Your emotion moves into my motor system

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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).
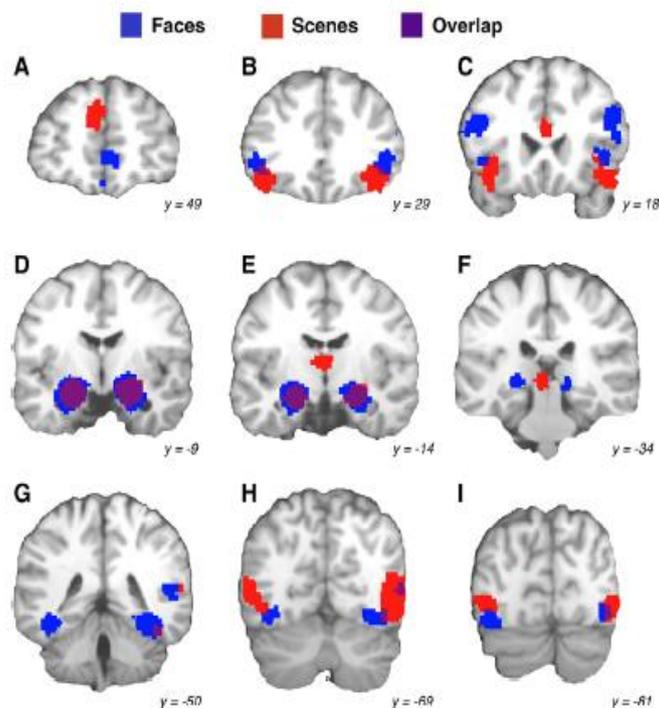
Sabatinelli et al.,2011; NeuroImage

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 occipito-temporal 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

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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 observed 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, threating 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 has 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 activated 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 convening 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 collegues (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

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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 neural 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 glutammatergic 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.