The multiform motor cortical output:

kinematic, predictive and response coding

Luisa Sartori[1,2], Sonia Betti[1], Eris Chinellato[3], and Umberto Castiello[1,2]

[1] Dipartimento di Psicologia Generale, Università di Padova, Padova, Italy.
[2] Center for Cognitive Neuroscience, Università di Padova, Padova, Italy.
[3] School of Computing, University of Leeds, Leeds, UK.

Corresponding Author:

Luisa Sartori

Dipartimento di Psicologia Generale,

Università di Padova.

Via Venezia 8, 35131, Padova, Italy.

Fax: +39 049 8276600.

mailto:luisa.sartori@unipd.it

Abstract

Observing actions performed by others entails a subliminal activation of primary motor cortex reflecting the components encoded in the observed action. One of the most debated issues concerns the role of this output: Is it a mere replica of the incoming flow of information (kinematic coding), is it oriented to anticipate the forthcoming events (predictive coding) or is it aimed at responding in a suitable fashion to the actions of others (response coding)? The aim of the present study was to disentangle the relative contribution of these three levels and unify them into an integrated view of cortical motor coding. We combined transcranial magnetic stimulation (TMS) and electromyography recordings at different timings to probe the excitability of corticospinal projections to upper and lower limb muscles of participants observing a soccer player performing: (i) a penalty kick straight in their direction and then coming to a full stop, (ii) a penalty kick straight in their direction and then continuing to run, (iii) a penalty kick to the side and then continuing to run. The results show a modulation of the observer's corticospinal excitability in different effectors at different times reflecting a multiplicity of motor coding. The internal replica of the observed action, the predictive activation, and the adaptive integration of congruent and non-congruent responses to the actions of others can coexist in a not mutually exclusive way. Such a view offers reconciliation among different (and apparently divergent) frameworks in action observation literature, and will promote a more complete and integrated understanding of recent findings on motor simulation, motor resonance and automatic imitation.

Keywords: action observation, motor resonance, transcranial magnetic stimulation, motor evoked potentials.

Introduction

The subliminal activation of the motor system while observing actions performed by others (i.e., motor resonance) is a widely investigated phenomenon (Grezes & Decety, 2001). A long-term debate on the level of motor coding carried out during action observation concerns whether it reflects the observed action's kinematics or its final goal. Findings supporting the hypothesis of a direct matching between another person's body movements and our own motor representations have been drawn from different methodological approaches. Single cell recordings demonstrated the existence of 'mirror neurons' which discharged both when a monkey actually grasped 3-D objects and when it observed that action being carried out (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). In humans, single-neuron responses were likewise recorded during both action execution and observation (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010) and neuroimaging studies provided evidence that the fronto-parietal system is implicated in coupling the representations of executed and observed actions (for review see Fabbri-Destro & Rizzolatti, 2008; Giorello & Sinigaglia, 2007; Keysers, 2009; Molenberghs, Cunnington, & Mattingley, 2012; Rizzolatti & Craighero 2004; Turella, Tubaldi, Erb, Grodd, & Castiello, 2012). A growing body of neurophysiologic studies have, moreover, demonstrated that action observation selectively activates the effector muscles involved in performing that action (for review see Fadiga, Craighero, & Olivier, 2005). The motor potentials (MEPs) evoked by transcranial magnetic stimulation (TMS) during action observation appear, in fact, to be specifically attuned to the muscles involved in the action being observed (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Sartori, Bucchioni, & Castiello, 2012a; Strafella & Paus, 2000; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006) and to its temporal pattern (Aglioti, Cesari, Romani, & Urgesi, 2008; Borroni, Montagna, Cerri, & Baldissera, 2005; Borroni & Baldissera, 2008; Janssen, Steenbergen, & Carson, 2013; Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004; Gangitano, Mottaghy, & Pascual-Leone, 2001; Montagna, Cerri, Borroni, & Baldissera, 2005; Urgesi, Maieron, Avenanti, Tidoni, Fabbro, & Aglioti, 2010). Behavioral studies have also demonstrated motor compatibility

