which emotional ratings and emotion recognition data were collected, resulting in a final selection of 15 fearful body expressions, 15 happy body expressions, 15 neutral movements and 15 static postures that were well recognized as prototypical representations of the different expressions (see Borgomaneri et al. 2012 for details). All the emotional and neutral movement stimuli represented a whole-body movement with a clear involvement of upper limbs (implied motion stimuli). In none of the stimuli did the model interact with objects or other individuals. To rule out that any differential modulatory effect in the left and right M1 was due to a different amount of implied motion of the models' left or right hands, mirror-reflected copies of the selected stimuli were also created. In each experiment, half the participants were tested with the original version of the stimuli, and the remaining half were tested with mirror-reflected copies.

Transcranial magnetic stimulation and electromyography recording

Both Exp1M1right and Exp2M1left started with the electrode montage, detection of optimal scalp position and measurement of resting motor threshold. To explore motor excitability, MEPs induced by TMS were recorded from the first dorsal interosseus (FDI) muscles with a Biopac MP-35 (Biopac, U.S.A.) electromyograph. In Exp1M1right and Exp2M1left, MEPs were recorded from the left and the right FDI, respectively (contralateral to the stimulated hemisphere). To check muscle relaxation during MEP recording, EMG activity was also recorded from the FDI muscle ipsilateral to the stimulated hemisphere. 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-eight coil connected to a Magstim Rapid2 stimulator (Magstim, Whitland, Dyfed, U.K.) was placed over M1. The intersection of the coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle from the midline. With this antero-medial coil orientation, biphasic TMS pulses elicited eddy currents in the brain flowing in a posterior-anterior/anterior-posterior direction approximately perpendicular to the line of the central sulcus. The chosen coil orientation may require slightly greater (biphasic) pulse intensity to elicit MEPs relative to the opposite postero-lateral coil orientation (Kammer et al., 2001). However, the antero-medial orientation is best suited for recording MEPs during visual tasks because it does not require the experimenter holding the coil to stand in front of the participant during TMS. Detection of optimal scalp position and resting motor threshold was performed as it follows. Using a slightly suprathreshold stimulus intensity, the coil was moved over the target hemisphere to determine the optimal position from which maximal amplitude MEPs were elicited in the contralateral FDI muscle. The optimal position of the coil was then marked on the scalp with a pen to ensure correct coil placement throughout the experiment. In Exp1M1right and Exp2M1left, the intensity of magnetic pulses was set at 125% of the resting motor threshold (rMT), defined as the minimal intensity of stimulator output that produces MEPs with an amplitude of at

least 50 μ V from the muscle with 50% probability (using about 20 pulses). Mean stimulation intensity (mean % of maximal stimulator output \pm S.D) was statistically comparable in Exp1M1right (72.0 % \pm 10.2) and Exp2M1left (67.7 % \pm 5.2; $t(38) = 1.70$, $p = 0.1$). The absence of voluntary contraction was visually verified continuously throughout the experiments. When muscle tension was detected the experiment was briefly interrupted and the subject was invited to relax. In Exp3Sham, no electrophysiological preparation was necessary since no EMG signal and MEPs were recorded. Sham stimulation was performed by placing the coil tilted at 90° over the vertex. In all subjects, stimulation intensity was set at 70 % of the maximal stimulator output, corresponding to mean intensity used in Exp1M1right and Exp2M1left. Although during sham no current was induced in the brain, TMS pulses produced some scalp sensations and auditory click comparable to active stimulation.

Procedure and experimental design

The experiments were programmed using Matlab software to control picture presentation and to trigger TMS pulses. In Exp1M1right and Exp2M1left, MEPs were collected in two separate blocks of 60 trials. In each block, subjects performed an emotion recognition task: they were presented with a picture and were asked to categorize it as either a happy, fearful, neutral dynamic or static body posture. Trial sequence was as follows: a gray screen (1 s duration) indicated the beginning of the trial, and it was followed by the test picture projected at the center of the screen (Fig. 1b). In half the trials, stimuli were presented for 160 ms and TMS was delivered at 150 ms from stimulus onset. In the remaining trials, stimuli were presented for 310 ms and TMS was delivered at 300 ms from stimulus onset. Stimulus duration was randomly distributed in the two blocks. 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 1 s. Then the question “What did you see?” appeared on the screen, and the subject provided a verbal response (forced choice). Possible choices were: happy, fearful, neutral, static. An experimenter collected the answer by pressing a computer

key. To avoid changes in excitability due to verbal response (Tokimura et al., 1996), participants were invited to answer only during the question screen, a few seconds after the TMS pulse (Tidoni et al., 2013). After the response, the screen appeared black for 4-6 s, ensuring an inter-pulse interval greater than 10 s and thereby avoiding changes in motor excitability due to TMS per se (Chen et al., 1997). To reduce the initial transient-state increase in motor excitability, before each block two magnetic pulses were delivered over the targeted M1 (inter-pulse interval >10 s). Each block lasted about 10 min. To provide control conditions for comparing behavioral performance in Exp1M1right and Exp2M1left we carried out Exp3Sham in which instead of active M1 stimulation, sham TMS was performed. In this third control experiment, stimuli (and their duration, 160 and 310 ms), task and trial structure were the same as in Exp1M1right and Exp2M1left, but no MEPs were recorded. Only behavioral performance at the emotion recognition task was collected.

Subjective measures

After TMS, only subjects in Exp1M1right and Exp2M1left were presented with all the stimuli (shown in a randomized order) and asked to judge arousal, valence and perceived movement using a 10 cm electronic visual analogue scale (VAS). To avoid building up artificial correlations between the different judgments, each rating was collected separately during successive presentation of the whole set of stimuli (Avenanti et al., 2009a). Finally, subjects completed the IRI questionnaire (Davis 1996), a 28-item self-report survey that consists of four subscales, namely Perspective Taking (PT, that assess the tendency to spontaneously imagine and assume the cognitive perspective of another person), Fantasy Scale (FS, that assess the tendency to imaginatively transpose oneself into fictional situations), Empathic Concern (EC, that assess the tendency to feel sympathy and compassion for others in need) and Personal Distress (PD, that assess the extent to which an individual feels distress in emotional interpersonal contexts). PT and FS assess cognitive components of empathy, while EC

and PD correspond to the notions of other-oriented empathy reactions and self-oriented emotional distress, respectively (Davis 1996).

Data analysis

Neurophysiological and behavioral data were processed off-line. Mean MEP amplitudes in each condition were measured peak-to-peak (in mV). MEPs associated with incorrect answers were discarded from the analysis (less than 6%). Since background EMG is known to affect motor excitability (Devanne et al., 1997), MEPs with preceding background EMG deviating from the mean by more than 2 S.D. were removed from further analysis (less than 6%). To compare motor excitability in Exp1M1right and Exp2M1left we computed MEP contrast indices by subtracting the mean MEP amplitudes recorded in the static body posture condition from the MEP amplitudes recorded in the three dynamic conditions (happy, fearful, neutral movements). MEP contrasts (dynamic-static) were first analyzed by means of a three-way mixed model ANOVA with Area (2 levels: Exp1M1right and Exp2M1left) as a between-subjects factor, and Time (2 levels: 150 and 300ms) and Movement type (3 levels: happy, fearful and neutral) as within-subjects factors. To test whether the TMS pulse had interfered with visual recognition of body expressions, we compared behavioral performance in the emotion recognition task across the three experiments. Accuracy (i.e., % correct responses) was analyzed by means of a mixed model two-way ANOVAs with Area (3 levels: Exp1M1right, Exp2M1left and Exp3Sham) as a between-subjects factor, and Time (2 levels: 150 and 300ms) as within-subjects factors. A preliminary ANOVA including also the factor Movement type (see Supplementary Table 1), did not reveal any interaction between Movement Type and Area ($p > 0.27$) and thus data were collapsed across the Movement type factor. Mean VAS ratings for arousal, valence and implied movement were analyzed by means of mixed model two-way ANOVAs with the factors Area (2 levels: Exp1M1right and Exp2M1left) and Movement type (4 levels: happy, fearful, neutral and static). Because subjective ratings in the various experimental conditions were slightly correlated ($-0.16 < r < 0.50$, with Pearson coefficients computed across the

experiments), and therefore not independent we then corrected the p-level for the number of ANOVAs. In all the ANOVAs, post-hoc comparisons were carried out by means of the Newman-Keuls test. Finally, to test the relation between behavioral performance, dispositional empathy and motor excitability, standard regression and correlational analyses were performed. In these analyses, MEP contrasts were entered as dependent variables whereas indices of performance accuracy in the emotion recognition task (accuracy drop contrast: mean % accuracy at 150 ms – mean % accuracy at 300 ms) and the four subscales of the IRI questionnaire were entered as predictors.

Results

Subjective measures

The Area x Movement type ANOVAs carried out on valence, arousal and implied motion scores showed only a main effect of Movement type (all $F > 123.43$, $p < 0.0001$). No other main effects or interactions were significant in the ANOVAs (all $p > 0.43$; see Table 1).

Exp1M1right	Static	Happy	Neutral	Fearful
Arousal	1.50 ± 1.32	5.84 ± 1.49	3.91 ± 1.86	6.13 ± 1.25
Valence	4.71 ± 0.22	8.04 ± 0.83	5.16 ± 0.55	1.52 ± 0.71
Perceived motion	0.46 ± 0.44	6.03 ± 1.61	5.96 ± 1.18	5.10 ± 1.51

Exp2M1left	Static	Happy	Neutral	Fearful
Arousal	1.04 ± 1.17	5.56 ± 1.70	3.46 ± 1.79	6.32 ± 1.15
Valence	4.82 ± 0.16	7.87 ± 0.93	5.27 ± 0.53	1.42 ± 0.75
Perceived motion	0.42 ± 0.44	6.09 ± 1.57	5.98 ± 1.45	5.18 ± 1.93

Table 1 Mean ± standard deviation subjective evaluations (arousal, valence and perceived implied motion) of stimuli used in first (Exp1M1right) and the second experiment (Exp2M1left).

Post-hoc analyses showed that valence ratings were lower for fearful movements relative to happy and neutral movements and static body postures (all $p < 0.001$); moreover, valence ratings were higher for happy relative to neutral movements and static postures (all $p < 0.001$); neutral movements were considered more positive than static postures ($p = 0.004$). Arousal scores were greater for happy and fearful movements relative to neutral movements and static postures (all $p < 0.001$). Moreover, arousal ratings were not significantly different between fearful and happy movements ($p = 0.07$) whereas neutral movements were considered more arousing than static postures ($p < 0.001$). Implied motion scores were greater for happy, neutral and fearful movements relative to static postures (all $p < 0.001$); moreover, scores were higher for happy and neutral movements relative to fearful movements (all $p < 0.002$). Happy and neutral movements contained the same amount of implied motion ($p = 0.69$).

Behavioral performance in the emotion recognition task

The ANOVA on accuracy data showed a main effect of Time ($F(1,53) = 19.50, p < 0.0001$) and, importantly, a significant Time x Area interaction ($F(2,53) = 3.57, p = 0.035$). This was accounted for by the lower accuracy in the early (150 ms) relative to the late (300 ms) temporal condition (mean % of correct responses \pm S.D.: $92.8 \% \pm 4.0$ vs $95.8 \% \pm 2.9, p = 0.0006$) found in the Exp1M1right only. Indeed, the same comparison between temporal conditions was not significant in Exp2M1left ($94.1 \% \pm 4.7$ vs $94.7 \% \pm 3.8; p = 0.37$) or Exp3Sham ($94.1 \% \pm 2.3$ vs $95.5 \% \pm 2.8; p = 0.12$). These data indicate that in Exp1M1right there was a small but significant drop in accuracy in the 150 ms relative to the 300 ms condition ($-2.9 \% \pm 2.4$; Fig. 2), whereas the drop was not significant in Exp2M1left ($-0.6 \% \pm 3.2$) and Exp3Sham ($-1.5 \% \pm 2.7$). Planned comparisons also showed that accuracy drop was greater in Exp1M1right than in the other two experiments ($p = 0.018$; Fig. 2) which in turn did not differ from one another ($p = 0.39$). These findings suggest that TMS administered over right M1 at 150 ms from stimulus onset selectively interfered with visual recognition of body expressions. This interference was similar across body expressions (see Supplementary Table 1).

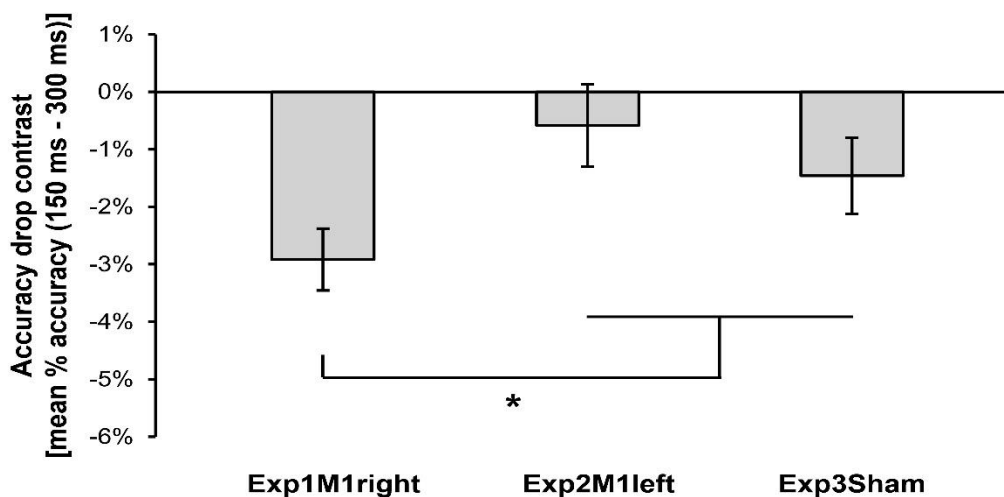


Fig. 2 Effect of TMS on recognition accuracy. Accuracy drop contrast (mean drop in % of accuracy found in the early relative to the late temporal condition) computed in the three experiments. Only in Exp1M1right there

was a significant drop in accuracy in the early temporal condition (see main text). The drop in accuracy detected in Exp1M1right was greater than that found in the other two experiments. Error bars indicate s.e.m. Asterisks (*) denote significant comparisons ($p < 0.05$).

Neurophysiological data

The Area x Time x Movement type ANOVA on MEP contrasts (happy-static, fearful-static and neutral-static) showed a significant triple interaction ($F(2,76) = 3.67, p = 0.03$). This interaction seems to be driven by the fact that MEP suppression generated by the display of emotional (happy and fearful) when compared to neutral bodies, which is only significant at 150 ms in the right hemisphere (Fig. 3a), decreases from 150 ms to 300 ms in the right hemisphere, while the opposite is true for the left hemisphere $\{[\text{mean (happy and fearful) – neutral}]_{150\text{M1right}} - [\text{mean (happy and fearful) – neutral}]_{300\text{M1right}} > [\text{mean (happy and fearful) – neutral}]_{150\text{M1left}} - [\text{mean (happy and fearful) – neutral}]_{300\text{M1left}}; \text{two sample } t\text{-test}, p = 0.03\}$. To further explore the triple interaction we carried out two separate ANOVAs, one for each Area. The Time x Movement type ANOVA on MEP contrasts collected in Exp1M1right showed a Time x Movement type interaction ($F(2,38) = 3.35, p = 0.046$). The post-hoc analysis showed that when TMS was administered at 150 ms after stimulus onset, observation of happy and fearful expressions brought about lower MEP values relative to observation of neutral movements ($p = 0.049$ and $p = 0.03$, respectively), indicating a reduction of motor excitability for emotional body stimuli. This inhibitory response was comparable for emotionally positive and negative body expressions ($p = 0.55$).

In contrast, when TMS was administered at 300 ms after stimulus onset, MEPs were facilitated in a similar way during observation of emotional and neutral movements (all comparisons $p > 0.32$). No other effects were significant in the ANOVA (all $F < 2.33, p > 0.14$; Fig. 3a).

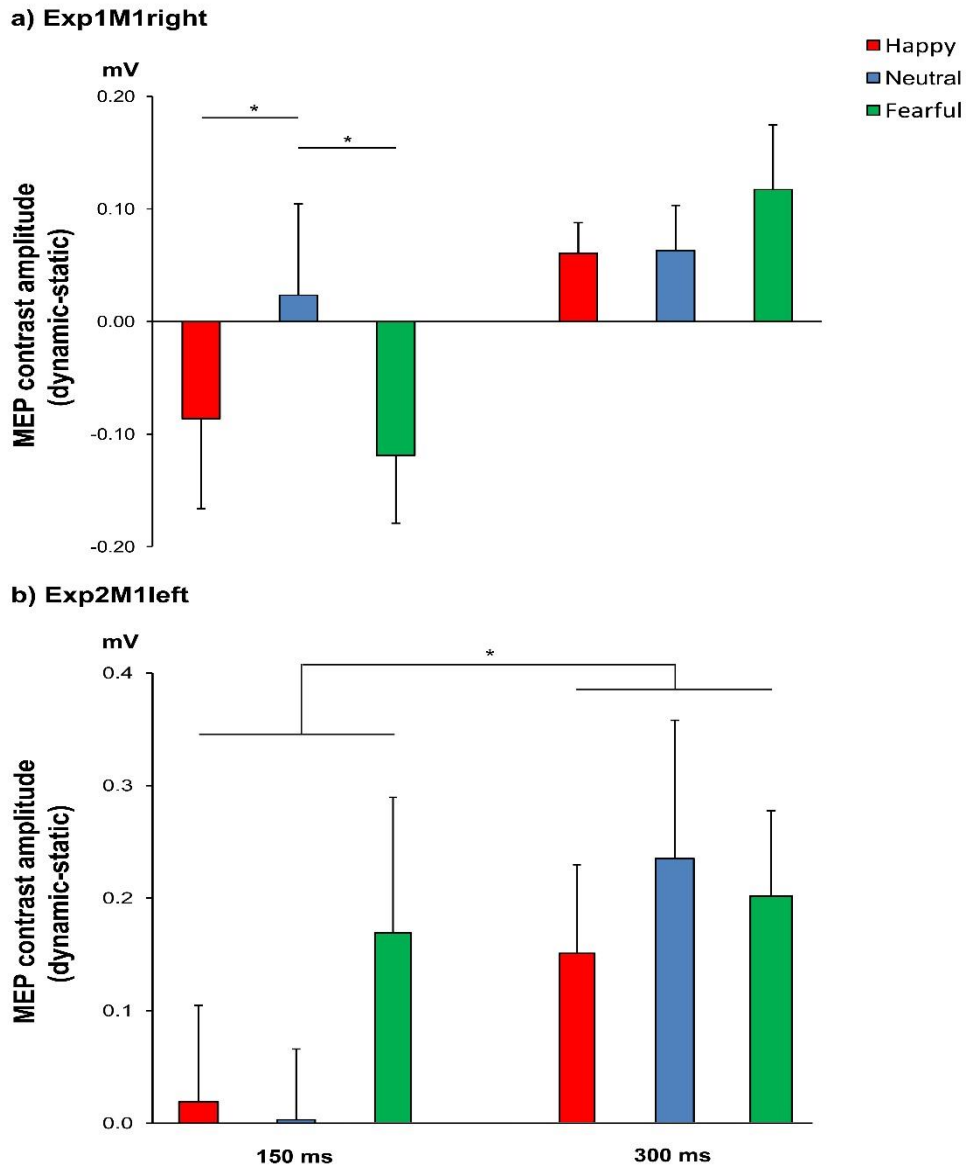


Fig. 3 Neurophysiological modulations during the emotion recognition task. MEP amplitude contrasts (dynamic - static) during perception of happy, neutral and fearful body postures at 150 and 300 ms from the stimulus onset. a Data from the first (Exp1M1right) experiment showing an early suppression of MEPs for emotional bodies and a later increase of MEPs for the three dynamic expressions. b Data from the second experiment (Exp2M1left), showing greater MEPs for the three dynamic expressions in the late relative to the early temporal condition. See main text for further statistical results. Error bars indicate s.e.m. Asterisks (*) denote significant comparisons ($p < 0.05$).

