Your emotion moves into my motor system

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Chapter 4

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

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 threating 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; Hagenaars 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 poostures rapidly reduces exitability of the observer's M1. To this aim, we used TMS ovor M1 to non-invasively asses 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; Hagenaars 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 glutammatergic 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 ± S.D.: 22.8 y ± 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 ± S.D.: 23.3 y ± 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).
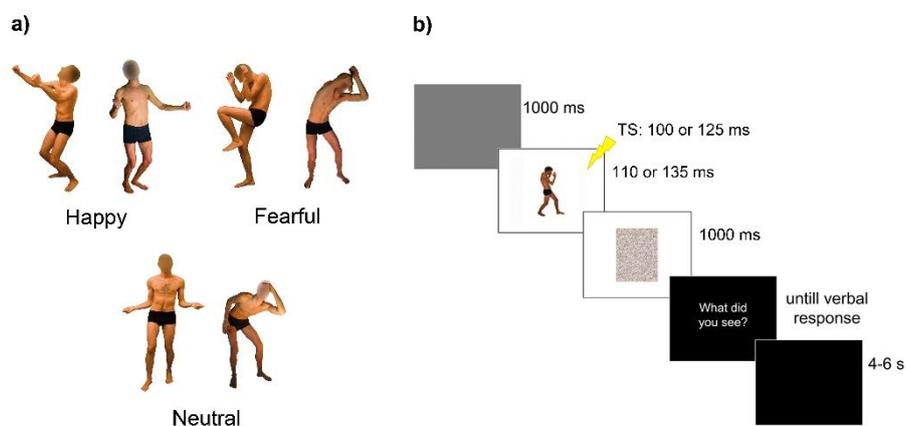

Fig. 1a Examples of visual body stimuli. **b** Trial sequence.

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 controlateral 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 µV 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 trails, 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 eta^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 ± S.D.: 90.7% ± 5.3%; SICI: 89.5% ± 6.7% and ICF: 90.5% ± 5.3%; Exp2M1left: SP mean accuracy ± S.D.: 92.7% ± 5.5%; SICI: 91.7% ± 5.2% and ICF: 90.6% ± 4.9%). The Experiment x 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 x 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 x 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 x Movement type ANOVAs carried

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out on implied motion ratings (Table 1) did no 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 ± 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 ± SD in Exp1M1right: 1.11 mV ± 0.26; in Exp2M1left: 1.02 mV ± 0.22) and last block of baseline (Exp1M1right: 1.15 mV ± 0.43; Exp2M1left: 1.09 mV ± 0.38) were entered in an Experiment x 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% ± 23) relative to the SP (118% ± 37; $p < 0.001$; $d = 1.99$) and ICF sessions (195% ± 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% ± 90) than for happy (198% ± 116; $p = 0.003$; $d = 0.30$) and neutral bodies (200% ± 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).
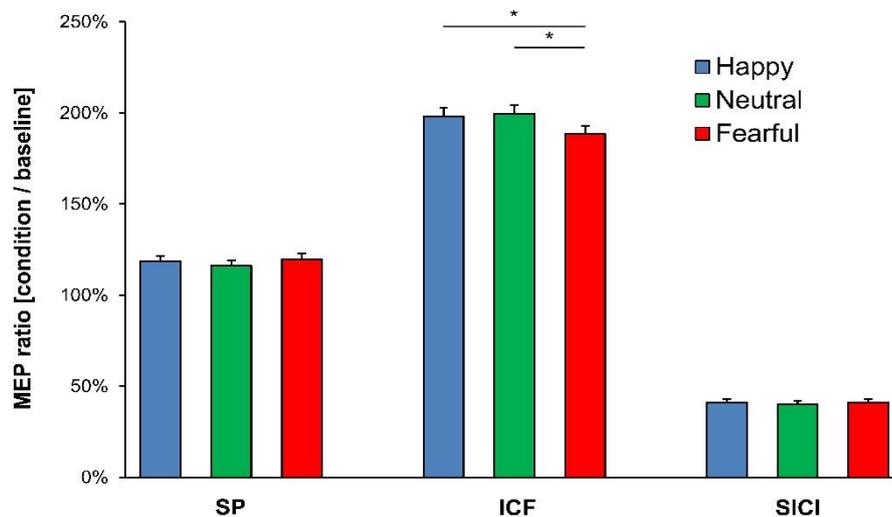
Fig. 2 Neurophysiological modulations during the emotion recognition task. MEP amplitude ratio (condition / baseline) during perception of happy, neutral and fearful body postures during single pulse (SP), intracortical facilitation (ICF) and short intracortical facilitation (SICI) sessions. Data show 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% ± 112 vs 0.38% ± 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% ± 101) relative to the happy (182% ± 111; $p = 0.03$; $d = 0.32$) and neutral body conditions (191% ± 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 ($F < 1.28$, $p > 0.29$).
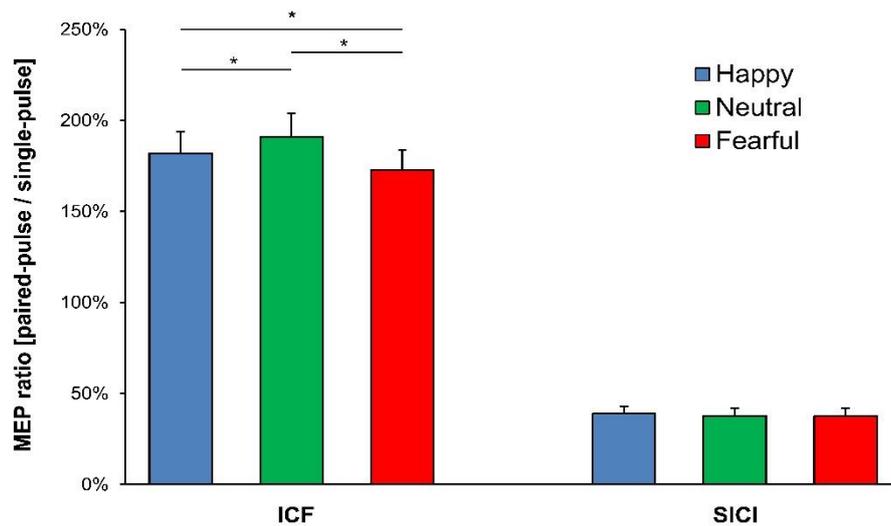

Fig. 3 Cortical motor modulations during the emotion recognition task. MEP amplitude ratio (paired-pulse / single-pulse) during perception of happy, neutral and fearful body postures during intracortical facilitation (ICF) and short intracortical facilitation (SICI) sessions. Data show the interaction 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 biologically 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; Hagenaars 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; Hagenaars 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 glutammatergic 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 closed 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).