3

effects, showing how the observation of a finger movement that corresponds to the instructed finger movement can facilitate the response (Brass & Heyes, 2005; Liepelt, Prinz, & Brass, 2010). On the other hand, the simulation theory specifically argues that observing another person's action is not simply a reconstruction of visual input, but an intrinsically predictive activity (Gallese & Goldman, 1998). When we observe another person's actions, we automatically anticipate their future ones. At the most basic level, humans can predict how a movement will evolve simply by watching how it was begun. For example, by observing how a person throws a dart at a dartboard, an observer can predict where the dart will land (Knoblich & Flach, 2001). An observer can likewise anticipate the type of tennis or volleyball serve that is about to be made (Abernethy, Zawi, & Jackson, 2008), predict the success of a basketball shot (Aglioti et al., 2008), foresee if a player is about to launch a real or a mimic throw (Sebanz & Shiffrar, 2009), and forecast if an action heralds a competitive or cooperative interaction (Sartori, Becchio, & Castiello, 2011a). When observing action sequences, infants as well as adults show anticipatory fixations to the target areas of the displayed actions (Hunnius & Bekkering, 2010). Some studies designed to assess cortical activity of the primary motor cortex (M1) during action observation have shown that there is an anticipatory bias also in the motor response to observed actions (Candidi, Vicario, Abreu, & Aglioti, 2010; Kilner et al., 2004; Urgesi et al., 2010). For instance, motor facilitation has been found to be greater for images depicting hand actions in their initial-middle phases than for their final stages (Gangitano et al., 2001; Urgesi et al., 2010). In this perspective, predicting another person's behavior could have immediate implications for one's own action selection system because, depending on the output of action simulation, a suitable action can be selected from a multiplicity of possible alternatives (Bekkering, De Bruijn, Cuijpers, Newman-Norlund, Van Schie, & Meulenbroek, 2009; Sartori, Xompero, Bucchioni, & Castiello, 2012c).

Notably, recent findings speak in favor of both the hypothesis that motor activations provide a literal copy of the observed action (Cavallo, Sartori, & Castiello, 2011; Sartori et al., 2012a) and the hypothesis that the observer's motor system codes the distal goal of the observed acts, irrespective

4

of the actual movements (Cattaneo, Maule, Barchiesi, & Rizzolatti, 2013). These two hypotheses

are only seemingly contrasting, and can be reconciled considering that the relative influence of goal

and kinematics might depend on the amount of information available to the observer (Mc Cabe,

Villalta, Saunier, Grafton, & Della-Maggiore, 2014), on the time at which motor facilitation is

measured (Cavallo, Bucchioni, Castiello, & Becchio, 2013a; Janssen et al., 2013; Lago &

Fernandez-del-Olmo, 2011), and on the distinct components of the motor system which are

measured (Alaerts, Heremans, Swinnen, & Wenderoth, 2009; Barchiesi, Wache, & Cattaneo, 2012).

A growing interest on dynamic human interactions has also led to the discovery of divergent forms

of motor activations in effectors not primarily involved in the observed actions (Hamilton, 2013). In

particular, recent evidence suggests that compatibility effects in automatic imitation can be

overridden by social response preparation (Liepelt et al., 2010; Sartori, Bucchioni, & Castiello,

2013c).

Overall, these findings suggest that different coding levels develop during action observation.

The kinematic coding, which operates at a simple motor level; the predictive coding, which

anticipates the incoming actions; and the response coding, which allows an observer to prepare a

response that is compatible with task demands. Crucially, an integrated view of these three levels

has never been proposed, since a single effector can only be activated in one or the other modality

in a given moment. The present experiment was specifically designed to disentangle the relationship

between these levels and their relative contribution by measuring corticospinal excitability in

multiple effectors at different timings. We adopted a paradigm involving the observation of a soccer

player performing : (i) a penalty kick straight in the onlooker's direction and then coming to a full

stop (Fig. 1a), (ii) a penalty kick straight in the onlooker's direction and then continuing to run (Fig.