The Time x Movement type ANOVA on MEPs recorded in Exp2M1left showed a main effect of Time ($F(1,19) = 4.65, p = 0.044$) but no main effect of Movement type or Time x Movement type interaction (all $F < 1.46, p > 0.24$). MEPs were larger at 300 ms relative to those recorded at 150 ms from stimulus onset (Fig. 3b). To specifically test whether observation of emotional and non-emotional movements induced motor resonance, a series of planned comparisons were performed. These showed that, collapsing across hemispheres, MEPs recorded at 300 ms during observation of happy (mean amplitude \pm S.D.: $1.52 \text{ mV} \pm 0.92$), fearful ($1.58 \text{ mV} \pm 0.96$) or neutral movements ($1.57 \text{ mV} \pm 1$) were larger than those recorded when seeing static body postures ($1.42 \text{ mV} \pm 0.75$, all comparisons $p < 0.03$), indicating that seeing emotional and neutral implied motion stimuli brought about an increase of motor excitability relative to static controls. These motor facilitations for emotional and neutral movements were comparable in the two hemispheres (all $p > 0.19$). To further test motor excitability in the early time window an additional analysis was performed. A previous study showed that seeing emotionally negative scenes increases the excitability of the left M1 at 150 ms after stimulus onset (Borgomaneri et al. 2013). Although the Time x Movement type interaction was not significant in Exp2M1left, visual inspection of the MEPs in Fig. 2b suggests a possible increase in M1 activity for fearful expressions in the 150 ms condition. However, a planned *t-test* comparing fearful with neutral movements at 150 ms revealed only a non-significant trend ($p = 0.067$).

Relation between changes in motor excitability and behavioral performance

In sum, we found that seeing both emotionally positive and negative movements reduced motor excitability at 150 ms relative to neutral movements in Exp1M1right. No similar modulation of motor excitability was found in Exp2M1left at the same temporal delay. In addition, behavioral performance suggests an accuracy drop for the early temporal condition only in Exp1M1right. To test the relation between neurophysiological and behavioral data, we computed a simple correlation between the MEP

contrast at 150 ms [mean (happy and fearful) – neutral movement] and an index expressing the drop in accuracy in the early timing [accuracy drop contrast: (average % accuracy at 150 ms) – (average % accuracy at 300 ms)]. We found that the MEP contrast was strongly negatively correlated with the accuracy drop contrast found in Exp1M1right ($r = -0.57$, $p = 0.008$; Fig. 4) with stronger inhibitory response associated with lower drop in accuracy and smaller inhibition with greater accuracy drop. The same analysis conducted on Exp2M1left was not significant ($r = 0.12$, $p = 0.61$). These findings suggest a close link between visual recognition of body expressions and early changes in the excitability of the right M1.

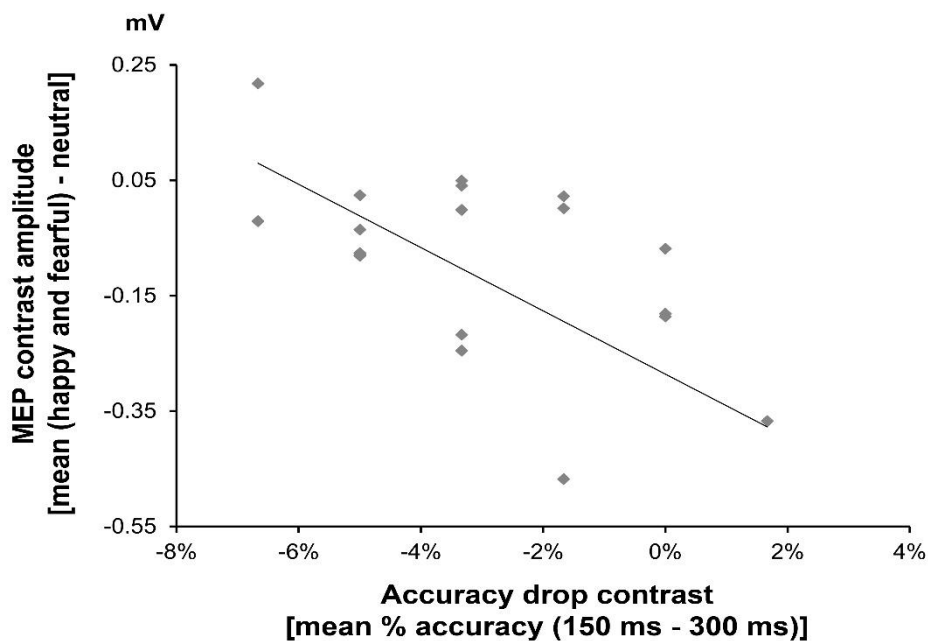


Fig. 4 Simple correlation between neurophysiological and behavioral changes in Exp1M1right. MEP contrast index, representing the early changes in motor excitability (mean amplitude during happy and fearful body postures minus mean amplitude during neutral body postures) significantly correlated with the index representing the early interferential effect of right M1 stimulation on visual recognition of body expressions,

i.e., the accuracy drop contrast (mean drop in % of accuracy found in the early relative to the late temporal condition of Exp1M1right).

Relation between changes in motor excitability and dispositional empathy

While early motor reactivity in the right hemisphere consisted in a reduction of excitability for emotional bodies, at 300 ms, we found a motor facilitation for all dynamic bodies. This motor facilitation was comparable for emotional and neutral movements and was similar in the two hemispheres. To test whether these two neurophysiological effects were related to individual differences in dispositional empathy, two multiple regression analyses were carried out. MEP contrasts, computed based on the results of the ANOVAs, were entered as dependent variables in the regression models, and individual scores from the IRI subscales (Fantasy, Perspective Taking, Empathic Concern and Personal Distress) were entered as predictors.

In the first analysis we considered the contrast representing early inhibition found in Exp1M1right [mean (happy and fearful) – neutral movement]. The regression model was non-significant ($R^2 = 0.08$, $F(4,15) = 1.41$, $p = 0.28$; no statistical outliers with residual >2 sigma were present in the data set). However, Personal Distress (PD) was a significant negative predictor of the emotion-related MEP change ($\beta = -0.52$, $t(15) = -2.33$, $p = 0.03$), showing greater MEP reduction in participants with higher PD scores. No other predictors were significantly related to the neurophysiological index. Simple correlations confirmed that emotion-related MEP reduction correlated with PD ($r = -0.49$, $p = 0.029$; Fig. 5a) but not with other IRI subscales (all $p > 0.77$). These findings suggest that subjects who scored high in PD showed greater early motor inhibition in the right hemisphere when seeing emotional bodies.

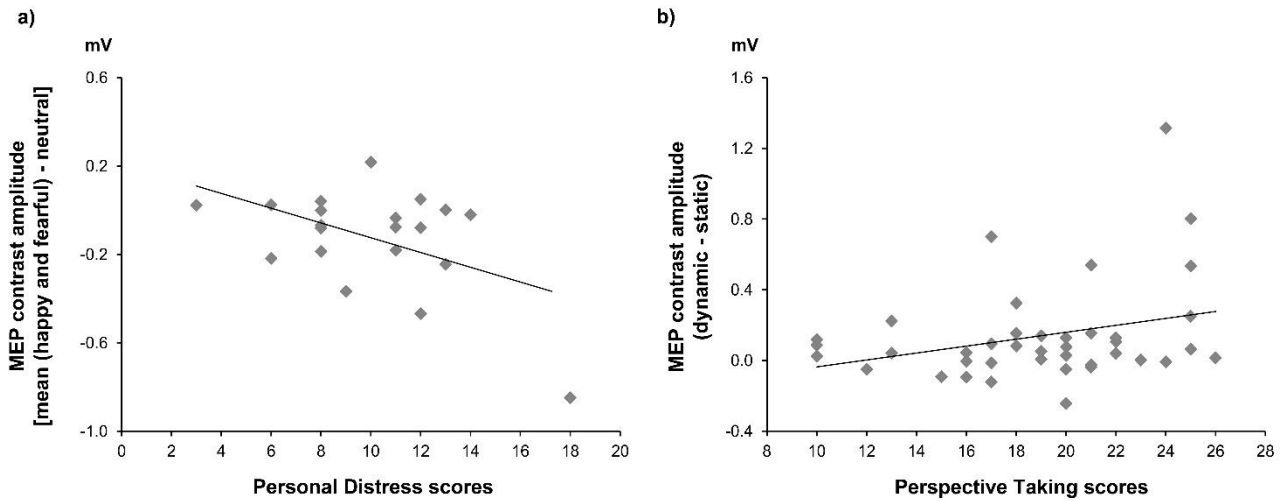


Fig. 5 Simple correlations between early and late neurophysiological effects and personality dispositions. **a** Simple correlation between early MEP contrasts in Exp1M1right (mean amplitude during happy and fearful body postures minus mean amplitude during neutral body postures) and the Personal Distress subscale of the Interpersonal Reactivity Index. **b** Simple correlation between late MEP contrasts in Exp1M1right and Exp2M1left (mean amplitude during dynamic body postures minus mean amplitude during static body postures) and the Perspective Taking subscale of the Interpersonal Reactivity Index.

Since early motor reactivity in Exp1M1right correlated with both PD scores and behavioral performance (see previous paragraph), we also explored the relation between these two variables as a control analysis. The simple correlation was not significant in this case ($r = -0.01$, $p = 0.96$), suggesting that interindividual differences in PD scores were not associated with the magnitude of the interferential effect of right M1 stimulation. Additionally, we computed a regression model in which PD scores and the index of accuracy drop were entered as predictors of early motor reactivity. The regression was significant ($R^2 = 0.57$, $F(2,17) = 11.49$, $p = 0.0007$; no statistical outliers with residual >2 sigma were present in the data set) and both PD scores ($\beta = -0.50$, $t(17) = -3.15$, $p = 0.006$) and the drop in accuracy ($\beta = -0.58$, $t(17) = -3.67$, $p = 0.002$) were significant independent predictors of early motor reactivity.

Finally, we tested whether the bilateral motor facilitation we found at 300 ms for emotional and neutral movements was related to dispositional empathy. Since participants in Exp1M1right and Exp2M1left showed very similar motor responses to dynamic stimuli and scored similarly on all the IRI subscales (all $p > 0.49$), we pooled the two groups together to increase statistical power. A MEP modulation index reflecting the late motor facilitation for dynamic bodies was computed by averaging the MEP contrasts computed for happy, fearful and neutral movements at 300 ms. This index was entered as a dependent variable in a standard regression model and the IRI subscales were entered as predictors. The regression model was non-significant ($R^2 = 0.01$, $F(4,35) = 1.05$, $p = 0.39$; no statistical outliers with residual >2 sigma were present in the data set), and no predictors were found to be significant (all $p > 0.16$). Based on previous studies showing a relation between cognitive empathy and imitative behavior (Chartrand and Bargh, 1999) and motor resonance (Gazzola et al., 2006; Keysers and Gazzola, 2006; Cheng et al., 2008; Avenanti et al., 2009a) we specifically tested the bivariate relation between late motor facilitation and scores on the IRI Perspective Taking (PT) subscale. The Pearson coefficient showed a marginally significant positive correlation ($r = 0.30$, $p = 0.06$; Fig. 5b). This suggests that individuals who reported higher levels of PT tended to show stronger motor resonance when seeing emotional and neutral movements.

Discussion

It is well established that the motor system is recruited during emotion processing (Lang, 1993; Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009). However, the nature of motor cortex activations in the perception of emotional body language is a matter of debate. According to embodied simulation accounts, neural activity in the observer's motor system reflects motor resonance, i.e., simulation of the motor features of the observed emotional expression (Carr et al., 2003; Leslie et al., 2004; Oberman et al., 2007; Bastiaansen et al., 2009; Niedenthal et al., 2010; Gallese and Sinigaglia,

2011). On the other hand, early motor reactivity may reflect different non-simulative processing, including fast motor reactions to emotional cues (i.e., fight/flight reactions) or neural processing that facilitates visual perception (e.g., orienting responses) (Tamietto et al., 2009; de Gelder et al., 2010; LeDoux, 2012). Here we tested the hypothesis that motor responses to emotional cues and motor resonance are both implemented in the motor system but at different times. We found that seeing emotional body movements reduced MEP amplitude at 150 ms, only after stimulation of the right M1. This early inhibition of motor excitability, which may reflect an orienting response toward emotional cues, was comparable for fearful and happy expressions and larger than for neutral movements. Moreover, at 150 ms, TMS over right M1 interfered with accuracy in the emotion recognition task. No similar effects were found with sham or left M1 stimulation. Greater TMS interference on task accuracy correlated with reduced changes in motor excitability, suggesting a link between neural activity reflecting early orienting and visual recognition of body expressions. In addition, orienting responses, correlated with the participants' scores on the Personal Distress scale of the IRI. At 300 ms, greater MEP amplitudes were measured for negative, positive and emotionally neutral movements relative to static body postures for both hemispheres. This later increase in motor excitability indexed the presence of body motion in the stimulus rather than its emotional content. Indeed, MEP facilitation was comparable for the three dynamic conditions and possibly reflected motor simulation of the body movements implied in the pictures. The magnitude of this putative simulative response marginally correlated with the IRI Perspective-Taking subscale. Our findings reveal two possibly distinct functional stages of motor cortex involvement during perception of emotional body language: an initial stage (~150 ms) reflecting early orienting responses that would actively support visual recognition of body expressions; and a later stage (~300 ms) in which the motor cortex implements resonance to any observed movements independent of their emotional content. Moreover, our study shows that distinct personality traits influence these two neural phenomena. These results shed new light on the temporal relation between the motor processes hypothesized by simulative and non-simulative theories of emotion processing and their causal role

in perception. In particular, our study demonstrates that early motor activity is critical for visual perception of body expressions but this motor activity appears to reflect an orienting response rather than motor resonance. These findings may provide some support to general proposals of embodied simulation suggesting that motor (and somato-motor) activity facilitates social and emotion perception. However, they speak against theoretical accounts that have maintained that motor resonance is an early and *necessary* step for the attribution of emotional meaning to visual signals (Carr et al., 2003; Iacoboni, 2009). Indeed, we provide evidence that motor resonance occurs in M1 *after* the signals discriminating between emotional and non-emotional bodies (i.e., reflecting the orienting response) have been already processed, and thus suggests that at this level motor resonance may not be a prerequisite for processing the emotional features of body expressions. These findings have implications for constraining embodied simulation theories of emotion perception.

Early orienting supporting visual perception in the right motor cortex

The major point of novelty in our study is the demonstration that the motor system is transiently modulated during perception of emotional body language, with an early and transient suppression of motor excitability in the right M1. This motor modulation reflects neural signals discriminating between emotional and non-emotional bodies and, remarkably, these signals appear critical for visual perception of body expressions.

The early right M1 modulation occurred at 150 ms, thus not only before the occurrence of the neural signature of motor resonance (i.e., the increase in motor excitability for dynamic bodies that we detected at 300 ms after stimulus onset), but even before the typical latency of occipito-temporal components of event-related potentials (ERPs), which are supposed to reflect the visual processing underlying the structural encoding of bodies (i.e., the N170/N190 component; Gliga and Dehaene-Lambertz, 2005; Thierry et al., 2006; Righart and de Gelder, 2007) Previous studies have shown that,

relative to neutral actions, fearful body expressions affected the ERP response in the earliest stage of visual perception, i.e., the P1 component (van Heijnsbergen et al., 2007). This occipital component, which typically occurs in the 100-150 ms window, also showed sensitivity to emotional congruence of the body and the face (Meeren et al., 2005). Our study significantly expands these previous ERP findings on several fronts. First, it shows that, in approximately the same temporal window, the brain response to emotional bodies involves not only the visual cortex but also motor structures. Second, it characterizes one of the components of such brain responses as an inhibitory modulation of the right M1. Third, it shows that such modulation is detected not only for emotionally negative but also for positive body expressions. Fourth, it shows that the magnitude of the inhibitory response correlates with the disposition to feel personal distress. Lastly, this response appears to reflect neural processing causally involved in the visual perception of body expressions.

We suggest that this early inhibitory modulation reflects the motor counterpart of an orienting response toward a salient stimulus, like an emotional body, that would manifest as a fast inhibition of the motor response. In support of this interpretation are TMS studies showing that freezing-like inhibitory modulations of M1 are detected when processing salient stimuli in different modalities, including noxious stimuli (Farina et al., 2001; Tamburin et al., 2001; Urban et al., 2004), loud acoustic stimuli (Furubayashi et al., 2000), salient auditory stimuli presented close to the body (Serino et al., 2009; Avenanti et al., 2012a), unexpected visual flashes (Cantello et al., 2000), approaching visual stimuli (Makin et al., 2009) and visual stimuli depicting pain in others (Minio-Paluello et al., 2006; Fecteau et al., 2008; Avenanti et al., 2009b). Our study adds to these previous findings by showing that during observation of emotional body language, early modulations in the right M1 are not an epiphenomenon of perception. Rather, they appear to play an active and casual role in the visual recognition of body expressions, as evidenced by the small but significant drop in task accuracy found in Exp1M1right (but not in Exp2M1left or Exp3Sham) and the close relation between the magnitude of TMS interference and orienting responses.

It should be noted that our paradigm was optimized for assessing motor excitability during accurate perception of emotional body language. For this reason, on each trial we delivered TMS at the end of picture's presentation, collected recognition accuracy of the picture, and considered only MEPs associated to correct recognition (Borgomaneri et al., 2012, 2013). This implied however that the two temporal conditions (150 and 300 ms) differed not only for the latency of TMS pulse relative to picture onset, but also for the duration of visual stimuli. Thus, to correctly interpret behavioral data in Exp1M1right and Exp2M1left, we carried out Exp3Sham that clarified that recognition accuracy in the two temporal conditions was comparable when no active stimulation of the right M1 was performed. These findings demonstrate a causal link between early right (but not left) M1 activity and visual perception. This link fits with the notion that sensorimotor networks in the right hemisphere support emotion and attention processing (Adolphs et al., 2000; Pourtois et al., 2004b; Tamietto et al., 2006; Beraha et al., 2012) and appears also in line with the study of Pitcher and colleagues (2008) who found that TMS interference with early right somatosensory cortex activity (~100-170 ms) impaired visual recognition of facial expressions. While this latter study has been interpreted as strong evidence for embodied simulation accounts, it should be noted that the paradigm used by of Pitcher and colleagues (2008) could not directly demonstrate the nature of somatosensory activation during emotion perception, because only behavioral data were acquired. In contrast, here we were able to show that when the right *M1* appears critical for visual perception (~150 ms), no signs of motor resonance can be detected in that region, speaking against a major role of motor resonance processes – at least those that can be detected at 300 ms in bilateral M1 – in the visual recognition of body expressions.

Early orienting versus fight/flight motor reactions

While we found clear evidence for an early (~150 ms) orienting response in the right M1, in the same time window the left M1 showed a weak and marginally significant facilitation. This facilitation was

specific for observation of fearful body expressions and did not correlate with accuracy in the emotion recognition task. In addition, the effect of left M1 stimulation on task performance did not differ from that of sham stimulation. While the non-significance of these findings needs to be interpreted with caution, the increase of left M1 excitability fits with previous work showing that watching threatening emotional scenes facilitates the excitability of the motor representation of the dominant hand in the left M1 (Borgomaneri et al. 2013), possibly reflecting the preparation of fast fight/flight motor reactions as hypothesized by evolutionary non-simulative accounts of emotion perception (Morris et al., 1999; Luo et al., 2007; Tamietto et al., 2009, 2012; de Gelder et al., 2010; LeDoux, 2012). This may suggest that early in time, the right and left M1 may implement different neural processing supporting visual perception and adaptive fight/flight motor reactions, respectively. It should be considered that TMS effects are site-specific but not site-limited (Fox et al., 1997; Ishikawa et al., 2007; Siebner et al., 2009; Avenanti et al., 2012a, 2012b). Thus, it is possible that TMS modulated activity not only locally in M1, but also in other interconnected sensorimotor regions and these regions contributed to the observed neurophysiological and behavioral effects. Nevertheless, our study suggests a dissociation between right and left sensorimotor networks in supporting perception of body expressions and implementing motor reactions to negative cues, respectively.