1b), (iii) a penalty kick to the side and then continuing to run (Fig. 1c). Single-pulse TMS was used

to assess CS excitability of participants' arm and leg muscles as they watched the videos.

We hypothesized that if motor coding purely reflects what is observed, then a motor resonant

activation should be found in the observers' leg muscles in all the conditions (Fig. 2a). On the other

5

hand, if a predictive coding is performed, then leg activation should not be found when the soccer player is going to stop (Fig. 2b). Finally, if motor coding reflects the preparation of an effector-specific response, then activation should be found in the upper limb muscles, but only during the final phase of the action sequences showing the approaching ball (Fig. 2c). That is, modulations between the still and run condition should reflect either kinematic or predictive coding (as these two conditions differ only in what the soccer player does after kicking), while modulation between the run and side condition would be due to response coding (since what differentiate the two conditions is whether the ball is directed or not toward the observer).

Materials and Methods

Participants

Thirty individuals were recruited. The data from one participant could not be used in the analysis due to technical problems. Twenty nine participants (21 female; M_{age} = 23 years, SD = 2.24) were then included in the final analysis. All the participants were right-handed (Briggs & Nebes, 1975), reported right-foot dominance, and normal or corrected-to-normal visual acuity. As their greater action simulation abilities could have biased the results, individuals with any motor expertise in playing soccer were excluded from the experiment by means of a pre-screening procedure. Athletes, in fact, present superior abilities in predicting and anticipating other players' actions (Abernethy et al., 2008; Aglioti et al., 2008; Makris & Urgesi, 2014; Sebanz & Shiffrar, 2009; Tomeo, Cesari, Aglioti, & Urgesi, 2012; Urgesi et al., 2012; Weissensteiner et al., 2008). Notably, also observational practice may contribute to action prediction abilities (Urgesi, Savonitto, Fabbro, & Aglioti, 2012), and soccer is a quite familiar sport. But in this case we presented an action sequence observed from the goal, and this is quite an uncommon view. None of the participants had any neurological, psychiatric, or other medical problems, nor did they have any contraindication to TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2009; Wassermann, 1998). None were aware of the experiment's purpose and all gave their written informed consent at the time they were recruited.

6

The study protocol was approved by the Ethics Committee of the University of Padova and was carried out in accordance with the principles of the Declaration of Helsinki. None of the participants reported experiencing discomfort or adverse effects during the experiment.

Stimuli

The stimuli were three digital video clips recorded in an ecological setting showing a soccer player: (i) kicking a ball straight toward the camera and then coming to a full stop (still condition; Fig. 1a), (ii) kicking a ball straight toward the camera and then continuing to run (run condition; Fig. 1b), and (iii) kicking a ball to the left side of the camera and then continuing to run (side condition; Fig. 1c). All of the videos were taken from a frontal view. We specifically devised them so that the different position of the foot at T_2 could be used to infer the future course of action. As we know from an extensive literature on this topic, observers can predict the fate of an action by quickly reading body cues (Abernethy et al., 2008; Aglioti et al., 2008; Knoblich & Flach, 2001; Sebanz & Shiffrar, 2009; Sartori et al., 2011a). Moreover, all the videos implied the same amount of perceived movement and the leg at T_2 was equally lifted in all conditions (i.e., 20 cm from the ground). Since we recorded muscle activity from the quadriceps femoris, and leg extension best targets this muscle, no difference was expected across conditions. A 1800 ms sequence was extracted from each of the videos which included the player's initial run and the ball's trajectory until it disappeared. The player's foot made contact with the ball approximately 1350 ms after the video began and the ball trajectory reached its highest peak approximately 400 ms later (1750 ms after onset of the video). The ball was travelling at a velocity of approximately 10 m/s during its trajectory. An animation effect was obtained by presenting a series of single frames each lasting 25 ms (resolution 720 x 576 pixels, color depth 24 bits, frame rate 30 fps) following the first frame which lasted 500 ms. A preliminary pilot investigation, carried out with a questionnaire and the assistance of a group of participants with characteristics that were similar to those participating in the study experiment,

7

confirmed that only observing the approaching ball led in the onlooker an impulse to react with the

upper limbs (97% of positive responses).