Possible neural pathways supporting early orienting and perception of body expressions

We can only speculate about the neural networks supporting early motor orienting and visual perception of body expressions. On the one hand, right M1 may reflect the spill over of somatosensory activity associated with emotion perception (Winston et al., 2003; Leslie et al., 2004; Hennenlotter et al., 2005; Gazzola et al., 2012) and the drop in accuracy found in Exp1M1right could be due to the spread of the TMS interference to closely interconnected right somatosensory regions (Fox et al., 1997; Ishikawa et al., 2007; Keyzers et al., 2010) which in turn may have affected perception of emotional expressions (Adolphs, 2002; Pourtois et al., 2004b; Pitcher et al., 2008) and body

movements (Jacquet and Avenanti, 2013). More extensively, observation of emotional body expressions recruits a complex neural network which includes occipito-temporal and parieto-frontal somato-motor regions (de Gelder et al., 2004, 2010; Grèzes et al., 2007; Peelen et al., 2007; van de Riet et al., 2009; Tamietto and de Gelder, 2010; Kret et al., 2011a; Pichon et al., 2012), and these regions may provide a cortical pathway for fast orienting and visual perception of body expressions.

On the other hand, studies on brain damaged patients with permanent cortical blindness (Tamietto et al., 2009; Van den Stock et al., 2011) and imaging evidence on healthy individuals that subcortical structures (i.e., pulvinar, caudate nucleus and amygdala) are active during the perception of emotional bodies (van de Riet et al., 2009; de Gelder et al., 2010) suggest that the motor reaction to emotional bodies can be also implemented through subcortical routes (Tamietto and de Gelder, 2010). These subcortical structures are anatomically connected with different segments of the motor pathway and may additionally provide signals to M1 during the processing of emotional bodies (Tamietto and de Gelder, 2010; Tamietto et al., 2012). A possible role of subcortical networks in the perception of body expressions is also suggested by the evidence that TMS over visual cortex impairs visual recognition of neutral more than emotional body postures (Filmer and Monsell, 2013), in line with the idea that emotion recognition can be supported by subcortical emotion-processing routes bypassing processing in the visual cortex (Morris et al., 1999; Liddell et al., 2005; Tamietto and de Gelder, 2010; de Gelder et al., 2011). Thus, it possible that TMS disruption of the right M1 may have influenced subcortical activity critical for emotion processing resulting in reduced orienting and impaired visual perception of body expressions, although it should be noted that our data suggests that TMS over right M1 interferes with perception of emotional and non-emotional body to a similar extent (Supplementary Table 1). Thus, if early orienting in the right M1 is mediated by subcortical networks, the causal involvement of such networks for visual recognition might be similar for emotional and non-emotional body expressions.

Motor resonance with emotional and non-emotional body movements occurs later

As mentioned in the introduction, at 300 ms after stimulus onset the motor system is likely involved in action simulation: left M1 is modulated by action observation in the 250-350 ms range (Nishitani et al., 2004; Catmur et al., 2011; Barchiesi and Cattaneo, 2013) and evidence indicates that motor resonance effects in M1 are mediated by those sectors of the premotor and parietal cortex that are recruited during action execution (Avenanti et al., 2007, 2013a, 2013b; Koch et al., 2010; Catmur et al., 2011) and where mirror neurons were first recorded in the macaque brain (e.g. di Pellegrino et al., 1992; Gallese et al., 1996; Fogassi et al., 2005; Casile, 2013). In a previous study using left M1 stimulation, we showed that MEPs recorded at 300 ms after stimulus onset increased in amplitude when a similar set of emotional and non-emotional body stimuli was presented (Borgomaneri et al. 2012). Confirming and extending this result, we found that, at 300 ms, seeing not only happy and fearful but also neutral body expressions increased the amplitude of MEPs relative to observing static neutral postures, and this was true not only for the left but also for the right M1, providing neurophysiological support for the notion that simulation-related activity is largely bilateral (Molnar-Szakacs et al., 2005; Keysers and Gazzola, 2009; Caspers et al., 2010). The motor facilitation detected during observation of neutral body movements did not significantly differ from that found with emotional bodies. Pictures of neutral gestures received lower emotional ratings than pictures of emotional body movements but, like the emotional expressions, were perceived as dynamic body postures. At 300 ms, motor excitability thus appears to be related to a simulation of the dynamic features of the observed expressions (Nishitani et al., 2004; Catmur et al., 2011; Barchiesi and Cattaneo, 2013). It should be noted, however, that fearful expressions received slightly lower implied motion ratings than happy or neutral movements, whereas motor facilitation was statistically comparable in the three dynamic conditions. This suggests that, at this stage, our measure of motor excitability is not sensitive to small differences in perceived implied motion and instead reflects a coarse categorization of the observed body posture as a dynamic or static body configuration. Our

study suggests that late markers of motor resonance in M1 do not play a major role in visual perception of body expressions. However, in this context, it is important to entertain the possibility that resonance processes necessary for perception could occur earlier in time (e.g., after 150 ms and before 300 ms) or in other anatomical locations (e.g., in premotor or parietal regions) without being immediately evident in M1 activity as measured by TMS (Jabbi and Keysers, 2008; Avenanti and Urgesi, 2011; Cattaneo et al., 2011; Tidoni et al., 2013; Urgesi et al., 2014). Thus, further studies are needed to test the chronometry and causal involvement of motor resonance in visual perception of emotional body language.

Influence of personality traits on orienting and motor resonance

The two sequential stages of motor system modulation were dissociated not only functionally and in time – with motor orienting occurring earlier and being more involved in visual perception – but also in respect to the influence that personality traits exert on such distinct stages of processing. The early right hemisphere reduction of motor excitability for emotional bodies was related to inter-individual differences in personal distress (PD) but not to the other IRI subscales (Davis 1996), which reflect more mature empathic dispositions. Personal distress is a self-focused aversive reaction to the negative state of another, and it is considered in line with our finding of a correlation with PD only at 150ms an early and rudimentary form of empathy, like emotional contagion (Davis 1996). Imaging studies have reported that participants who score high on the PD scale show enhanced reactivity of the insula when seeing happy and disgusted facial expressions (Jabbi et al., 2007) and when seeing painful expressions (Saarela et al., 2007), suggesting increased emotional reactivity to the emotions displayed by others. These findings are in line with ERP and imaging evidence that interpersonal anxiety-related dispositions are associated with a stronger visual cortex response to social and emotional information (Kolassa and Miltner, 2006; Rossignol et al., 2012; Schulz et al., 2013). A relation between inter-individual differences in PD and increased neural reactivity at the motor level

was also reported during observation of complex negative scenes (Borgomaneri et al. 2013) and others receiving painful stimulation (Avenanti et al., 2009a). Ferri and colleagues (2010) additionally showed that greater PD scores correlate with weaker motor control when facing emotional expressions. Taken together, these findings support the idea that interpersonal anxiety-related dispositions are associated with greater orienting and freezing-like responses to emotional cues, and that anxiety-related traits influence the way in which social and emotional signals are processed in the brain (Kret et al., 2011a; Azevedo et al., 2013; Borgomaneri et al., 2013). That the magnitude of early motor orienting in Exp1M1right correlated with both TMS interferential effect and PD scores may raise the concern that unspecific factors (e.g., distractibility due to TMS that may be in principle greater in participants prone to experiencing personal distress) explain participants' performance. However, PD scores did not correlate with the interferential effect and indeed these two variables were independent predictors of early motor orienting. This finding, together with the lack of interference with sham and left M1 stimulation, assures that unspecific effects cannot explain our results. We additionally found that motor resonance in the two hemispheres marginally correlated with scores on a cognitive empathy subscale of the IRI, namely the Perspective Taking scale, which taps the ability to take the psychological perspectives of others. Although this finding was only marginally significant and should therefore be interpreted with caution, the correlation with PT is in line with (i) social psychology studies indicating that subjects who score high on the PT scale show high levels of automatic mimicry of postures, mannerisms and facial expressions during interpersonal communication (Chartrand and Bargh, 1999); (ii) the fMRI study of Gazzola and colleagues (2006) showing that activity in premotor and somatosensory regions responsive to both action perception and execution was predicted by inter-individual differences in PT scores; and (iii) a series of other studies relating dispositional cognitive empathy to "resonant" activations (Pfeifer et al., 2008; Martínez-Jauand et al., 2012; Schaefer et al., 2012; Bolognini et al., 2013; see Bufalari and Ionta, 2013 for a review). Neurophysiological studies have additionally shown that participants with high levels of PT and cognitive empathy show greater modulation of sensorimotor excitability during

observation of actions (Lepage et al., 2010; Novembre et al., 2012) and pain (Cheng et al., 2008; Avenanti et al., 2009a, 2010; Minio-Paluello et al., 2009). Altogether, our findings and the above-mentioned results suggest that participants with greater cognitive empathy may show greater motor resonance during observation of others' emotional expressions. Thus, our study suggests that during observation of emotional body language, early motor orienting more than motor resonance would support visual recognition of body expressions. However, the finding of a relation between cognitive empathy and motor resonance may suggest that this later neural phenomenon – reflecting the embodiment of the observed body expression in the observers' motor system – is involved in more sophisticated empathy-related understanding that occurs *after* visual recognition and may provide the observer with a reading of the body expressions 'from the inside' (Rizzolatti and Sinigaglia, 2010).

Conclusions

We provided correlational and causative evidence for a two-stage involvement of the motor cortex during perception of emotional body language. Our study suggests that early in time, the right M1 actively supports perception of body expression through the implementation of orienting responses, whereas the left M1 may be involved in preparing potential fight/flight motor reactions to negative cues. At a later stage, bilateral motor cortex activity reflects motor resonance mechanisms possibly linked to more sophisticated empathy-related processing.

Supplementary Data

Visual recognition of body expressions was compared in the three experiments. Accuracy scores were analyzed by means of a mixed model three-way ANOVAs with Area (3 levels: Exp1M1right, Exp2M1left and Exp3Sham) as a between-subjects factor, and Time (2 levels: 150 and 300ms) and Movement type (4 levels: static, happy, neutral and fearful) as within-subjects factors (see Supplementary Table 1). The ANOVA showed a main effect of Time ($F(1,53) = 19.50, p < 0.0001$) and Movement Type ($F(3,159) = 19.22, p < 0.0001$). These main effects were qualified by a significant Time x Movement Type interaction ($F(3,159) = 3.07, p = 0.03$), suggesting that lower accuracy in the early (150 ms) relative to the late (300 ms) temporal condition for neutral (88.1% vs 91.5%, $p = 0.0007$) and fearful movements (95% vs 97.4%, $p = 0.006$), whereas accuracy for happy and static postures was similar in the two temporal conditions ($p > 0.41$). Notably, this interaction was not qualified by the triple interaction ($F(3,159) = 0.78, p = 0.59$) suggesting similar trends in the three experiments. Importantly, the Time x Area interaction was also significant ($F(2,53) = 3.57, p = 0.035$). This was accounted by the fact that in Exp1M1right, accuracy was lower in the early (150 ms) relative to the late (300 ms) temporal condition (92.8% vs 95.8%, $p = 0.0006$), whereas no similar change in performance was found in the other two experiments ($p > 0.12$). Thus, accuracy was generally lower in the early relative to the late conditions of Exp1M1right and this effect was similar across visual conditions.

150 ms**300 ms**

	Static	Happy	Neutral	Fearful	Static	Happy	Neutral	Fearful
Exp1M1right	99.0 ± 2.4	91.7 ± 12.2	87.0 ± 10.9	93.7 ± 6.7	98.7 ± 2.7	93.7 ± 9.3	93.3 ± 6.8	97.3 ± 4.0
Exp2M1left	99.7 ± 1.5	94.0 ± 12.0	86.3 ± 10.7	96.3 ± 5.9	100.0 ± 0.0	93.7 ± 10.0	88.0 ± 9.6	97.0 ± 4.0
Exp3Sham	98.8 ± 3.6	90.8 ± 6.8	91.7 ± 7.5	95.0 ± 5.7	98.8 ± 2.7	91.7 ± 8.6	93.8 ± 7.9	97.9 ± 5.3

Supplementary Table 1 Mean ± standard deviation of accuracy scores (% of correct responses) in the recognition task during observation of static, happy, neutral and fearful body postures in first (Exp1M1right), second (Exp2M1left) and third control experiment (Exp3Sham) after 150 or 300 ms from stimulus onset.

Chapter 4

Seeing fearful body language rapidly freezes the observer's motor cortex*

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Abstract:

Fearful body language is a salient signal alerting the observer about the presence of a potential threat in the surrounding environment. Although detecting potential threats may trigger extremely quick reduction of motor output in animals (e.g., freezing behavior), it is unclear at what point in time similar reductions occur in the human motor cortex and whether they originate from excitatory or inhibitory processes. Using single-pulse and paired-pulse transcranial magnetic stimulation (TMS), here we tested the hypothesis that the observers' motor cortex implements extremely fast suppression of motor readiness when seeing emotional bodies – and fearful body expressions in particular. Participants observed pictures of body postures and categorized them as happy, fearful or neutral while receiving TMS over the right or the left motor cortex at 100-125 ms after picture onset. In three different sessions, we assessed corticospinal excitability, short intracortical inhibition (SICI) and intracortical facilitation (ICF). Independently of the stimulated hemisphere and the time of stimulation, watching fearful bodies suppressed ICF relative to happy and neutral body expressions. Moreover, happy expressions reduced ICF relative to neutral actions. No changes in corticospinal excitability or short intracortical inhibition were found during the task. These findings show extremely rapid bilateral modulation of the motor cortices when seeing emotional bodies, with stronger suppression of motor readiness when seeing fearful bodies. Our results provide neurophysiological support for the evolutionary notions that emotion perception is inherently linked to action systems and that fear-related cues require motor reactions to be urgently mobilized.

Introduction

Different lines of evidence suggest that threat-related signals are rapidly and efficiently processed in the central nervous system (LeDoux, 1996; Öhman and Mineka, 2001; Adolphs and Tranel, 2003) and that attention tends to be prioritized towards threatening stimuli (Fox et al., 2000; Vuilleumier, 2002). Fearful body language is a salient emotional signal, easily observable from a distance and alerting the observer about the presence of a potential threat (de Gelder et al., 2004; Tamietto et al., 2007). Perceiving fearful expressions in others requires specific processing in an attempt to garner more information about the source of threat in the surrounding environment (Whalen et al., 1998). Indeed, behavioral studies have shown enhanced sensory acquisition (Lee et al., 2013), perceptual processing (Phelps et al., 2006) and attention (Davis and Whalen, 2001; Kret et al., 2013) when exposed to fearful expressions. Notably, electrophysiological studies have also reported a rapid bias in visual attention allocation with greater resources devoted to fearful expressions: they reported increased amplitude or shorter latencies of early (100-200 ms) occipito-temporal components of event-related potentials (ERPs) for fearful body (van Heijnsbergen et al., 2007; Jessen and Kotz, 2011) and face (Pourtois et al., 2005; Righart and de Gelder, 2006; Williams et al., 2006) expressions relative to emotionally positive and neutral expressions. Besides the requirement to increase sensory vigilance and allocate resources for the monitoring and detection of potential threats, the sight of fear expressions may affect the motor system. Animal research has shown that initial reactions to sudden stimuli - and potential threats, in particular - involve reducing motor output i.e., implementing freezing behavior or orienting immobility while monitoring the source of danger and similar phenomena have been suggested in humans (Fanselow, 1994; Frijda, 2010; Lang and Bradley, 2010; Hagens et al., 2014). In keeping with this notion, transcranial magnetic stimulation (TMS) studies have documented fast reductions of motor excitability following salient and potentially noxious stimuli like strong, unexpected or rapidly approaching auditory or visual stimuli (Cantello et al., 2000; Furubayashi et al., 2000; Makin et al., 2009; Serino et al., 2009; Avenanti et al., 2012a), painful

stimuli on self (Farina et al., 2001, 2003; Urban et al., 2004) or observed in others (Avenanti et al., 2006, 2009a, 2009b). Moreover, a reduction of activity in M1 has been reported during periods in which participants expect to receive painful stimuli relative to conditions without pain expectation (Butler et al., 2007). Remarkably, imaging studies have shown that observing fearful expressions in others activates subcortical (e.g., amygdala, superior colliculus) and cortical regions (e.g., cingulate cortex, supplementary motor area) known to be involved in emotional processing and motor control (Vuilleumier et al., 2001; Hadjikhani and de Gelder, 2003; de Gelder et al., 2004, 2010; Grèzes et al., 2007; Thielscher and Pessoa, 2007; Vuilleumier and Pourtois, 2007; Kret et al., 2011b). However, the nature of such activations is ambiguous because imaging can hardly distinguish between motor inhibition (which would support freezing-like, body immobilizations) and excitation (which would reflect increased action readiness) and cannot precisely determine when in time these modulations occur. On the other hand, the high temporal resolution of TMS and its possibility to distinguish between excitatory and inhibitory activity in motor areas allows effective exploration of motor dynamics during emotion perception. The goal of this study is to test whether exposure to fearful body postures rapidly reduces excitability of the observer's M1. To this aim, we used TMS over M1 to non-invasively assess motor excitability during perception of emotional body expressions. In previous studies, we started to investigate the dynamics of the human motor system by investigating corticospinal excitability in the observers' left and right M1 during an emotional recognition task (Borgomaneri et al., 2012, 2014). We recorded motor-evoked potentials (MEPs) at 150 and 300 ms after the presentation of fearful, happy and neutral expressions in which the body posture was presented in isolation, with no contextual or facial cues. In the earlier time window (150 ms) we found a weak increase in corticospinal excitability for fearful body postures in the left hemisphere, suggesting action preparation activity in the motor representation of the dominant hand (see also Schutter et al., 2008; Borgomaneri et al., 2013 for similar findings using fearful facial expressions and negative natural complex scenes). Remarkably, at the same timing, we found a consistent reduction of corticospinal excitability in the right hemisphere for both fearful and happy body

postures (Borgomaneri et al., 2014). Such motor response appeared also causally related to visual recognition of body postures: indeed, right M1 (but not left M1) stimulation that was used for recording MEPs at 150 ms also decreased the ability to recognize the observed body postures; moreover, the decrease in performance correlated with the reduction of corticospinal excitability, suggesting a close link between motor suppression in the right M1 and perceptual processing of body postures. At the later stage (300 ms), greater MEP amplitudes were measured for fearful, happy and emotionally neutral dynamic body postures relative to emotionally neutral static body postures. This later increase in motor excitability was similar in the two hemispheres. Moreover it was comparable for the three dynamic postures (see also Borgomaneri et al., 2012) and likely reflected motor resonance, i.e., the embodiment of the actor's movements into one's own motor system (Gallese et al., 2004; Oberman et al., 2007; Bastiaansen et al., 2009; Keysers and Gazzola, 2009; Niedenthal et al., 2010; Rizzolatti and Sinigaglia, 2010; Gallese and Sinigaglia, 2011) that is typically detected in similar time windows (200-400 ms) according to TMS and MEG evidence (Nishitani et al., 2004; Barchiesi and Cattaneo, 2013; Cavallo et al., 2014; Naish et al., 2014). Consistently with this interpretation, magnitude of later motor facilitation also correlated with dispositional cognitive empathy scores (Borgomaneri et al., 2014) as previously shown in a number of studies investigating motor resonance (e.g., Gazzola et al., 2006; Avenanti et al., 2009b, 2010; Minio-Paluello et al., 2009; Lepage et al., 2010). In contrast to what reported at 150 ms, the stimulation of right or left M1 that was employed to record MEPs at 300 ms, did not affect visual recognition of body postures. These findings indicated that at this stage of processing (300 ms), neural activity reflecting the motor resonance was stronger in highly empathetic participants who tend to take the psychological perspective of others in daily life but was not critical for visual recognition of emotional body postures. These results revealed two distinct functional stages of motor cortex involvement during perception of emotional body language: an initial stage (~150 ms) reflecting increased motor readiness in the left hemisphere and perceptual-related mechanisms in the right hemisphere; and a later stage (~300 ms) in which the motor cortices bilaterally implement motor resonance, which may

reflect more sophisticated and empathy-related reading of the observed body expression “from the inside” (Gazzola et al., 2006; Rizzolatti and Sinigaglia, 2010; Avenanti and Urgesi, 2011; Avenanti et al., 2013b). In the present study, we sought to further investigate motor responses to emotional bodies in the right and left hemispheres and to test the possible existence of an earlier additional stage of M1 involvement during perception of emotional bodies. Our previous studies suggest comparable motor reactivity for happy and fearful body expressions when motor excitability is tested in the 150-300 ms temporal window (Borgomaneri et al., 2012, 2014). Here, based on the evolutionary contentions that i) emotional and in particular threat-related stimuli should evoke extremely rapid motor reactions (Lang et al., 2000; Carretié et al., 2001b; Öhman and Mineka, 2001; Frijda, 2009; Costa et al., 2013); and that ii) fear-related signals might be particularly adept to reducing motor readiness (e.g., as during orienting immobility and freezing responses) in order to monitor the source of danger in the environment (Fanselow, 1994; Whalen et al., 1998; Frijda, 2010; Lang and Bradley, 2010; Hagenaaers et al., 2014), we tested the hypothesis that a transient suppression of motor reactivity can be detected for fearful bodies if motor excitability is assessed very early in timing. To this aim, we investigated motor excitability in the right and left M1 within the same temporal window in which fearful faces and bodies are known to induce the earliest modulation of occipito-temporal cortices (i.e., at 100-125 ms corresponding to the timing of the P1 component; Pourtois et al., 2005; Righart and de Gelder, 2006; Williams et al., 2006; van Heijnsbergen et al., 2007; Vuilleumier and Pourtois, 2007). Similarly to previous research on emotion perception, we used single-pulse TMS over M1 in order to record MEPs from the hand muscles and thus assess how visual perception affects the functional state of the observers’ corticospinal system. However, it should be noted that MEP amplitude obtained with single-pulse TMS reflects the net effect of excitatory and inhibitory inputs to the corticospinal pathway, allowing to assess cortical but also spinal excitability (Di Lazzaro et al., 2001). To directly assess modulations of excitability of intracortical circuitry within the right and left M1, in the present study, we used for the first time in emotion perception research the paired-pulse protocol, which allows to give pairs of TMS stimuli through a single coil placed over the target M1.

In paired-pulse TMS, a conditioning stimulus (CS) below the threshold intensity needed to elicit an MEP is followed at short interstimulus intervals (ISIs) by a suprathreshold test stimulus (TS). At ISIs of 1–5 ms, the CS results in MEP inhibition (i.e., so called “*short intracortical inhibition*”, SICI), while longer ISIs of 7–20 ms produce MEP facilitation (“*intracortical facilitation*”, ICF). This modulation of MEP size takes place at the cortical level and is thought to reflect the activation of separate populations of inhibitory and excitatory cortical interneurons without affecting spinal circuits (Kujirai et al., 1993). In particular it is held that SICI and ICF mainly reflect the activation of low threshold inhibitory interneurons mediated by gamma-aminobutyric acid (GABA) (Ziemann et al., 1996a; Di Lazzaro et al., 2000; Ilic et al., 2002) and glutamatergic interneurons (Nakamura et al., 1997; Ziemann, 2003), respectively. Therefore, paired-pulse TMS provides reliable indices of motor cortical activations. Here, taking advantage of these paired-pulse paradigms, we aimed to further investigate whether the excitatory or inhibitory intracortical neural circuits within the right and left M1 are modulated during observation of emotional body expressions. By comparing neurophysiological indices of intracortical and corticospinal excitability, we tested whether at early timing (100-125 ms) the sight of emotional bodies affected the observers’ M1, descending corticospinal pathways or both. This allowed to demonstrate that before the perceptual- and action-related processing that are implemented at 150 and 300 ms (see Borgomaneri et al., 2012, 2014), the motor system in both hemispheres implements fast suppression of motor reactions to emotional bodies with stronger suppression for fearful body expressions.

Methods

Participants

Twenty-eight healthy subjects took part in the study. Fourteen participants (6 men, mean age \pm S.D.: 22.8 y \pm 2.6) were tested in a first experiment in which the right M1 was stimulated (Exp1M1right),

whereas the remaining 14 participants (7 men, mean age \pm S.D.: 23.3 y \pm 2.6) were tested in a second experiment in which the left M1 was stimulated (Exp2M1left). All participants were right-handed according to a standard handedness inventory (Oldfield, 1971) and free from any contraindication to TMS (Rossi et al., 2009). They gave their written informed consent to take part in the study which was approved by the Department of Psychology ethics committee and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

Visual stimuli

Different types of pictures were presented on a 19-inch screen located 80 cm away from the participants. Forty-five pictures were selected from a validated database (Borgomaneri et al., 2012, 2014). Pictures depicted four different actors in emotional and neutral postures (Figure 1a). To focus specifically on body-related information, the face was blanked out in all pictures. Stimuli included pictures of emotionally positive (happy) and negative (fearful) movements and neutral movements (i.e., actions with implied movement comparable to emotional body expressions but with no emotional meaning).

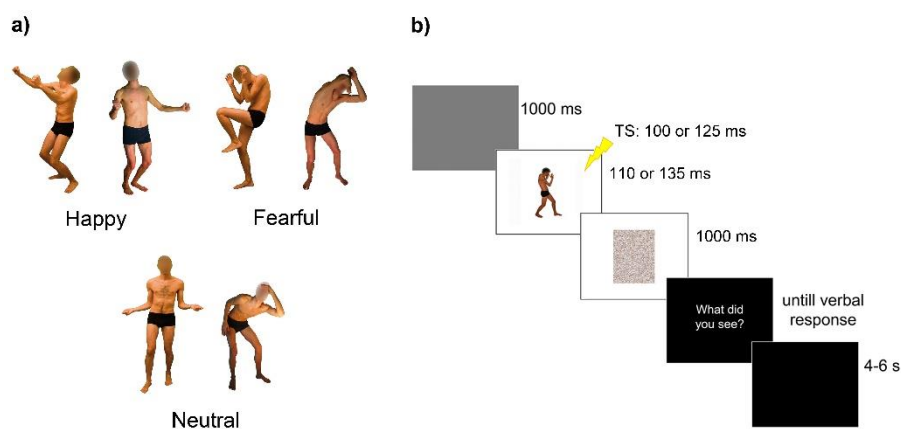


Fig. 1a Examples of visual body stimuli. **b** Trial sequence.

During the recording of neutral movements, instructions to the actors specified the action to be performed (e.g., jump rope). For emotional expressions, 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 at the actor). Stimuli were selected from an initial sample of about 1000 images based on two pilot studies in which emotional ratings and emotion recognition data were collected, resulting in a final selection of 15 fearful body expressions, 15 happy body expressions and 15 neutral movements that were well recognized as prototypical representations of the different expressions (see Borgomaneri et al., 2012 for details). All the emotional and neutral movement stimuli represented a whole-body movement with a clear involvement of upper-limbs (implied motion stimuli). In none of the stimuli did the model interact with objects or other individuals. To rule out that changes in right M1 excitability were due to a different amount of implied motion of the models' left or right hands, mirror-reflected copies of the selected stimuli were also created. Within each experiment, half the participants were tested with the original version of the stimuli, and the remaining half were tested with mirror-reflected copies.

Transcranial magnetic stimulation and electromyography recording

Both Exp1M1right and Exp2M1left started with the electrode montage, detection of optimal scalp position and measurement of resting motor threshold. To explore motor excitability, MEPs induced by TMS of the right and left M1 were recorded from the left and right first dorsal interosseus (FDI) muscle, respectively (contralateral to the stimulated hemisphere), using with a Biopac MP-35 (Biopac, U.S.A.) electromyograph. 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-eight coil connected to a Magstim Bistim2 stimulator (Magstim, Whitland, Dyfed, U.K.) was placed

over the target M1. The intersection of the coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline. In this way, the current induced in the neural tissue was directed approximately perpendicular to the line of the central sulcus, optimal for trans-synaptic activation of the corticospinal pathways (Brasil-Neto et al., 1992; Mills et al., 1992). Using a slightly suprathreshold stimulus intensity, the coil was moved over the target hemisphere to determine the optimal position from which maximal amplitude MEPs were elicited in the contralateral FDI muscle. The optimal position of the coil was then marked on the scalp with a pen to ensure correct coil placement throughout the experiment. The resting motor threshold (rMT) was defined as the minimal intensity of the stimulator output that produces MEPs with an amplitude of at least 50 μ V with 50% probability (using about 20 pulses) was assessed (Rossini et al., 1994). The absence of voluntary contraction was visually verified continuously throughout the experiment. When muscle tension was detected the experiment was briefly interrupted and the subject was invited to relax. In both experiments, MEPs were recorded in three sessions: Single pulse (SP), Short-interval intracortical inhibition (SICI) and Intracortical facilitation (ICF). During the SP session, intensity was set to evoke MEP with a peak-to-peak amplitude of ~1.0mV. During the paired-pulse TMS paradigm, SICI and ICF were measured using an established protocol (Kujirai et al., 1993; Ziemann et al., 1996b): the conditioning (CS) and test (TS) stimuli were given through the same coil. The intensity of the CS was 80% of the rMT, at which we confirmed that MEP could never be induced in the ipsilateral FDI. TS intensity was the same as that used in the SP session. We selected two interstimulus intervals (ISIs), 3 ms and 12 ms, which are typically used to investigate SICI and ICF circuits, respectively (Kujirai et al., 1993; Ziemann et al., 1996b).

Procedure and experimental design

The experiments were programmed using Matlab software to control pictures presentation and to trigger TMS pulses. In each experiment, MEPs were collected in three experimental sessions (SP,

SICI and ICF). Before and after these sessions, additional SP blocks were recorded and served as baseline: subjects held their eyes closed with the instruction to imagine watching a sunset at the beach (Fourkas et al., 2008; Borgomaneri et al., 2012) while receiving TMS over the right motor cortex (inter-pulse interval ~10 s). Ten trials were recorded for each of the baseline blocks. In the other three experimental sessions, subjects performed an emotion recognition task, in which they were presented with a picture and were asked to categorize it as happy, fearful or neutral body postures. Each emotional evaluation block included 90 trials (270 trials in total). In the emotion evaluation blocks, trial sequence was as follows: a gray screen (1 s duration) indicated the beginning of the trial and it was followed by the test picture projected at the center of the screen (Figure 1b). In half the trials, stimuli were presented for 110 ms and SP (or TS in the paired-pulse sessions) was delivered at 100 ms from stimulus onset. In the remaining trials, stimuli were presented for 135 ms and SP/TS was delivered at 125 ms from stimulus onset. Stimuli duration was randomly distributed in the two blocks and the session's order was counterbalanced across participants. 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 1 s. Then the question "What did you see?" appeared on the screen, and the subject provided a verbal response (forced choice). Possible choices were: happy, fear, neutral. An experimenter collected the answer by pressing a 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, a few seconds after the TMS pulse (Tidoni et al., 2013). After response, the screen appeared black for 4-6 s, ensuring an inter-pulse interval greater than 10 s and thereby avoiding changes in motor excitability due to TMS *per se* (Chen et al., 1997). To reduce the initial transient-state increase in motor excitability, before each block two SP (or two paired-pulses) were delivered over M1 (inter-pulse interval >10 s). 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 a

5 point 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 and behavioral data were processed off-line. Mean MEP amplitude values in each condition were measured peak-to-peak (in mV). MEPs associated to incorrect answers (less than 10% in both experiments) were discarded from the analysis. Since background EMG is known to affect motor excitability (Devanne et al., 1997), MEPs with preceding background EMG deviating from the mean by more than 2 S.D., were removed from further analysis (less than 6% in both experiments).

In a first analysis, MEPs recorded in the SP, ICF and SICI sessions were expressed relative to the baseline (% of the average of the two baseline blocks) and analyzed by means of a mixed-model four-way ANOVA with Experiment (2 levels: Exp1M1right and Exp2M1left) as between-subjects factor and Session (3 levels: SP, ICF and SICI), Time (2 levels: 100 and 125 ms) and Movement type (3 levels: happy, fearful and neutral) as within-subjects factors. Moreover, to quantify ICF and SICI effects, we expressed MEPs in the paired-pulse sessions relative to the SP session (to estimate the effects of subthreshold CS on the MEP elicited by the suprathreshold TS): for each experimental condition we calculated the ratio of the mean conditioned MEP over the mean unconditioned test MEP (Kujirai et al., 1993; Ziemann et al., 1996b). These data were analyzed by means of an Experiment x Session x Time x Movement type mixed-model ANOVA as the previous analysis but the factor Sessions had only 2 levels (ICF and SICI). Mean VAS ratings for arousal, valence and implied movement were analysed by means a two-way mixed-model ANOVA with Experiment (2 levels: Exp1M1right and Exp2M1left) as between subjects factor and Movement type as within-subjects factor (3 levels: happy, fearful and neutral). Accuracy in the emotion recognition task was analyzed by means of a two-way mixed-model ANOVA with Experiment (2 levels: Exp1M1right

and Exp2M1left) as between-subject factor and Session as within-subjects factor (3 levels: SP, ICF and SICI). In all the ANOVAs, post-hoc comparisons were carried out by means of the Newman-Keuls test. Moreover, effect size indices for main effects and interactions were computed using partial η^2 , whereas repeated measure Cohen's d were computed for post-hoc comparisons (Cohen, 1977; Wolf, 1986).

Results

Subjective measures

Mean task accuracy in the three sessions was high in both experiments (Exp1M1right: SP mean accuracy \pm S.D.: 90.7% \pm 5.3%; SICI: 89.5% \pm 6.7% and ICF: 90.5% \pm 5.3%; Exp2M1left: SP mean accuracy \pm S.D.: 92.7% \pm 5.5%; SICI: 91.7% \pm 5.2% and ICF: 90.6% \pm 4.9%). The Experiment \times Session ANOVA carried out on accuracy data showed no main effects or interactions (all $F < 0.95$; $p > 0.39$), suggesting similar accuracy across the two experiments and three TMS sessions. The Experiment \times Movement type ANOVAs carried out on valence ratings (Table 1) showed the main effect of Movement type ($F_{2,52} = 296.91$; $p < 0.001$; $\eta^2 = 0.92$), but no main effect of Experiment or interaction (all $F < 2.82$, $p > 0.11$). Post-hoc analysis showed that valence ratings were lower for fearful movements relative to happy and neutral movements (all $p < 0.001$; $d > 3.05$); moreover, valence was greater for happy relative to neutral movements (all $p < 0.001$; $d = 2.35$). The Experiment \times Movement type ANOVAs carried out on arousal ratings (Table 1) showed the main effect of Movement type ($F_{2,52} = 57.34$; $p < 0.001$, $\eta^2 = 0.69$), but no main effect of Experiment or interaction (all $F < 1.63$, $p > 0.21$). Post-hoc analysis showed that arousal scores were greater for happy and fearful movements relative to neutral movements (all $p < 0.001$; $d > 1.67$). Moreover, arousal ratings were not significantly different between fearful and happy movements ($p = 0.33$). The Experiment \times Movement type ANOVAs carried

out on implied motion ratings (Table 1) did not show significant main effects or interactions (all $F < 2.87$; $p > 0.07$), suggesting that the three movement types contain similar amount of implied motion.

Exp1M1right

	HAPPY	NEUTRAL	FEARFUL
Arousal	3.41 ± 0.78	2.21 ± 0.56	3.36 ± 0.93
Valence	4.18 ± 0.68	2.87 ± 0.58	1.39 ± 0.39
Perceived motion	3.32 ± 0.66	3.41 ± 0.59	3.07 ± 0.67

Exp2M1left

	HAPPY	NEUTRAL	FEARFUL
Arousal	3.57 ± 0.55	2.27 ± 0.55	3.90 ± 0.60
Valence	4.37 ± 0.33	3.15 ± 0.19	1.39 ± 0.19
Perceived motion	3.44 ± 0.47	3.36 ± 0.55	3.17 ± 0.66

Table 1 Mean \pm standard deviation subjective evaluations (arousal, valence and perceived implied motion) of stimuli used in first (Exp1M1right) and in the second experiment (Exp2M1left).

Neurophysiological data

MEPs recorded in the first (mean raw MEP amplitude \pm SD in Exp1M1right: $1.11 \text{ mV} \pm 0.26$; in Exp2M1left: $1.02 \text{ mV} \pm 0.22$) and last block of baseline (Exp1M1right: $1.15 \text{ mV} \pm 0.43$; Exp2M1left: $1.09 \text{ mV} \pm 0.38$) were entered in an Experiment \times Time ANOVA that showed no main effect or interactions (all $F < 1$, $p > 0.45$), confirming that the experiment did not alter participants' corticospinal excitability (Chen et al., 1997). In a first analysis, MEP collected in the various sessions and

experimental conditions were expressed relative to the average of the two baseline blocks. The Experiment x Session x Time x Movement type ANOVA on MEP amplitudes (% of baseline) showed a main effect of Session ($F_{2,52} = 51.59$; $p < 0.0001$; $\eta^2 = 0.66$) accounted for by the lower MEP amplitudes recorded in the SICI ($41\% \pm 23$) relative to the SP ($118\% \pm 37$; $p < 0.001$; $d = 1.99$) and ICF sessions ($195\% \pm 102$; $p < 0.001$; $d = 1.71$) and the greater amplitudes recorded in the ICF relative to the SP session ($p = 0.001$; $d = 0.75$). Critically, a significant Session x Movement type interaction was found ($F_{4,104} = 3.51$; $p = 0.01$; $\eta^2 = 0.12$; Figure 2). Post-hoc analysis showed that in the ICF session, MEP were lower for fearful bodies ($188\% \pm 90$) than for happy ($198\% \pm 116$; $p = 0.003$; $d = 0.30$) and neutral bodies ($200\% \pm 100$, $p = 0.002$; $d = 0.64$) which in turn did not significantly differ from one another ($p = 0.63$). No significant modulations were found in either the SP or SICI session (all $p > 0.44$). The Session x Movement type interaction was not qualified by a triple or quadruple interaction involving the factor Time (all $F < 1.01$; $p > 0.41$), suggesting that in the ICF session, MEP recorded at 100 and 125 ms were similarly reduced in the fearful body condition. Similarly, no interaction with factor Experiment was found to be significant (all $F < 2.48$; $p > 0.094$), suggesting that the reduction in ICF was similar in both motor cortices. No other significant effects were found in the ANOVA (all $F < 3.03$, $p > 0.09$). Results from the first analysis on MEP amplitudes (% of baseline) confirm the robustness of the paired-pulse protocol observed in both experiments, with lower MEP when CS preceded the TS by 3 ms and larger MEP when the CS preceded the TS by 12 ms (Kujirai et al., 1993; Ziemann et al., 1996b). Moreover, they show that emotional bodies significantly modulated MEP size in the ICF but not in the SP or SICI sessions.