Procedure

The participants were tested individually in a sound-attenuated Faraday room during a single

experimental session lasting approximately 40 minutes and consisting in two blocks (upper limb,

lower limb). Each participant was directed to sit in a slightly raised armchair with his/her legs

comfortably stretched, the right arm was positioned on an arm support and the head on a fixed head

rest. Each was instructed to remain as still and relaxed as possible and to watch the 4 video clips

that were presented on a 19" monitor (resolution 1280 x 1024 pixels, refresh frequency 75 Hz,

background luminance of 0.5 cd/m2) set at eye level (the eye-screen distance was 80 cm). To ensure

that the participants paid attention to the contents of the video clips, they were told that they would

be questioned at the end of the session about the visual stimuli presented. TMS-induced MEPs were

acquired from the participant's right flexor carpi ulnaris (FCU) muscle and from the participant's

right quadriceps femoris (QF) muscle. Upper and lower limb activity was recorded in separate

blocks to precisely identify the optimal scalp locations (OSP) for stimulating each muscle and avoid

loss of modulation involving the less stimulated muscle. The order in which the two blocks were

presented was counterbalanced across the participants. A single TMS pulse was released during

each video presentation at one of two specific time points: (i) during the frame showing the player's

foot making contact with the ball (T_1; 1350 ms) and (ii) during the frame showing the highest peak

of the ball's trajectory (T_2; 1750 ms). The same timing was applied to both the conditions. The first

time point (T_1) was chosen to evaluate the motor resonant response. As recently demonstrated by

Lago and Fernandez-del-Olmo (2011), an unspecific motor activation was found in the hand

muscles of participants observing an effector before it made contact with an object. When the

effector–object interaction was, instead, shown, the motor program activated via action observation

was muscle specific. The second time point (T_2) was set at the highest peak of the ball's trajectory

8

just before it disappeared as we intended to maximize the reaction to the stimulus. The order of the

three videos and of the two different TMS delays were randomized within each of the two blocks.

The observer could not know in advance whether the player would continue running or would stop,

neither whether the player would kick straight or to the side. A total of 120 MEPs (2 muscles $\times$ 3

conditions $\times$ 10 repetitions $\times$ 2 time points) was recorded for each participant. Prior to presenting

the videos, each participant's baseline was assessed by acquiring 10 MEPs per block while they

passively watched a white-colored fixation cross (10x10 mm) on a black background on the

computer screen. Ten more MEPs were recorded at the end of each block. By comparing MEP

amplitudes recorded during the two baseline series it was possible to check for any CS excitability

changes related to TMS per se in each block. The average amplitude of the two series was then

utilized to set each participant's individual baseline for data normalization procedure. An inter-

pulse interval lasting 10 s was presented between trials in order to minimize the potential risk of

carryover effect of a TMS pulse on the subsequent one. During the first 5 seconds of the rest period,

a message reminding the participants to keep their arms and legs still and fully relaxed appeared on

the screen. Stimuli presentation, EMG recordings and timing of TMS stimulation were managed by

E-Prime V2.0 software (Psychology Software Tools) running on a PC.

Data recording

Transcranial magnetic stimulation

Single-pulse TMS (pulse characteristics: 100μs rise time, 1ms duration) was delivered using a 70

mm figure-of-eight coil (Magstim polyurethane-coated coil) connected to a Magstim BiStim^2

stimulator (The Magstim Company, UK). Pulses were delivered to the left M1 corresponding to the

forearm and leg regions during the 'upper limb' and 'lower limb' blocks, respectively. The coil was

placed tangentially on the scalp, with the handle pointing laterally and caudally (Brasil-Neto et al.,

1992; Mills, Boniface, & Schubert, 1992). The OSP was determined by moving the intersection of

the coil in approximately 0.5 cm steps around the target area until a position was reached at which a

9

maximal MEP amplitude was produced in the target muscle with a minimal stimulation intensity.

This position was marked on a tight-fitting cap that each participant was asked to wear. During the

experimental sessions the coil was held by a tripod with an articulated arm. The position and

orientation of the coil over the OSP was recorded and loaded into the Brainsight 2.0

neuronavigation system (Rogue Research, Montreal QC) to maintain accurate placement of the coil

throughout the experiment. Defined as the minimum stimulation intensity on the OSP that induced

reliable MEPs ($\geq$ 50 µV peak-to-peak amplitude) in a relaxed muscle in five out of ten consecutive

trials, the resting motor threshold (rMT) was determined for each participant. rMT ranged from

34% to 59% (mean = 46%, SD = 6.19) of the maximum stimulator output in the upper limb block

and from 50% to 65% (mean = 57%, SD = 4.45) in the lower limb one. Stimulation intensity was

set at 120% of the rMT to record a clear and stable EMG signal and avoid floor or ceiling effects.

Electromyography

MEPs were recorded from the flexor carpi ulnaris (FCU) muscle of the right arm and from the

quadriceps femoris (QF) of the right leg. EMG activity was recorded through pairs of surface Ag-

AgCl cup electrodes (9 mm diameter) placed in a belly-tendon montage. The ground electrode was

placed over the dorsal part of the elbow during the upper limb block and over the patella of the leg

during the lower limb block. The skin impedance condition, evaluated at rest prior to beginning the

experimental session, was considered of good quality when below the threshold level (5 Ohm).

Electrodes were connected to an isolable portable ExG input box linked to the main EMG amplifier

for signal transmission via a twin fiber optic cable (Professional BrainAmp ExG MR). The raw

myographic signals were band-pass filtered (20 Hz – 1 kHz), amplified prior to being digitalized (5

KHz sampling rate), and stored on a computer for off-line analysis. EMG data were recorded for a

300 ms interval. The interval was time-locked to the delivery of the magnetic stimulation pulse and

began 100 ms prior to the onset of stimulation and ended 200 ms post-stimulation. Trials in which

any EMG activity was present in the time window preceding the TMS pulse were discarded to prevent contamination of MEP measurements by background EMG activity.

Data analysis

The CS excitability of FCU and QF muscles was quantified at each stimulation point during each experimental condition by the MEP peak-to-peak amplitude (mV). Those amplitudes deviating more than 3 standard deviations from the mean and the trials contaminated by muscular pre-activation were excluded as outliers (< 6%). A paired-sample t-test (2-tailed) was used to compare the amplitude of MEPs recorded during the two baseline trials carried out at the beginning and at the end of each block. Ratios were computed using the participants' individual mean MEP amplitude recorded during the two fixation-cross periods as baseline (MEP ratio = MEPobtained/MEPbaseline). We entered the MEP ratios in a repeated-measures ANOVA with muscle (FCU, QF), condition (still, run, side) and stimulation time (T_1, T_2) as within-subjects factors. The sphericity of the data was verified prior to performing statistical analysis (Mauchly's test, $p > 0.05$). Post-hoc pairwise comparisons were carried out using t-tests and Bonferroni correction was applied to control P-values for multiple comparisons. A significance threshold of $P < 0.05$ was set for all statistical analyses.