It should be noted that the index used in the first analysis (MEP amplitude relative to baseline) does not allow to rule out a possible contribution of spinal excitability in the observed motor modulations. Therefore to quantify SICI and ICF effects, a second Experiment x Session x Time x Movement type ANOVA was conducted on MEPs ratios computed for each condition separately (mean conditioned MEP relative to mean unconditioned test MEP) (Kujirai et al., 1993; Ziemann et al., 1996b).

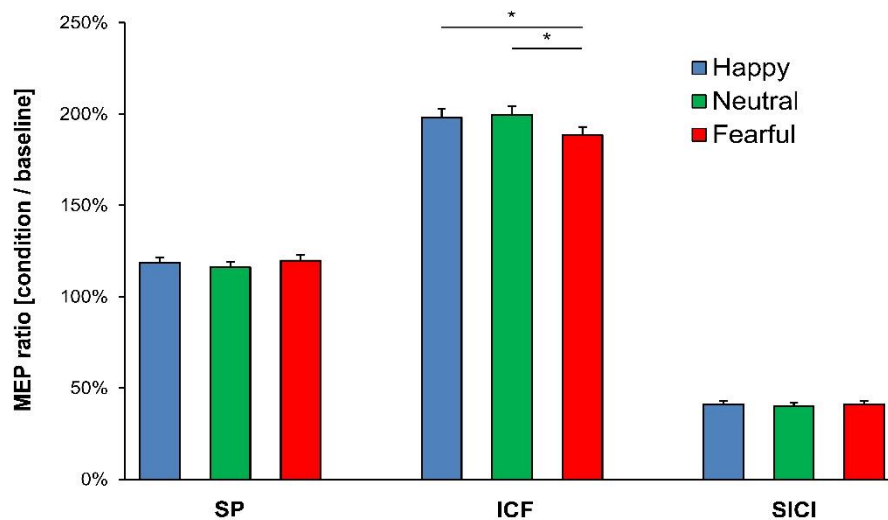


Fig. 2 Neurophysiological modulations during the emotion recognition task. MEP amplitude ratio (condition / baseline) during perception of happy, neutral and fearful body postures during single pulse (SP), intracortical facilitation (ICF) and short intracortical facilitation (SICI) sessions. Data show the interaction Session x Movement Type (average of the two experiments, Exp1M1right and Exp2M1left and time points, 100 ms and 125 ms). Error bars indicate s.e.m. Asterisks (*) denote significant comparisons ($p < 0.05$).

This second analysis revealed a main effect of Session ($F_{1,26} = 68.13$; $p < 0.0001$; $\eta^2 = 0.72$; larger MEPs in the ICF relative to the SICI session: $182\% \pm 112$ vs $0.38\% \pm 0.27$) and non-significant main effect of Movement type ($F_{2,52} = 2.48$, $p = 0.09$) and, importantly, a significant Session x Movement type interaction ($F_{2,52} = 4.70$; $p = 0.01$; $\eta^2 = 0.15$, Figure 3). The interaction was due to the lower ICF in the fearful body condition ($173\% \pm 101$) relative to the happy ($182\% \pm 111$; $p = 0.03$; $d = 0.32$) and neutral body conditions ($191\% \pm 128$; $p < 0.001$; $d = 0.48$); moreover, ICF was lower in the happy than in the neutral body condition ($p = 0.03$; $d = 0.24$). No significant modulation of the SICI index was found ($p > 0.79$). The ANOVA also showed non-significant trends for the main effect of Experiment and the Experiment x Session interaction (all $F < 3.85$, $p > 0.06$), suggesting that the gain in motor excitability in the ICF session tended to be larger in Exp2M1left than Exp1M1right. However, the

factor Experiment did not interact with other factors (all $F < 0.91$, $p > 0.41$), suggesting that the reduction of ICF for fearful body expressions was similar in Exp1M1right and Exp2M1left. No other main effects or interactions were significant in the ANOVA ($F < 1.28$, $p > 0.29$).

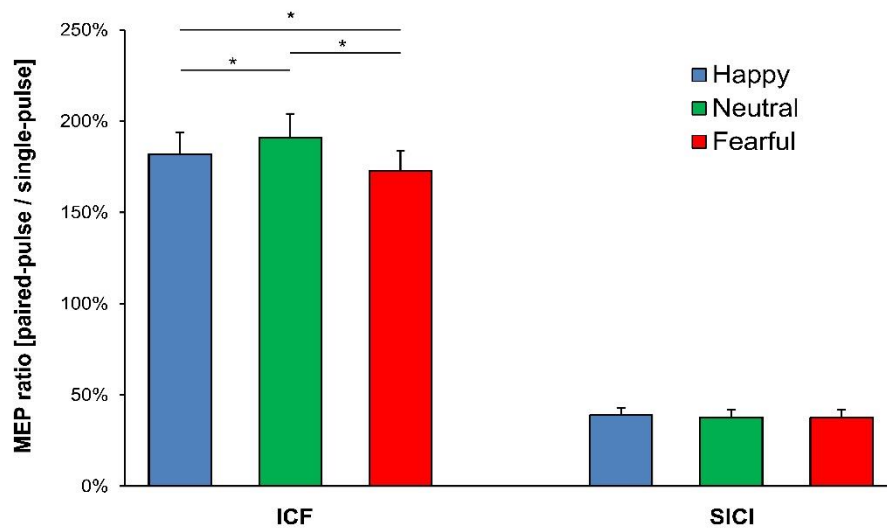


Fig. 3 Cortical motor modulations during the emotion recognition task. MEP amplitude ratio (paired-pulse / single-pulse) during perception of happy, neutral and fearful body postures during intracortical facilitation (ICF) and short intracortical facilitation (SICI) sessions. Data show the interaction Session x Movement type (average of the two experiments, Exp1M1right and Exp2M1left and time points, 100 ms and 125 ms). Error bars indicate s.e.m. Asterisks (*) denote significant comparisons ($p < 0.05$).

Discussion

Emotional body language represents a powerful vehicle of interpersonal communication (Darwin, 1872) and it is widely assumed that processing emotional language can prime the body for action (Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009). However, little is known about how the sight of emotional bodies affects the observers' M1. Using the high-temporal resolution of TMS, here, we tested the hypothesis that seeing emotional body expressions – and fearful expressions in particular

– triggers very early reduction of excitability in the observers' motor system. We used single-pulse TMS to characterize the functional state of the corticospinal system, and, for the first time in emotion perception research, the paired-pulse protocol to investigate the excitability of intracortical facilitatory (ICF) and inhibitory (SICI) circuits in the right and left M1. We tested M1 excitability in a time window (100-125 ms), corresponding to the latency of the P1, i.e., the earliest cortical component of the ERPs that is modulated by emotional expressions (Pourtois et al., 2005; Righart and de Gelder, 2006; Williams et al., 2006; van Heijnsbergen et al., 2007; Vuilleumier and Pourtois, 2007). Results supported our initial prediction and allowed to characterize a local neurophysiological mechanism in bilateral M1 involved in processing emotional bodies. In particular, we found that seeing fearful bodies reduced the magnitude of the ICF effect relative to watching happy or neutral bodies. Moreover, happy bodies reduced ICF relative to neutral bodies. No similar modulations were found for SICI or corticospinal excitability in the 100-125 ms range. These findings show a surprisingly early cortical motor mechanism during processing of emotional body postures. We propose this decrease in ICF reflects the cortical motor counterpart of a fast orienting response toward emotionally salient body postures that would manifest as a quick and transient reduction in motor readiness that may ultimately favor perception and subsequent motor reaction to the emotional cues.

The stronger motor suppression for fearful relative to happy body posture may be accounted for by the greater biological salience of the former relative to the later expression. Indeed, fearful expressions signal the presence of potential threats in the environment and these may require that neural resources are more strongly mobilized very early in time. More specifically, it is held that because the source of danger is not clearly signaled, detection of fearful expressions increase sensory vigilance in the observers and prompt monitoring of threats in the surrounding environment (Whalen et al., 1998; Davis and Whalen, 2001; Phelps et al., 2006; Kret et al., 2013; Lee et al., 2013). Thus, the suppression of excitatory activity in M1 may reflect a quick reduction in motor readiness that may favor such monitoring processes. Our findings are in keeping with animal research showing a

reduction in motor output when animals face novel or emotionally salient stimuli (and threatening stimuli in particular) (Fanselow, 1994; Whalen et al., 1998; Frijda, 2010; Lang and Bradley, 2010; Hagedaars et al., 2014). Moreover, they fit with studies in humans reporting that during passive observation the observers' body freezes upon the perception of aversive and arousing stimuli (Hillman et al., 2004; Azevedo et al., 2005; Facchinetti et al., 2006; Roelofs et al., 2010; Horslen and Carpenter, 2011; Stins et al., 2011; Eerland et al., 2012; Lelard et al., 2013). Remarkably, our study significantly expands these observations by providing a possible early cortical mechanism for the implementation of such motor reactions to emotional and, in particular, fear-related cues in humans. However, as we clarify below, our findings are suggestive of a transient reduction in motor readiness more than a complete and sustained body immobilization (Fanselow, 1994; Whalen et al., 1998; Frijda, 2010; Lang and Bradley, 2010; Hagedaars et al., 2014).

Local neurophysiological mechanisms supporting early motor suppression to emotional bodies

Our findings consisted in a modulation of ICF but not of corticospinal excitability or SICI. Single-pulse MEPs, SICI and ICF reflect at least partially distinct neurophysiological mechanisms (Liepert et al., 1998; Ziemann et al., 1998). Modulations of corticospinal excitability as measured by means of single-pulse MEPs reflect the net effect of excitatory and inhibitory inputs to the descending corticospinal pathway whereas SICI is thought to reflect a measure of intracortical GABA-ergic inhibition in M1 through GABA_A receptors. The ICF reflects a measure of intracortical excitation whose mechanisms are more complex, as it is thought to be influenced by glutamate-ergic facilitation through N-methyl-d-aspartate (NMDA) receptors (Ziemann et al., 1998) but also GABA-ergic inhibition through GABA_A receptors (Tandonnet et al., 2010). Moreover, ICF results from the recruitment of local M1 circuits related to the activation of long-range connections originating from remote areas (Ziemann et al., 1998; Ziemann, 2004). Hence, our data indicate that emotional bodies induce a fast modulation of cortical motor excitability in the two hemispheres, with a comparatively

stronger reduction of intracortical excitatory activity when perceiving fearful bodies and a weaker reduction for happy bodies. The fact that fearful and happy postures modulated the ICF but not the SICI suggests that processing of emotional bodies is mainly associated to a reduction in the input to excitatory glutamatergic interneuronal networks in M1 originating from interconnected regions, while it does not conspicuously modulate GABAergic cortical circuits. Similar changes in ICF in the absence of SICI modulations have been reported immediately after the administration of painful stimuli and have been interpreted as reflecting a role of intracortical glutamatergic networks in limiting the execution of body movements in the acute phase of pain (Schabrun and Hodges, 2012). However, differently from what we have found here, painful stimulations induced sustained suppression of ICF that was accompanied by a reduction in corticospinal excitability and also affected SICI at later timing (Schabrun and Hodges, 2012), thus indicating a massive and prolonged reduction of motor output when processing pain. In contrast, the motor modulation we report here: i) is not associated with changes in SICI and corticospinal excitability; and ii) is likely transient. The fact that emotional bodies modulated ICF, but not corticospinal excitability, suggests that the suppressive motor response we detected in our study occurs at the cortical level and does not immediately influence descending pathways. These features support the idea that ICF modulation reflects a reduction in the propensity to move the body i.e., a reduction in motor readiness while processing visual stimuli, more than a complete motor inhibition, which might be supported by additional modulation of GABAergic cortical circuits and corticospinal excitability (Reis et al., 2008; Stinear et al., 2009). Secondly, it should be considered that while the present study shows similar ICF suppression at the two tested timing (100 and 125 ms) and in the two hemispheres, previous research suggests that these fast motor responses might be transient as very different modulations are observed when motor excitability is tested at 150 ms and 300 ms after the presentation of emotional bodies (Borgomaneri et al., 2012, 2014; see below). The transient feature of the reduction in motor activity we detected in our study suggests it may favor early perceptual processing (e.g., threat monitoring)

without counteracting subsequent implementation of adaptive motor responses (e.g., fight/flight reactions).

Possible networks supporting early motor suppressions to emotional bodies

While our study indicates that visual processing of emotional bodies transiently reduces the input to excitatory glutamatergic interneuronal networks in M1 originating from interconnected regions, we can only speculate about the specific pathway supporting such early motor suppression. Studies have suggested that visual processing of affective stimuli could influence motor output via subcortical routes bypassing the cortex (Morris et al., 1999; Liddell et al., 2005; Tamietto et al., 2009; Tamietto and de Gelder, 2010; de Gelder et al., 2011; Filmer and Monsell, 2013): imaging evidence indicates that perception of emotional bodies activates subcortical structures (i.e., pulvinar, caudate nucleus and amygdala; van de Riet et al., 2009; de Gelder et al., 2010) even in cortically blind patients with striate cortex damage (Van den Stock et al., 2011), suggesting that subcortical structures receive an input from the retina that bypass the damaged visual cortex. Notably these structures also possess upstream projections influencing not only the visual system (Pourtois et al., 2013) but also M1 (Tamietto et al., 2012; Grèzes et al., 2014) and may thus have a role in influencing ICF when processing emotional body postures. On the other hand, ICF modulation may occur through a fast activation of a cortical route initially including regions involved in visual processing (e.g., occipito-temporal areas), lateral parieto-premotor circuits involved in action execution (de Gelder et al., 2004, 2010; de Gelder, 2006) and mesial regions of the frontal cortex that are involved in emotional processing and are densely connected to M1 via the supplementary motor area (SMA) (Morecraft et al., 1993; Cavada et al., 2000; Oliveri et al., 2003). In particular, a possible key involvement in the suppressive response to fearful (and happy) bodies could be played by the inferior frontal cortex (IFC, which includes the inferior frontal gyrus and the ventral premotor cortex; Avenanti et al., 2013a, 2013b; Urgesi et al., 2014). Monkey studies suggest this region implements fast flicking reactions to

unpleasant stimuli (Graziano et al., 2002; Cooke and Graziano, 2004; Gharbawie et al., 2011). Moreover, IFC is critically involved in inhibiting motor behavior (Chambers et al., 2007; Aron et al., 2014) and provides inhibitory input to M1 either directly or via SMA (Davare et al., 2009; Cattaneo and Barchiesi, 2012; Zandbelt et al., 2013). Moreover, IFC suppression appears to disrupt inhibitory responses to salient auditory stimuli presented close to the body (Avenanti et al., 2012), suggesting a possible role of IFC not only in voluntary outright action stopping and inhibitory control (Aron et al., 2014), but also in driving automatic inhibitory reactions. Future studies are needed to directly test the role of IFC in reacting to emotional body cues.

Motor dynamics during perception of emotional bodies

The early timing of the physiological modulation identified in our study is in keeping with the evidence that not only fearful facial expressions (Pourtois et al., 2005; Righart and de Gelder, 2006; Williams et al., 2006; Vuilleumier and Pourtois, 2007) but also fearful body expressions affect the ERP response in the earliest stages of visual perception, i.e., the P1 and N1 components (van Heijnsbergen et al., 2007; Jessen and Kotz, 2011). However, these ERP studies did not include positive emotional expressions and thus it unclear whether early ERP modulation for fearful bodies merely reflected an arousal response. Our data significantly expand ERP evidence by showing that in the very same time window of the P1 component, clear evidence of a ‘negative bias’ for fearful bodies can be found in cortical motor areas, with a stronger response for fearful than for happy or neutral expressions and a weaker response for happy expressions. Notably, in our study, fearful and happy expressions were matched for arousal. Moreover, fearful, happy and neutral conditions did not differ for implied motion (i.e., the quantity of movement perceived in the body posture), suggesting that these factors did not influence our results. However, some limitations of our design should be considered. Our stimuli depicted only male actors and the relatively small sample size prevented reliable analysis of sex-dependent effects. Moreover, we used a limited number of emotional

expressions and therefore it is unclear whether the reduction of ICF can be detected with other expressions. Therefore, further studies are needed to test motor excitability using larger number of stimuli and participants. Nevertheless, our study indicates a clear reduction in bilateral cortical motor activity when seeing particular emotional body postures. Together with our previous studies on emotional body perception (Borgomaneri et al., 2012, 2014), the present experiments suggest a specific dynamics of neural activity in the motor system during perception of emotional bodies. A negative bias in the form of a cortical suppressive response is initially detected in bilateral M1 (but not in the corticospinal system) at about 100-125 ms post-stimulus onset and may reflect a fast and transient reduction in motor readiness, with stronger reduction for fearful relative to happy and neutral body expressions. In a second stage (150 ms) the sight of emotional bodies starts to influence the corticospinal system and it does so in a different way for the two hemispheres, with the left hemisphere being more involved in preparing a potential motor response when exposed to fearful expressions and the right hemisphere being critically involved in perceptual recognition of any body posture (Borgomaneri et al., 2014). Finally, at around 300 ms, the corticospinal motor system appears to implement motor resonance processes which appear to reflect a mapping of the observed body movements that *follows* the visual recognition of the body posture (Borgomaneri et al., 2012, 2014). Thus, our studies support a three-stage model of motor cortex engagement during processing of emotional body language. Overall, the specific dynamics we observed across these studies support the notions that emotional cues trigger motor-related activity in the brain and that potential threats require particularly quick motor reactions to secure the survival of the organism (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009).

General Discussion

Social animals have evolved a whole range of communicative abilities on which their well-being and survival in a society hinges. Foremost amongst the cognitive skills of social species is the ability to monitor each others' behavior and emotions and to adapt continuously to the social signals of others. Therefore, appropriately understanding and reacting to the emotions of others appear to be fundamental abilities for survival.

Our motor system has a fundamental role in these processes: it is involved in internally simulate others' behaviors (motor resonance), probably in order to better understand them and to prepare the most opportune motor reaction.

However, to date, only few Transcranial Magnetic Stimulation (TMS) studies have investigated the effect of emotional stimuli observation on the observers' motor system activity (Baumgartner et al., 2007; Hajcak et al., 2007; Schutter et al., 2008; Coombes et al., 2009). These studies mainly tested the left motor cortex excitability relatively late in time (> 300 ms) specifically during emotional faces or complex scenes observation. Moreover, very few studies have tested the role of sensorimotor system on visual recognition of emotional signs (Adolphs et al., 2000; Pitcher et al., 2007; Balconi and Bortolotti, 2013). Therefore, it remains unclear whether observers' motor system, and the motor cortex (M1) in particular, is specifically modulated by the observation of others' emotion. In particular, to date, there were no TMS studies on M1 modulation during emotion observation, conveyed by body postures (i.e., when processing body expressions presented in isolation, with no additional contextual or facial cues).

In the present thesis, we employed TMS in order to test possible cortico (using paired-pulse TMS, ppTMS) and corticospinal (using single-pulse TMS, spTMS) modulations while subjects were requested to actively categorize emotional (positive and negative) or neutral complex scenes, or

emotional (happy and fear), neutral (depicting the mimic of an action) or static (at rest) human body postures presented in isolation. We also tested the different role of the left and the right primary motor cortices in facing such stimuli, using different times of stimulation, namely from 100 to 300 ms after the stimulus onset. Due to the high temporal resolution of the TMS and its ability to temporarily interfere with the activity of a specific area of the brain, we get a clear picture about the time course and the causal role of the motor system when faced with emotional and neutral stimuli.

1. Fast fear-related freezing reaction: a cortical origin.

Data from Chapter 4 revealed that the motor system of an observer is recruited very fast (100-125 ms) when facing emotional potentially dangerous stimuli. We presented emotional (happy and fear), dynamic neutral (depicting the mimic of an action) or static (at rest) body postures to test the hypothesis that a negative bias can be detected for fearful bodies if motor excitability is assessed very early in time. We assessed motor excitability in both left and right M1 using single-pulse TMS to characterize the functional state of the corticospinal system, and, for the first time in emotion perception research, the paired-pulse protocol to investigate the excitability of intracortical facilitatory (ICF) and inhibitory (SICI) motor circuits. We found that, regardless of the stimulated hemisphere, seeing fearful bodies strongly reduced the magnitude of the ICF relative to watching happy or neutral bodies. Moreover, observing happy bodies reduce ICF relative to neutral bodies. Our data indicate that emotional bodies induce a fast modulation of cortical motor excitability, with a stronger reduction of intracortical excitatory activity when perceiving fearful bodies. The fact that emotional bodies modulated ICF but not SICI suggests that processing of fearful bodies was mainly associated to a reduction in the input to excitatory glutamatergic interneuronal networks in M1 originating from interconnected regions. Moreover, the fact that emotional bodies modulated ICF, but not corticospinal excitability (no effect in the single pulse session), suggests that the negative bias we detected has a predominantly cortical origin. We propose this decrease in ICF reflects the cortical

motor counterpart of an orienting response toward emotionally salient body postures that would manifest as strong suppression of motor readiness when seeing fearful bodies. From our data, we can conclude that, only after 100-125 ms, our motor cortex, together with the visual system (Smith et al., 2003; Williams et al., 2004; Pourtois et al., 2005; van Heijnsbergen et al., 2007; Jessen and Kotz, 2011), is recruited during fearful body stimuli observation, supporting the notion that emotional cues drive action preparation in the brain and that potential threats require particularly quick motor reactions to secure the survival of the organism (Lang et al., 2000; Öhman and Mineka, 2001; Carretié et al., 2009; Frijda, 2009). Moreover, we observed that this effect has a cortical origin, which suggest that probably, at this time, our motor system is not already sending signals to the motor output in order to prepare an appropriate motor reaction but, probably, because what we found is an inhibition of facilitatory signals, M1 is planning to prevent potentially dangerous reaction toward harmful stimuli of the contralateral hand.

2. Different motor reactions toward emotionally salient stimuli in the two hemispheres: motor orienting *before* motor simulation.

Immediately after (at 150 ms) the aforementioned fear-related motor reaction, we observed (Chapter 3), using single-pulse TMS, that the right motor cortex continued to show an inhibitory modulation but, this time, the effect we record was related to corticospinal modulations and was independent of the emotional meaning of the stimuli (similar inhibitory reaction during observation of fearful and happy body postures).

We interpreted this inhibitory motor response as the sight of a rapid orienting reaction toward emotionally relevant stimuli. Additionally, since applying spTMS at variable times during task execution it is possible to investigate with a temporal resolution of a few tens of milliseconds (ms) at what exact time point neural activity at the stimulation site is critical for successful task performance

(chronometry of functional relevance), we also tested whether the TMS pulse interfered with recognition performance at any time or stimulation side. If early motor activity reflects neural processing necessary for perceiving body expressions, we might expect that right M1 stimulation at 150 ms would impair task performance. We found that, at 150 ms, TMS over right M1 interfered with accuracy in the emotion recognition task. No similar effects were found with sham or left M1 stimulation. Greater TMS interference on task accuracy correlated with reduced changes in motor excitability, suggesting a link between neural activity reflecting early orienting and visual recognition of body expressions. This effect provides a strong evidence of the fact that the corticospinal orienting reaction we found in the right motor cortex at 150 ms is necessary to the perception of the observed body stimuli.

In contrast, recording at the same time (150 ms) from the left motor cortex, we found a slightly activation of the corticospinal system (bigger MEPs) during fearful stimuli observation relative to happy and neutral body postures observation. This activation seems to reflect the preparation of a motor reaction specifically towards negative pictures (we found that it was stronger when facing negative complex scenes than isolated fearful body postures, Chapter 1). This effect was stronger when subjects were asked to observe violent, disgusting or fearful complex dynamic scenes relative to fearful human body postures, probably because these latter stimuli are not threatening enough to elicit such facilitatory motor reaction. This facilitatory effect seems to reflect the sign of motor preparation towards negative stimuli and it is independent of perceptual-related processes (no accuracy drop when TMS was applied over left M1).

Finally, we explored the relation between changes in motor excitability (found in Chapter 1 and 3) and individual scores of dispositional empathy using the Interpersonal Reactivity Index (IRI) (Davis 1996) and we observed that either the early right hemisphere reduction of motor excitability for emotional bodies (Chapter 3) and the facilitatory effect we found from the left M1 when facing threatening stimuli (Chapter 1) were related to inter-individual differences in personal distress (PD)

but not to the other IRI subscales (Davis 1996), which reflect empathic dispositions. Taken together, these data suggest that anxiety-related traits influence the way in which social and emotional signals are processed in the brain (Kret et al., 2011a; Azevedo et al., 2013; Borgomaneri et al., 2013). In particular, the more participants showed disposition to feel personal distress in interpersonal contexts the bigger were the effects we found at 150 ms in both hemispheres: on the right side, more orienting response to emotional cues, on the left side, more motor preparation when facing threatening complex scenes.

3. Bilateral motor simulation for complex scenes and dynamic body postures

As mentioned in the *incipit* of this discussion, most of the existing single pulse TMS studies have typically reported that the left motor cortex excitability is sensitive to emotional processing. In particular, they found larger motor evoked potentials (MEPs) when participants were asked to observe pleasant and unpleasant stimuli (Baumgartner et al., 2007; Hajcak et al., 2007; Coombes et al., 2009). Authors interpreted this facilitatory effect as the sign of the preparation of a motor reaction towards arousing stimuli. However, in all these studies, motor excitability was tested in a very late time window (after 3-7 sec of the stimulus onset). If the role of the motor system is to program the most adaptive reaction towards emotionally relevant stimuli, then 3-7 seconds after the stimulus appearance can be probably too late for implementing a motor reaction and secure the survival of an organism. In Chapter 2 we replicated previous findings, discovering that the same facilitatory effect facing emotionally complex scenes could be detected, from the left motor cortex, already after 300 ms. However, embodied simulation theorists posit that, during observation of emotional stimuli, our motor system is involved, not to react, but rather to internally simulate (motor resonance) the unobservable emotional states of others by embodying their observable motor behavior (Carr et al., 2003; Leslie et al., 2004; Gallese, 2007; Oberman et al., 2007; Jabbi and Keysers, 2008; Bastiaansen et al., 2009; Niedenthal et al., 2010; Gallese and Sinigaglia, 2011). Most radical “motoric-centric”

versions of these theories contend that motor resonance occurs prior to the activity in emotion-related regions (thus very early in time) and is necessary for assigning emotional meaning to visual signals (thus they would play a causal role in visual perception) (e.g., Carr et al., 2003; Iacoboni, 2009).

In order to test whether such facilitatory effect reflected the sign of a motor reaction towards arousing stimuli or motor resonance process (more implied motion conveyed by emotional complex scenes relative to the static ones) we used human body postures in isolation.

Using human bodies, we investigated motor system modulations observing emotional dynamic, static (no emotion and no implied movement) but also neutral body postures, which contain implied movement without conveying emotions. Indeed, it is important to notice that in the pool of stimuli we previously used (the same used in previous TMS studies, IAPS database) most of the positive and negative scenes depicted humans involved in dynamic complex situations while most of the neutral stimuli depicted static objects or landscapes with no humans. Therefore using our set of human dynamic body postures, we tested whether this facilitation was present, not only for fearful and happy body expressions, but also for also observing dynamic neutral body postures. Our data suggested that the facilitatory effect we found in Chapter 1 (and reported in the other TMS studies) is probably reflecting the internal simulation of the implied motion conveyed by the dynamic stimuli (i.e., the facilitatory modulation was present also observing neutral bodies, which convey no emotional meaning) rather than their emotional content.

Additionally, we observed the very same motion-related facilitatory effect recording MEPs from both hemispheres. Lastly, since studies suggest that participants with a greater tendency to take the psychological perspective of another may show stronger resonant activations (Gazzola et al., 2006; Cheng et al., 2008; Avenanti et al., 2009b; Minio-Paluello et al., 2009; Martínez-Jauand et al., 2012; Schaefer et al., 2012) and different empathy traits may modulate neural activity during social perception (Singer et al., 2004; Lamm et al., 2007a, 2010; Borgomaneri et al., 2013; Melloni et al., 2013), we explored the relation between changes in motor excitability and individual scores of

dispositional empathy using the Interpersonal Reactivity Index (IRI) (Davis, 1996). We found that this later motor facilitation marginally correlated with participants' tendency to assume the psychological perspectives of others (Perspective Taking subscale) and reflected simulation of the body movement implied in the emotional and neutral dynamic stimuli.

We can conclude that, at 300 ms, the motor system is bilaterally involved in responding to the emotional pictures and that its activation is reflecting the motor simulation of the implied stimuli rather than the preparation of a motor reaction (as posit by the embodied simulation theory; Carr et al., 2003; Iacoboni, 2009). However, our results demonstrated that motor resonance take place *after* the emotional signals have been already perceived in the right motor cortex and therefore our data suggested that motor resonance is not necessary, at least during simple emotion recognition tasks. However, in this context, it is important to entertain the possibility that resonance processes necessary for perception could occur earlier in time (e.g., after 150 ms and before 300 ms) or in other anatomical locations (e.g., in premotor or parietal regions) without being immediately evident in M1 activity as measured by TMS (Jabbi and Keysers, 2008; Avenanti and Urgesi, 2011; Cattaneo et al., 2011; Tidoni et al., 2013; Urgesi et al., 2014). Thus, further studies are needed to test the chronometry and causal involvement of motor resonance in visual perception of emotional body language. Moreover, we found a relation between cognitive empathy and motor resonance. This may suggest that this later neural phenomenon – reflecting the embodiment of the observed body expression in the observers' motor system – is involved in more sophisticated empathy-related understanding that occurs after visual recognition and may provide the observer with a reading of the body expressions 'from the inside' (Rizzolatti and Sinigaglia, 2010).

Conclusions

Data in this thesis shed new light on the temporal relation between the motor processes hypothesized by simulative and non-simulative theories of emotion processing. On the one hand, embodied simulation theories have suggested that the motor system's involvement in inferring the emotional states of others may be "imitative" in nature (i.e., motor resonance) (Carr et al., 2003; Leslie et al., 2004) and related to perceptual processes (Oberman et al., 2007). On the other hand, other scholars have proposed a different account based on the well established notion that emotions may prime the human body for action (Ekman and Davidson, 1994; Izard, 1994; Frijda, 2009). According to this view, the perception of emotional bodies would trigger the preparation of emotionally appropriate motor reactions, which is supposed to be independent of perception-related processes (Tamietto et al., 2009; de Gelder et al., 2010). In this vein, changes in motor reactivity are not action simulation processes triggered by the sight of corresponding actions but potential motor reactions stemming from the fast processing of emotional features in visual and subcortical-cortical emotional circuits (Tamietto et al., 2009; de Gelder et al., 2010). Our studies demonstrate that motor resonance occurs in M1 at 300 ms *after* the signals discriminating between emotional and non-emotional bodies have been already processed (at 150 ms in right M1). Moreover, we found that the orienting reaction we found at 150 ms in the right M1 was positively correlated to body postures recognition accuracy and that it was *necessary* for body postures recognition. We can conclude that, at this level, motor resonance is not a prerequisite for body posture's perception.

Therefore, the processes hypothesized by the two theories are not mutually exclusive. Our data demonstrated that, as supposed by evolutionary theorists, fast reactions towards threat-related stimuli, were detectable in the motor system (Chapter 4), however we found that such fast reactions are not merely involved in planning motor reactions but they have a role in more cognitive processes, such as supporting visual recognition of the observed bodies (Chapter 3). On the other side, as supposed by embodied simulation theorists, our data showed motor resonance processes when subjects were

asked to observed dynamic body stimuli (Chapter 1, 2 and 3), however, in contrast to what supposed by most radical “motoric-centric” versions of these theories, our data showed that motor resonance processes are not necessary to extract the emotional meaning of such stimuli: first, before the occurrence of motor resonance processes at 300 ms, we found that right M1 showed different modulations from emotional relative to neutral body observation then, importantly, we demonstrated that selectively interfering with motor resonance processes does not disrupt recognition performance. Anyway, this later neural phenomenon may be involved in more sophisticated empathy-related understanding that occurs after visual recognition and may provide the observer with a reading of the body expressions ‘from the inside’ (Rizzolatti and Sinigaglia, 2010).

Future directions

Our data suggest that very fast (100-150 ms) motor reactions take place in both motor cortices when observers have to actively categorize emotionally salient stimuli. First, future studies are necessary to deeper investigate these fast motor reactions, since it is possible to speculate that these fast inhibitory responses can be even more organized than what we interpreted as a general negative bias. It is possible to speculate that, even in this early time window, the functional meaning of this consistent inhibitory response we found in muscles normally involved in approaching action (grasping), is preventing non-adaptive actions. To test this hypothesis, future studies will test whether different hand-arm muscles, typically involved in opposite valence-related actions (i.e., approach or avoidance movements) show different modulations relatively to the valence of the observed body. Particularly, it is possible to speculate that muscles, which are typically recruited during avoidance reactions, will show an inhibitory reaction only when happy positive, approach-induced stimuli were presented. Opposite results will be expected in the muscles typically involved in approach reactions, namely inhibition of motor activity only when fearful, negative, avoidance-induced stimuli were

presented. These results will shed a new light over the functional meaning of this inhibitory effect suggesting that not only the emotional meaning is processed very fast (150 ms) in a sensorimotor or even cortico-subcortical network, but also that, at approximately the same time window (or even before, 100-125 ms), the primary motor cortices have already organize adaptive reactions toward arousing relevant stimuli in the environment.

Samenvatting

Dieren die in sociaal verband leven (ook mensen) hebben ten behoeve van hun welzijn en overlevingskansen in de maatschappij in de loop van de evolutie een hele reeks communicatievaardigheden ontwikkeld. De belangrijkste cognitieve vaardigheid van een sociale soort is de kunst om elkaars gedrag en emoties te herkennen en zich voortdurend aan te kunnen passen aan de sociale signalen die anderen afgeven. Als het om overleven gaat lijkt het van fundamenteel belang te zijn om emoties van anderen te herkennen en daarop te reageren.

Neurowetenschappers hebben nog niet zo heel lang geleden een speciaal soort neuronen ontdekt, de zogenaamde spiegelneuronen, die als eigenschap hebben dat ze gaan vuren wanneer iemand een handeling uitvoert, maar ook wanneer iemand diezelfde handeling ziet uitvoeren door een ander. Dit heeft geleid tot het idee dat het begrijpen van andermans innerlijke gevoelens afhangt van inherente motorsimulatie, namelijk, de activatie van motorprogramma's die wij zelf zouden gebruiken om dezelfde handelingen te verrichten die we een ander zien uitvoeren. Zo zou bijvoorbeeld het zien van een vriend die een glas water vasthoudt en wil gaan drinken in degene die hier naar kijkt het motorprogramma opstarten om hetzelfde te gaan doen, een glas vasthouden. Aangezien het logisch is dat dorst de reden is voor de vriend om het glas te pakken, zal in de toeschouwer tevens de neurale representatie worden geactiveerd die de achterliggende gedachte bij deze handeling weergeeft.

Recentelijk is geopperd dat hersengebieden die betrokken zijn bij het verwerken van emoties ook betrokken zouden kunnen zijn bij simulatieprocessen: ze zouden een 'emotionele simulatie' kunnen zijn van de ervaringen van een ander, waarbij ze activatie vertonen, niet alleen wanneer we zelf positieve of negatieve emoties ervaren, maar ook wanneer we zien dat anderen ze ervaren. Zo roept het zien van een glimlach bij een ander vergelijkbare neurale hersenactiviteit op bij de toeschouwer, én de bijbehorende gezichtsuitdrukking. Deze motorrepresentatie, alsmede de hiermee gepaard gaande somatosensorische representatie, worden verondersteld betrokken te zijn bij het begrip van de betekenis van andermans gezichtsuitdrukking. Het zien van handbewegingen bij een ander activeert

dus de motorische representatie van die handeling bij de toeschouwer, het zien van een emotie bij de ander activeert de neurale representatie van die emotie.

De huidige sociale cognitiemodellen houden daarom rekening met het idee dat gebieden die betrokken zijn bij onze motoriek en onze emoties kunnen bijdragen aan hoe anderen onze acties en emoties ervaren (belichaamde simulatietheorie). Echter, een andere onderzoekslijn oppert dat het motorsysteem tijdens de verwerking van emoties voornamelijk betrokken is bij de reacties op emotionele *cues*, door de meest bijpassende motoriek voor te bereiden.

Dit tweede idee wordt ondersteund door diverse gedragsstudies, bijvoorbeeld studies waarbij deelnemers sneller een hendel van zich weg duwen als ze negatieve woorden aangeboden krijgen en sneller een hendel naar zich toe trekken bij positieve woorden, hetgeen laat zien dat het motorsysteem een rol speelt bij het voorbereiden van de geschiktste motorreactie, namelijk toenadering in geval van positieve stimuli en ontwijking in geval van negatieve stimuli. Deze laatste theorie zou er op wijzen dat het motorsysteem niet zozeer een rol speelt bij het begrijpen van een situatie, maar slechts wordt ingezet om op gepaste wijze te kunnen reageren op de waargenomen stimuli.

Met inachtneming van het (voor het overleven van de soort) fundamentele belang van het begrijpen en adequaat reageren op sociale signalen, proberen we in dit proefschrift, door middel van het peilen van het motorsysteem op verschillende momenten - terwijl we de acties en emoties van anderen waarnemen - te ontdekken of de twee bovengenoemde theorieën met elkaar in strijd zijn of dat de kloof tussen die twee overbrugd kan worden. Dit doen we door het motor systeem te onderzoeken op verschillende momenten in tijd terwijl iemand de acties en emoties van een derde aanschouwt.

Onze interacties in een sociale context voorzien ons van informatie omtrent andermans gevoelens en emoties, vooral via gezichtsuitdrukking en lichaamshouding. Desondanks concentreert het merendeel van de emotieperceptiestudies zich op hersenactiviteit gegenereerd door gezichtsuitdrukkingen. Het aspect lichaamshouding wordt doorgaans genegeerd. Naar gezichten kijken wordt gezien als een meer automatische en directe manier om de gevoelens en bedoelingen van anderen te achterhalen. Echter, gezichten en lichamen kunnen niet los van elkaar gezien worden, maar zijn nauw

verwante entiteiten, zozeer zelfs dat het herkennen van emoties via gezichtsuitdrukkingen belemmerd wordt door lichaamstaal die hier niet mee overeenstemt. Dit ondersteunt het idee dat we in het dagelijks leven onze informatie betrekken uit gezichtsuitdrukkingen én uit lichaamstaal.

Zo biedt het observeren van een lichaam de mogelijkheid om signalen op te vangen en emoties waar te nemen van een grotere afstand dan het observeren van een gezicht. Bovendien verschuift dit de aandacht weg van de identiteit van de persoon dat normaliter zou vertoond worden door het gezicht, wat voor snelle ontcijfering van uitdrukkingen niet steeds noodzakelijk is. Een van de vragen die in het proefschrift behandeld wordt is daarom of de emotionele informatie die de lichaamstaal aangeeft volstaat voor het opstarten van de reeks hersenactiviteiten betrokken bij emotieverwerking. Helaas zijn er maar enkele imaging studies over emotionele lichaamsobservatie, en die suggereren dat het waarnemen van emotionele lichaamstaal hersengebieden die ook betrokken zijn bij de uitvoering van handelingen aanzet tot handelen. Wat echter niet duidelijk is, is of de aard van een dergelijke motorische activering een bijdrage levert wat betreft de planning van een reactie op of een codering van de waargenomen emotie. In dit proefschrift verzochten we de deelnemers om actief emotionele (blijdschap en angst), neutrale (uitbeelding van de mimiek van een handeling) en statische (rust) lichaamshoudingen geïsoleerd te categoriseren voor ons onderzoek naar het motorsysteem.

Voor het motorsysteemonderzoek hebben we gebruik gemaakt van Transcraniale Magnetische Stimulatie (TMS), een niet-invasieve techniek die onderzoekers in staat stelt bepaalde hersengebieden te stimuleren door middel van korte magnetische pulsen op de schedel via een spiraal. Na een enkele pulse stimulans (spTMS) in de primaire motorische cortex kan uit de spieren die hiermee verbonden zijn een motorische twitch vastgesteld worden, genaamd *motor-evoked potential* (MEP): de amplitude hiervan (in millivolts) vertegenwoordigt de hoofdmeting van de corticospinale excitabiliteit. Een voorbeeld dat de bovengenoemde belichaamde simulatietheorie ondersteunt betreft een TMS onderzoek waarbij handelingen geobserveerd werden. De onderzoekers troffen een vrijwel identiek patroon aan spiersamentrekkingen (vergelijkbare MEP amplitudes) tijdens het uitvoeren van dezelfde handeling. Deze bevindingen tonen duidelijk aan dat er een neurale systeem is dat het zien

en uitvoeren van handelingen met elkaar in overeenstemming brengt. TMS lijkt daarom het ideale middel om direct, en met hoge temporele resolutie, potentiële, snelle modulaties van het motorsysteem tijdens het ondergaan van emotionele stimuli te behandelen. Tot op heden hebben slechts enkele TMS studies het effect van emotionele stimulusobservatie op de motorsysteemactiviteit van toeschouwers onderzocht, en deze studies testen vooral de excitabiliteit van de linker motorcortex, relatief laat in het proces (> 300 ms) tijdens het observeren van gezichten die emoties vertonen of bij het zien van complexe scenes. Door de temporele volgorde en de duur van de puls op de scalp te veranderen kan TMS ook gebruikt worden om de causale rol van het doelbrein in een gegeven proces (zoals het waarnemen van emotie) te beoordelen. Bijvoorbeeld, bij een TMS studie over het waarnemen van handelingen veranderden de onderzoekers tijdelijk de activiteit in het *extrastriate body area* en ontdekten dat dit het vermogen van de deelnemers om lichaamsvormen te onderscheiden belemmerde, terwijl tijdelijke verandering van de activiteit in de ventrale premotorische cortex het vermogen om lichaamsacties van elkaar te onderscheiden verstoorde. Deze resultaten laten zien dat, terwijl het *extrastriate body area* vooral de lichaamsidentiteit verwerkt van degene die een handeling uitvoert, de premotorische cortex van cruciaal belang is voor het visueel onderscheiden van handelingen.

Tot op heden, en belangrijk voor de relevantie van mijn proefschrift, zijn er geen studies die de rol van het motorsysteem bij visuele herkenning van emotionele signalen behandelen.

In de vier hoofdstukken van dit proefschrift willen we de rol van het motorsysteem bij de herkenning van emotionele lichaamshoudingen onderzoeken. We willen voornamelijk testen of het motorsysteem simulatieprocessen implementeert of motorreacties op de stimuli voorbereidt, zoals de twee hierboven genoemde theorieën voorstellen. Om onze vraagstelling te onderzoeken combineren we twee verschillende TMS protocollen om de verschillende rollen van de linker- en rechter primaire motorische cortex tijdens het observeren van dynamische lichaamsstimuli te testen. Belangrijk voor dit onderzoek is of de verschillende processen op verschillende tijdstippen plaatsvinden. TMS stimulatie wordt toegepast tussen 100 tot 300 ms na aanvang van de stimulus. Vanwege de hoge

temporele resolutie van de TMS - en omdat TMS tijdelijk de activiteit van een specifiek hersengebied kan beïnvloeden – levert dit een duidelijker beeld op van het tijdsverloop en de causale rol van het motorsysteem wanneer we geconfronteerd worden met emotionele en neutrale stimuli.

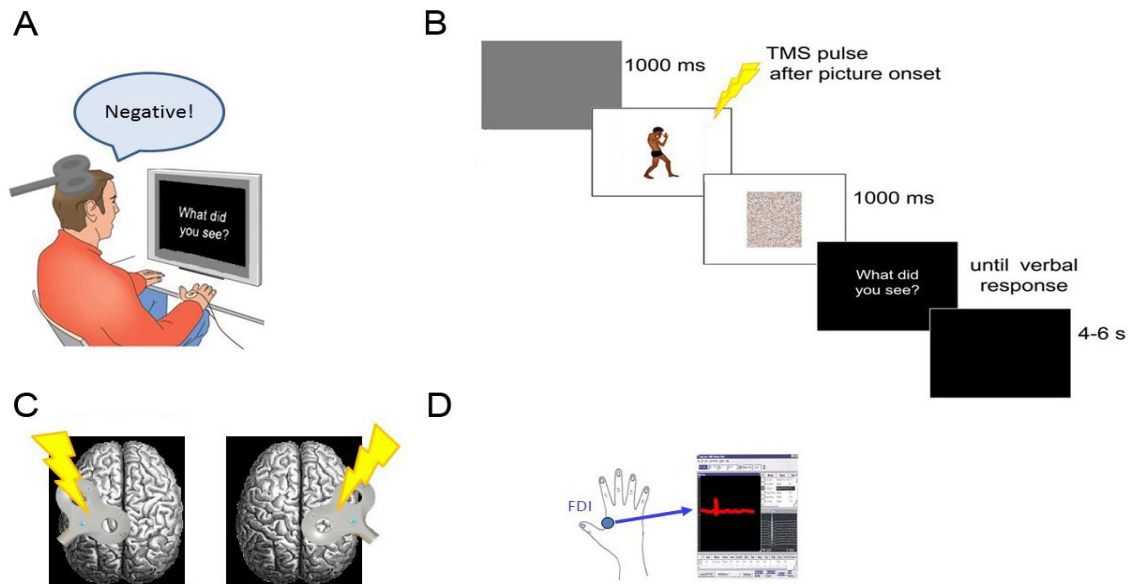


Fig 1A. De man achter de computer laat de algemene experimentele set-up zien; **B.** Voorbeeld van een trial; **C.** De twee renderings laten de plaats van de TMS stimulatie zien d.m.v. verschillende experimenten (linker of rechter primaire motorcortex); **D.** de hand laat een voorbeeld zien van de plek van de MEP recording (FDI spier) en een voorbeeld van hoe het EMG signaal eruit ziet.

Onze bevindingen schetsen de 3-lagige betrokkenheid van het motorsysteem bij het verwerken van de lichamelijke expressie van andermans emoties. In het allereerste stadium (in het timeframe tussen 100 en 125 ms) zien we dat, onafhankelijk van de hersenhelft die we stimuleren, de intracorticale verbindingen geremd worden wanneer deelnemers beangstigende en potentieel gevaarlijke stimuli te zien krijgen. Dit resultaat is een indicatie voor het bestaan van snelle motorreacties die we interpreteren als een onderdrukking van de motorreactie in het geval we angstige lichaamshoudingen waarnemen, en die mogelijk verkeerde handelingen voorkomen wanneer we iets potentieel

schadelijks in onze nabijheid waarnemen. Dit resultaat ondersteunt tevens het idee dat voorbereidingen voor actie in het brein gedreven worden door emotionele *cues* en dat potentiële dreigingen bijzonder snelle motorreacties vereisen als het organisme wil overleven.

Na 150 ms, onmiddellijk na de bovengenoemde snelle angst-gerelateerde motorreacties, zien we bewijs voor stadium twee, waarin de twee hersenhelften ieder een eigen rol lijken te spelen: de rechter motorcortex blijft remmende modulaties zien, maar ditmaal onafhankelijk van de emotionele lading van de stimuli (vergelijkbare remmende motorreactie gedurende het observeren van angstige en blijde lichaamshoudingen). We interpreteren de motorrespons die we zagen in de rechter M1 als een teken van een snelle oriëntatiereactie in de richting van emotioneel relevante stimuli. De data wijzen voorts op een positieve correlatie tussen de oriëntatiereactie en de herkenning van de emotie die de lichaamshouding laat zien: hoe meer deelnemers de oriëntatiereactie vertoonden, hoe beter zij in staat waren om de waargenomen lichaamshoudingen correct te identificeren. Deze correlatie wijst erop dat deze oriëntatiereactie die het motorsysteem gebruikt perceptie-gerelateerde processen ondersteunt. De resultaten van het selectief interfereren met de activiteit van de rechter M1 ondersteunen onze interpretatie dat de deelnemers minder accuraat presteren bij 150 ms (geen effect na stimuleren van de linker M1 of de sham groep). Samen wijzen deze bevindingen erop dat de oriëntatiereactie die we uit de rechter motorcortex verkrijgen niet alleen verband lijkt te houden met de perceptie van de waargenomen stimuli, maar hier zelfs noodzakelijk voor is. Op hetzelfde moment (150 ms) lijkt de linker motorcortex niet geremd maar juist geactiveerd te worden door de beangstigende stimuli (in lichaamshouding of complexe scenes) in vergelijking met de corresponderende blijde of neutrale stimuli. Dit effect is nog sterker wanneer deelnemers gevraagd wordt te kijken naar gewelddadige, walgelijke of beangstigende dynamische scenes in relatie tot angstige menselijke lichaamshoudingen, waarschijnlijk omdat deze laatste stimuli minder krachtig waren dan de geselecteerde scenes. We interpretern deze sterkere helpende respons van de linker hemisfeer op negatieve stimuli als een weerspiegeling van het signaal van de motorische voorbereiding van de dominante hand op de negatieve stimuli.

Als laatste rapporteren we dat zowel de rechterkant voor emotionele lichaamshoudingen als de linkerkant voor assistentie bij negatieve stimuli verband houden met angst-gerelateerde interindividuele trekken: hoe angstiger de deelnemers waren, hoe meer zij de bovengenoemde motoreffecten vertoonden. Samengevat wijzen deze data erop dat angst-gerelateerde trekken de manier beïnvloeden waarop sociale en emotionele signalen in de hersenen verwerkt worden.

In het derde en laatste stadium (300 ms), zien we dat zowel linker- als rechter motorische cortex gelijke betrokkenheid vertonen (facilitatie) bij het reageren op emotionele afbeeldingen en dat het waarschijnlijker is dat hun activatie de motorsimulatie van de impliciete stimuli weerspiegelt en niet zozeer de voorbereiding op een motorreactie. Zoals gesteld door de belichaamde simulatie-aanhangers, doen we aan interne simulatie (bij 300 ms vanaf de aanvang van de stimulus) wanneer we de acties en emoties van anderen waarnemen. Echter, onze resultaten duiden erop dat motorresonantie plaatsvindt nadat de emotionele signalen al in de rechter motorcortex zijn verwerkt. Hoewel onze data erop wijzen dat motorresonantie niet noodzakelijk is voor perceptie, althans niet voor simpele emotie-herkenningstaken, moeten we de mogelijkheid open houden dat resonantieprocessen die nodig zijn voor perceptie zich eerder zouden kunnen voordoen (bv. al vóór 150 ms) of in andere anatomische locaties (bv. in premotorgebieden of pariëtale gebieden) zonder dat dit onmiddellijk duidelijk is in de primaire motorcortexactiviteit zoals gemeten met TMS. Er zullen dus vervolgstudies moeten komen die de chronometrie en de causale betrokkenheid van motorresonantie bij visuele perceptie van emotionele lichaamstaal testen.

Conclusie: de data in dit proefschrift werpen nieuw licht op de temporele relatie tussen motorprocessen zoals gesteld door simulatie- en non-simulatietheorieën over emotieverwerking. Enerzijds wijzen simulatietheorieën in de richting van een ‘imitatieve’ betrokkenheid van het motorsysteem bij het afleiden van de emotionele toestand van anderen (= motorresonantie) en dat dit verband zou kunnen houden met perceptuele processen. Aan de andere kant zeggen andere wetenschappers, zich baserend op het idee dat emoties het menselijk lichaam aanzetten tot actie, dat de perceptie van emotionele lichaamshoudingen iemand voorbereidt op de bijpassende emotionele

motorreactie, die verondersteld wordt los te staan van perceptie-gerelateerde processen. Veranderingen in motorreactiviteit zouden dan dus geen actie-simulatieprocessen vertegenwoordigen, teweeggebracht door het zien van corresponderende handelingen, maar potentiële motorreacties, die voortkomen uit de snelle verwerking van emotionele aspecten in visuele en subcorticale-corticale emotionele circuits. Onze data laten zien dat, zoals verondersteld door evolutie-theoretici, snelle reacties op dreigende stimuli kunnen worden aangetoond in het motorsysteem (hoofdstuk 4). Dergelijke snelle modulaties zijn echter niet enkel betrokken bij de planning van motorreacties, maar lijken ook een rol te spelen bij meer cognitieve processen, zoals het ondersteunen van visuele herkenning van de waargenomen lichamen (hoofdstuk 3). Anderzijds, zoals verondersteld door belichaamde simulatie-theoretici, laten onze data motorresonantieprocessen zien, waarbij deelnemers wordt gevraagd dynamische lichaamsstimuli te bekijken (hoofdstuk 1, 2 en 3). In tegenstelling tot wat de meest radicale ‘motor-centrische’ versies van deze theorieën veronderstellen, laat onze data zien dat motorresonantieprocessen niet noodzakelijk zijn om de emotionele betekenis van dergelijke stimuli te kunnen extraheren: de rechter M1 laat andere modulaties zien van de emotionele lichaamsobservatie ten opzichte van de neutrale (ruim 150 ms) voordat de motorresonantie zich voordoet (300ms), en hoewel interferentie met de motoractiviteit van de rechter motorcortex op 150 ms positief correleerde met lichaamshoudingen, herkenningsaccuratesse en herkenning, verstoort selectieve inmenging met motorresonantieprocessen de herkenningsprestaties niet.

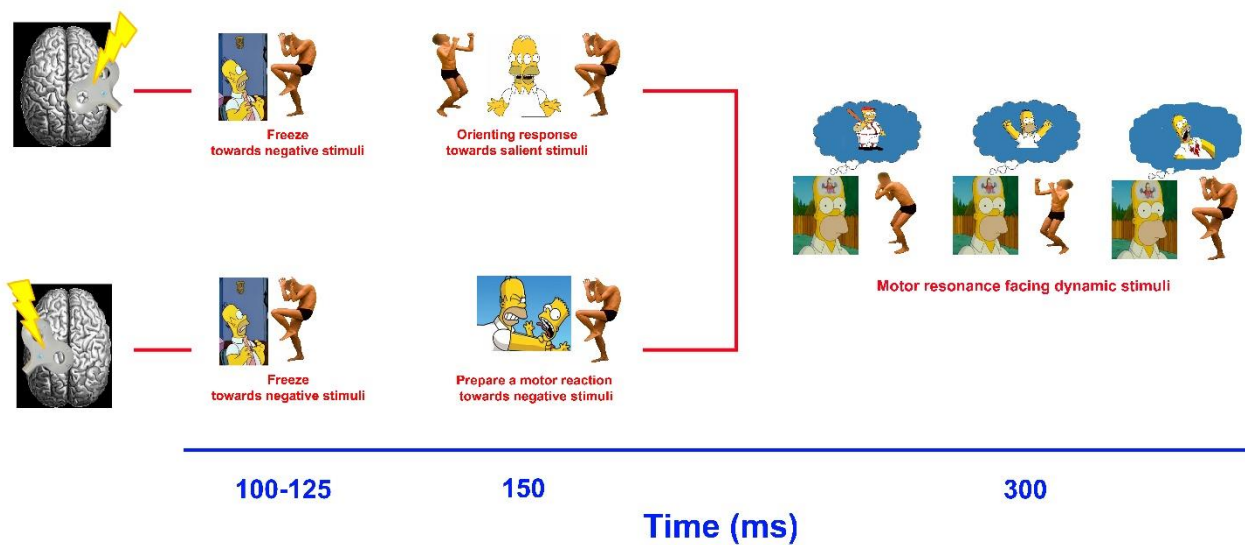


Fig 2. Verschillende stadia van betrokkenheid van het motorsysteem gedurende het kijken naar menselijke lichaamshoudingen. De bovenste rij laat de respons zien na stimulatie van de rechter hersenhelft, de onderste rij die van de linker hersenhelft.

Summary

Social animals have evolved a whole range of communicative abilities on which their well-being and survival in a society hinges. Foremost amongst the cognitive skills of social species is the ability to monitor each other's behavior and emotions and the ability to adapt continuously to the social signals of others. Therefore, appropriately understanding and reacting to the emotions of others appear to be fundamental abilities for survival.

In recent years, neuroscientists discovered a particular class of neurons called "mirror-neurons" that have the property of firing both during the execution of an action and while observing (or listening to) a similar action when performed by another agent. This has led to the idea that understanding the inner state of other individuals relies on implicit motor simulation, that is, the activation of motor programs that we would use to perform similar actions when witnessing another individual perform a motor act. For example, seeing a friend grasping a glass of water in order to drink it would activate in the observer the motor program necessary for the observer to perform the same action, namely grasping the glass. Because when thirsty, the observer will grasp a glass of water using the same neural representation activated whilst observing her friend's action.

More recently, it has been suggested that brain areas involved in emotion processing might also be involved in simulative processes: they might perform an 'emotional simulation' of other individuals' experiences, showing activity not only when we experience positive and negative emotions but also when we witness those of others. For example, observing another person smiling evokes a similar neural brain activity as well as the corresponding facial motor representation in the observer. This motor representation and its associated somatosensory consequences are supposed to be involved in understanding the meaning of the other person's facial expression. Thus, as observing hand actions activates the observer's motor representation of that action, observing an emotion activates the neural representation of that emotion.

Current models of social cognition, therefore, incorporate the notion that regions involved in processing our motor acts and emotions can contribute to the perception of others by simulating their actions and emotions (embodied simulation theory). Another line of research has instead suggested that during emotional processing the motor system is merely involved in reacting to emotional cues by preparing the most appropriate motor act.

In support of this second notion, different behavioral studies found, for example, that participants were faster to pull a lever towards themselves for positive words and to push for negative words, demonstrating the role of the motor system in preparing the most opportune motor reaction (approach in case of positive stimuli, avoid in case of negative, dangerous stimuli). As a consequence of this last theory, the motor system would not play a role in comprehension but it would be merely recruited in appropriately reacting to the observed stimuli.

Taking into account the fundamental importance that understanding and appropriately reacting to social signals may hold for the survival of the species, in this thesis we try to investigate whether the two above-mentioned theories are competing alternatives or whether they can be bridged. We do this by probing the motor system at different time windows while facing someone else's actions and emotions.

When we interact in a social environment, we can capture information about others' feelings and emotions mainly by observing their facial expressions and body postures. Nevertheless, to date, most investigations of emotion perception have focused on brain activity generated by the perception of facial expressions and neglected the body, in comparison. Face observation is seen as a more automatic and direct way to investigate another person's feelings and intentions. However, faces and bodies are not separate but closely linked entities, to the point that the recognition of emotions through facial expressions is impaired if the emotions expressed by the body are incongruent. This evidence supports the idea that, in everyday life, we collect information from both the face and the body. Observing bodies, for example, offers the possibility to capture signals and perceive emotions over a

longer distance than observing faces. This also shifts the attention away from the personal identity shown by the face, which may not always matter for rapid decoding of the expressions. One of the questions tackled in the thesis is therefore whether the emotional information carried out by body postures is sufficient to start the chain of brain activity involved in emotion processing. Unfortunately, only few imaging studies exist on emotional body observation and they suggest that perceiving emotional bodies recruits brain regions also involved in action execution. However, the nature of such a motor activation, i.e., whether the activation represents a contribution to the planning of a reaction to or the encoding of the observed emotion, remains unclear. In this thesis, we therefore investigate the role of the motor system while subjects are requested to actively categorize emotional (happy and fear), neutral (depicting the mimic of an action) or static (at rest) human body postures presented in isolation.

We tested the motor system using Transcranial Magnetic Stimulation (TMS), which is a noninvasive technique that allows researchers to stimulate discrete brain areas delivering brief magnetic pulses to the scalp through a coil. After a single pulse stimulation (spTMS) of the primary motor cortex, a motor twitch named the *motor-evoked potential* (MEP) can be recorded from the connected muscles: its amplitude (in millivolt) represents the main measure of the corticospinal excitability. One example in support of the aforementioned embodied simulation theory in the action domain comes from a TMS study during action observation. Authors found a very similar pattern of muscle contraction (similar MEPs amplitude) during the execution of the same action. These findings clearly indicate that there is a neural system matching action observation and execution. Therefore, Transcranial Magnetic Stimulation seems to be the ideal tool to directly address possible fast motor system modulations during emotional stimuli perception, with a high temporal resolution. To date, only a few TMS studies investigated the effect of emotional stimuli observation on an observer's motor system activity and these studies mainly tested the left motor cortex excitability relatively late in time (>300 ms), specifically during emotional faces or complex scenes observation. By changing the

temporal sequence and the duration of the pulses delivered to the scalp, TMS can also be used to assess the causal role of the targeted brain area in a given process (such as emotion perception). For example, in a TMS study on action perception, authors have temporally interfered with activity in the extrastriate body area, driving impaired discrimination of bodily forms; in contrast, temporarily interfering with the activity in the ventral premotor cortex impaired the discrimination of bodily actions. These results demonstrate that, whereas the extrastriate body area mainly processes actors' body identity, premotor cortex is crucial for visual discrimination of actions.

Importantly for the relevance of my thesis, there are no existing studies that have tested the role of the motor system on visual recognition of emotional signs.

In the four chapters of this thesis, we aim to investigate the role of the motor system in the recognition of emotional body postures. In particular, we aim to test whether the motor system is implementing more simulative processes or preparing motor reactions to the stimuli, as suggested by the two mainstream theories proposed above. In order to investigate our questions we combined different TMS protocols to test the different role of the left and the right primary motor cortices in the observation of dynamic body stimuli. Importantly, to investigate whether different processes occur at different time points, TMS stimulation is applied from 100 to 300 ms after the stimulus onset. Due to the high temporal resolution of the TMS and its capacity to temporarily interfere with the activity of a specific area of the brain, we can get a clearer picture about the time course and the causal role of the motor system when faced with emotional and neutral stimuli.

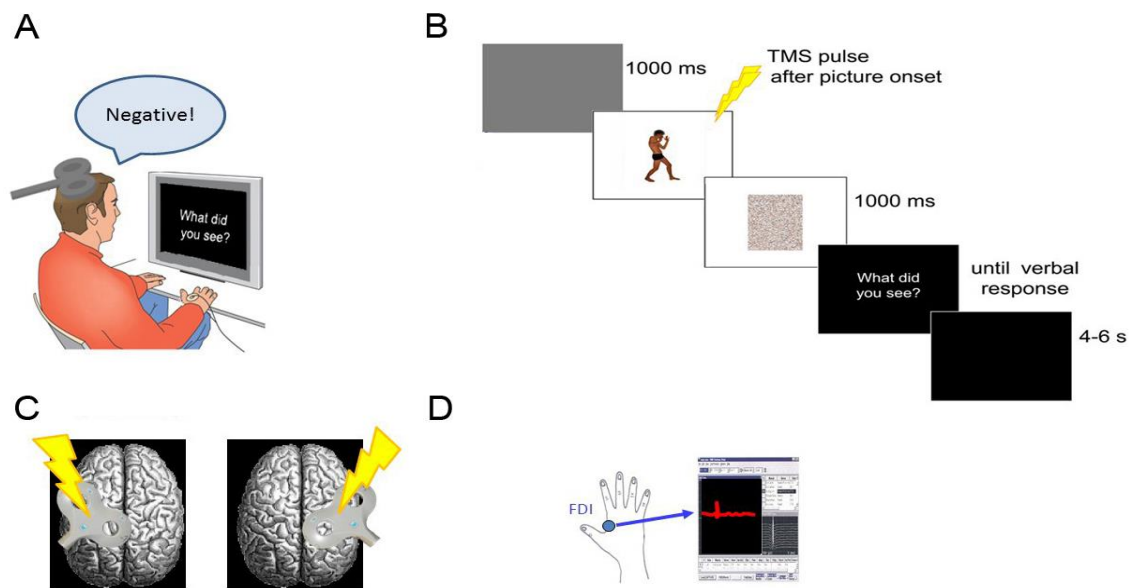


Fig 1A. The man in front of the computer shows the general experimental set-up; **B.** Trial example; **C.** The two renderings show the location of the TMS stimulation through different experiments (left or right primary motor cortex); **D.** the hand shows an example of the location of the MEP recording (FDI muscle) and an example of what the EMG signal looks like.

Our findings outline a 3-stage model of the motor system’s involvement in processing another person’s bodily expression of emotions. In the very first stage, happening at a time window of 100-125 ms, we find that, regardless of the stimulated hemisphere, the facilitatory intracortical connections are inhibited when subjects observe fearful, potentially dangerous, stimuli. This result suggests the existence of fast motor reactions that we interpret as suppression of motor readiness when seeing fearful bodies in order to prevent possibly erroneous actions when we are observing something potentially harmful in the environment. This result additionally supports the notion that emotional cues drive action preparation in the brain and that potential threats require particularly quick motor reactions to secure the survival of the organism.

At 150 ms, immediately after the aforementioned fast fear-related motor reactions, we observe evidence for a second stage, in which the two hemispheres seem to play different roles: the right motor cortex continues to show inhibitory modulation but, this time, it is independent of the emotional meaning of the stimuli (similar inhibitory motor reaction during fearful and happy body postures observation). We interpret the motor response we observe from the right M1 as the sign of a rapid orienting reaction toward emotionally relevant stimuli. The data further suggest a positive correlation between the orienting reaction and the recognition of the emotion carried out through the body posture: the more subjects exhibit the orienting reaction, the more they were able to accurately identify the observed body postures. This correlational result suggests that this orienting response implemented by the motor system supports perception-related processes. In support of this interpretation, we find that when we selectively interfere with the activity of the right M1, subjects show worse accuracy performance at 150 ms (no effect when stimulating either left M1 or in the Sham group). Altogether, these findings suggest that the orienting reaction we record from the right motor cortex appears to be, not only related to, but also necessary for the perception of the observed stimuli. At the same time (150 ms) the left motor cortex appears to be more activated, rather than inhibited, by the observation of fearful stimuli (bodies or complex scenes) compared to the observation of the corresponding happy or neutral stimuli. This effect is stronger when subjects are asked to observe violent, disgusting or fearful complex dynamic scenes relative to fearful human body postures, probably because these latter stimuli were less powerful than the selected scenes. We interpret this stronger facilitatory left hemisphere response to negative stimuli as a sign of a motor preparation of the dominant hand towards negative stimuli.

Finally, we report that both the orienting for emotional bodies and the facilitatory effect for negative stimuli are related to anxiety-related interpersonal traits: the more anxiety traits the subjects exhibit, the more they show the aforementioned motor effects. Taken together, these data suggest that anxiety-related traits influence the way in which social and emotional signals are processed in the brain.

In a third last stage (300 ms), we find the two motor cortices are equally involved (facilitation) in responding to the emotional pictures and that their activation is more likely to reflect the motor simulation of the implied motion of the stimuli than the preparation of a motor reaction. As posited by the embodied simulationists, at 300 ms from the stimulus onset, when we observe the actions and emotions of others, we internally simulate them. However, our results indicate that motor resonance takes place *after* the emotional signals have already been perceived in the right motor cortex. Although our data suggest that motor resonance is not necessary for perception, at least not during simple emotion recognition tasks, it is important to entertain the possibility that resonance processes necessary for perception could occur earlier in time (e.g., before 150 ms) or in other anatomical locations (e.g., in premotor or parietal regions) without being immediately evident in the primary motor cortex activity as measured by TMS. Thus, further studies are needed to test the chronometry and causal involvement of motor resonance in the visual perception of emotional body language.

To conclude, data present in this thesis shed new light on the temporal relation between the motor processes hypothesized by simulative and non-simulative theories of emotion processing. On the one hand, embodied simulation theories suggest that the motor system involvement in inferring the emotional states of others may be “imitative” in nature (i.e., motor resonance) and related to perceptual processes. On the other hand, based on the notion that emotions may prime the human body for action, other scholars propose that the perception of emotional bodies triggers the preparation of emotionally appropriate motor reactions, which is supposed to occur independent of perception-related processes. In this vein, changes in motor reactivity would not represent action simulation processes triggered by the sight of corresponding actions but rather potential motor reactions stemming from the fast processing of emotional features in visual and subcortical-cortical emotional circuits. Our data demonstrate that, as proposed by evolutionary theorists, fast reactions towards threat-related stimuli are detectable in the motor system (Chapter 4). However, such fast modulations are not merely involved in planning motor reactions, but seem to have a role in more

cognitive processes, such as supporting visual recognition of the observed bodies (Chapter 3). On the other hand, as proposed by embodied simulation theorists, our data show motor resonance processes when subjects are asked to observed dynamic body stimuli (Chapter 1, 2 and 3). In contrast to what is supposed by most radical “motoric-centric” versions of these theories, our data show that motor resonance processes are not necessary to extract the emotional meaning of such stimuli. The right M1 shows different modulations from emotional relative to neutral body observation well before (150 ms) the occurrence of motor resonance (300 ms). Furthermore, interfering with the motor activity of the right motor cortex at 150 ms was positively correlated to body posture recognition accuracy and recognition, whereas selectively interfering with motor resonance processes does not disrupt recognition performance.

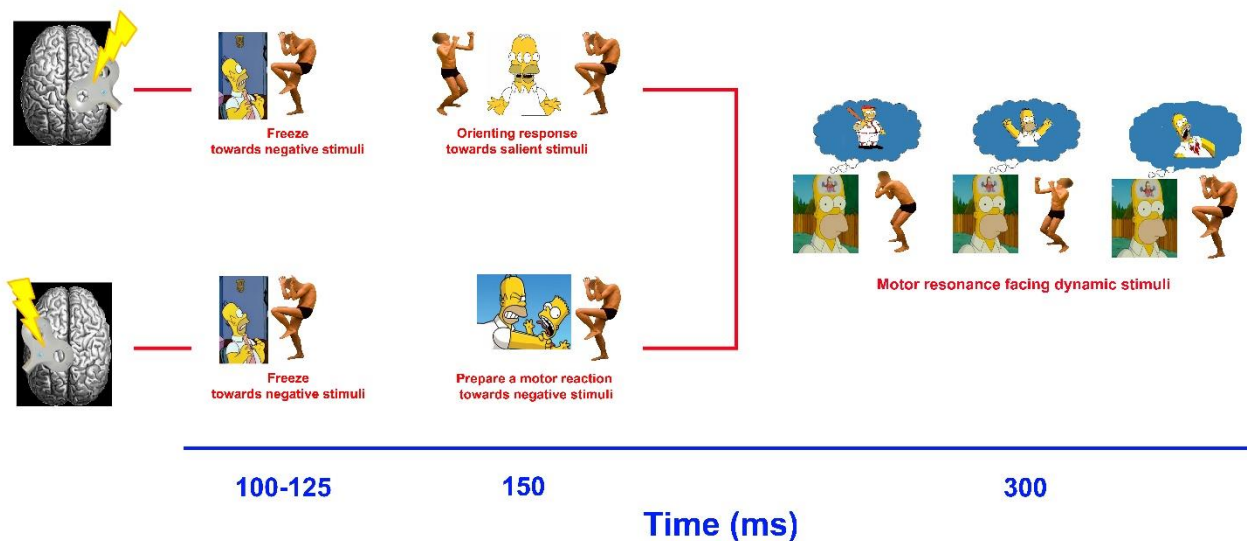


Fig 2. Different phases of motor system involvement during human body posture observation. The top row represents the responses following right hemisphere stimulation, the bottom row following stimulation to the left hemisphere.

Curriculum Vitae et studiorum

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Current Positions:

- Since 2011, PhD Student University of Groningen (The Netherland).
- 2011-2015, Fellowship (Assegno di ricerca) at Centro studi e ricerche in Neuroscienze Cognitive, University of Bologna.

Education

- 2004, Diploma Liceo Classico “E. Cairoli” (VA), score: 80/100
- 2007, Bachelor’s Degree in Psychological Sciences and Techniques, University of Milan “Vita-Salute S. Raffaele”, score: magna cum laude.
Title of the thesis: “*DEEPASHRAM (INDIA): COGNITIVE STIMULATION IN CHILDREN WITH PHYSICAL AND MENTAL DISORDERS*”.
- 2009, Master Degree in Neuropsychology , University of Bologna, score: magna cum laude.
Title of the thesis: “*ROLE OF SENSORIMOTOR CORTICES IN MAPPING OTHERS’ PAIN*”.

Courses and workshops

- 2010, Advanced technical course on tDCS, Fatebenefratelli, Brescia, Italy (11-12 November).
- 2011, Congress of Functional Neuroradiology, San Raffaele Hospital, Milano.
- 2012, Brain dissection Summer School, Bangor; UK.
- 2013, 5th International conference of non invasive brain stimulation, Leipzig, Germany.

Scientific experiences

- 2007 November - 2008 February, Neuropsychological testing in “Ville Turro”, Neuropsychiatric clinic, San Raffaele Hospital in Milan, Italy.
- 2007 March-April, Neuropsychological testing, Indian Spinal Injury Hospital, New Delhi, India.
- Since 2008, Research assistant at Non invasive Brain Stimulation lab at Centro studi e ricerche in Neuroscienze Cognitive, Cesena – University of Bologna under the supervision of Prof. Alessio Avenanti.

- 2010 April-June, Research assistant at Neuroimaging Center, University Medical Center of Groningen, The Netherlands, under supervision of Prof. Valeria Gazzola and Christian Keysers. TMS and fMRI experiments investigating the neural correlates of action observation and weight judgments (in progress).

Visiting

- 2013 June-July Combined TMS and fMRI experiment on action observation at University of Maastricht under the supervision of Prof Alexander Sack.

Publications

- **Borgomaneri S.**, Gazzola V., Avenanti A. (2012). Motor mapping of implied actions during perception of emotional body language. *Brain Stimulation*. 5:70-6. (**IF: 4.538**)
- Tidoni E., **Borgomaneri S.**, di Pellegrino G., Avenanti, A. (2013). Action Simulation Plays a Critical Role in Deceptive Action Recognition. *Journal of Neuroscience*. 33:611-23. (**IF: 6.91**)
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Posters

- **Borgomaneri S.**, Aglioti S., Avenanti A. Perturbing and measuring neural activity in the pain resonance network: TMS studies. CAOS, Concepts, Actions and Objects. Functional and Neural Perspectives. Rovereto, 19-22.05.2011.
- Avenanti A., Tidoni E., **Borgomaneri S.**, di Pellegrino G. The inferior frontal gyrus is critical for recognition of deceptive intentions. IBRO. International Brain Research Organization. Firenze, 14-18.07.2011.
- **Borgomaneri S.**, Aglioti S., Avenanti, A. Perturbing and measuring neural activity in the pain resonance network: TMS studies. IBRO. International Brain Research Organization. Firenze, 14-18.06.2011.
- **Borgomaneri S.**, Avenanti, A. Causative connectivity of sensorimotor regions during embodied empathy for pain as revealed by perturb-and-measure TMS. 2011 TMS Summer School_Oxford.

- **Borgomaneri S.**, Gazzola V., Avenanti A. Motor mapping of implied actions during perception of emotional body language. CAOs, Concepts, Actions and Objects: Functional and Neural Perspectives. Rovereto, 24-27.05.2012.
- **Borgomaneri S.**, Avenanti A. Temporal dynamics of motor cortex excitability during perception of emotional IAPS stimuli. 2012 Magstim Neuroscience Conference & Workshop 2012. Oxford, UK. 12-13.05.2012.
- **Borgomaneri S.**, Avenanti A. Motor resonance and motor reactions during emotional body perception. 2013 Magstim Neuroscience Conference & Workshop 2013. Oxford, UK. 04-05.05.2013.
- Makris S., Mele S., **Borgomaneri S.**, Avenanti A., Urgesi, C. "I know you are going to grasp a cactus". 2013 Magstim Neuroscience Conference & Workshop 2013. Oxford, UK. 04-05.05.2013.
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Talk

- **Borgomaneri S.**, Anelli F., Aglioti S., Avenanti, A. (2011). Causal connectivity in the cortical network underlying embodied empathy for pain: a perturb-and-measure TMS study. Riunione Autunnale della Società Italiana di Neuropsicologia (SINP 2011), Bologna, 18-19.11.2011.
- **Borgomaneri S.**, Avenanti A. (2012). Perception of emotional bodies triggers fast motor reactions and motor resonance in the human corticospinal system. Congresso Annuale SINP 2012. Roma, 9-10.11.2012.
- **Borgomaneri S.**, Avenanti A. (2013). Che emozione è? Chiedi al Solco Temporale Superiore: uno studio di TMS-priming. AIP - SEZIONE PSICOLOGIA SPERIMENTALE, Roma, Italy.
- **Borgomaneri S.**, Avenanti A. (2014). Comprendere e reagire al linguaggio emotivo del corpo, prima della simulazione motoria. AIP - SEZIONE PSICOLOGIA SPERIMENTALE, Pavia, Italy.

Award

- Premio casa editrice Pearson, best scientific oral presentation, AIP - SEZIONE PSICOLOGIA SPERIMENTALE, Roma, Italy.

Research Grant/Fellowship

- 2011-2014, Grant Fellowship (Assegno di ricerca, ~20k € per year) at Centro studi e ricerche in Neuroscienze Cognitive, University of Bologna, Italy.
- October 2010, Research grant for a Phd position University of Rome, La Sapienza, Italy (~12k € per year, renounced).
- April 2010, Small grant fellowship, University Medical Center of Groningen, The Netherland (~2k €).
- March 2010, Travel Grant for students working on Masters thesis, University of Bologna, Italy (~1k €).
- 2011-2012, Assegno di Ricerca, Polo scientifico didattico di Cesena, Università di Bologna.
- 2012-2013, Assegno di Ricerca, Polo scientifico didattico di Cesena, Università di Bologna.
- 2013-2014, Borsa di studio, IRCCS Fondazione Santa Lucia, Roma.

Scientific and technical skills

- Transcranial Magnetic Stimulation: single pulse, paired-pulse, dual coil and repetitive TMS protocols. Cortico-cortical paired associative stimulation paradigm.
- Offline and Online Transcranial Magnetic Stimulation and Electroencephalography study, Online combined TMS and fMRI.
- Analysis of physiological signals (electromyography, motor-evoked potentials): Biopac MP 35/36, 150.
- Neuronavigation systems: Polaris Vicra and SofTaxis Neuronavigation.
- Programming behavioural and neurophysiological experiments using Matlab.

Computer skills

- Operating Systems: Windows
- Programming Languages: MATLAB
- Research software applications: MATLAB Psychtoolbox, Statistica, SPSS.
- Packages: Office, Photoshop, Premiere.

Other language: English

- Writing: good
- Speaking: good
- Comprehension: good

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