Results

The mean raw MEP amplitudes during the pre- and post- experimental session were not significantly different in the FCU ($t_{28} = 1.416$, $p > 0.05$) or the QF ($t_{28} = -0.037$, $p > 0.05$) muscles. We can thus conclude that TMS per se did not induce any significant, nonspecific change in motor corticospinal excitability during the study that could have confounded the results. The ANOVA on normalized MEP amplitudes showed a statistically significant 2-way interaction of muscle × condition ($F(2,56) = 6.415$, $p < 0.05$, $\eta^2_p = 0.186$), a significant 2-way interaction of muscle × time

11

$(F(1,28) = 9.669, p < 0.05, \eta^2_p = 0.257)$, and a significant 3-way interaction of muscle × time ×

condition $(F(2,56) = 5.193, p < 0.05, \eta^2_p = 0.156)$. The results obtained from the post-hoc contrasts

exploring the source of the significant 3-way interaction are outlined as follows.

Kinematic coding

Post-hoc comparisons during the initial part of the action sequence (T_1) showed a significant

activation in the QF muscle, compared to the final part of the action sequence (T_2), but only when

the player was shown in a still position $(p < 0.05$; Fig. 2a). This seems to reflect a muscle-specific

motor resonant effect, in line with the observed movement. Interestingly, post-hoc comparisons

confirmed that the same activation was found at T_1 in the QF muscle when comparing the 'still'

with the 'run' $(p = 0.941)$ and the 'side' $(p = 0.771)$ conditions (Table 1).

Predictive coding

Post-hoc comparisons during the final part of the action sequence (T_2) showed instead a higher leg

activity both in the 'run' $(p < 0.001)$ and the 'side' conditions $(p < 0.05)$ compared to the 'still'

condition (Table 1). The same muscular activity was found across time points (T_1, T_2) for the 'run'

condition $(p = 0.604$; Fig. 2b) and the 'side' condition $(p = 0.381$; Fig. 2c). Notably, TMS was

delivered at the same time point in all these videos (i.e., right after kicking the ball, during the

maximum extension of the player's leg). This seem to suggest that the increase in corticospinal

excitability for the 'run' and the 'side' conditions was finely tuned to the following phase of the

observed action (i.e., continuing to run).

Response coding

As concerns the pattern of variation observed for the upper limbs, the mean MEP amplitude of the

FCU muscle was higher when the ball was approaching the observer (T_2) compared to the initial

part of the action sequence (T_1) both for the 'still' and the 'run' conditions $(p_s < 0.05$; Fig. 1a,b), but

12

not for the 'side' condition, in which the ball was kicked to the left ($p = 0.561$; Fig. 1c). This indicates that the observer's upper limb activation only occurred when the perceived action directly involved him/her. Post-hoc comparisons at T_2 confirmed this effect showing that in the 'run' and the 'still' conditions the activation in the FCU muscle was higher than in the 'side' condition ($p_s < 0.05$), while at T_1 the same low MEP amplitude was found across all conditions ($p_s < 0.05$; Fig. 1a,b,c).

Discussion

The aim of this study was to disentangle the relative contribution and combination of different levels of motor coding during action observation. The experimental design allowed us to determine whether, as the action unfolded, modulation of corticospinal output either proceeded in conformity with the action that was seen, was modulated by what was expected, or was altered in accordance with the appropriate response to carry out. The results showed a modulation of output to quadriceps femoris consistent with the observed kick during the first phase of the action sequence in all the three videos - thus supporting the kinematic coding. However, only observing the soccer player before performing the run protracted motor resonance in the lower limb muscle to the final phase of the action sequences - thus indicating the existence of a predictive coding. Crucially, motor activation in flexor carpi ulnaris was evident only during the terminal phase of the motion sequence showing the approaching ball - thus pointing to the existence of a mechanism specifically tailored for preparing an effector-specific response (i.e., blocking the ball). Our experimental findings show for the first time that observing another person's body movements can prompt three different levels of motor coding in a not mutually exclusive way: the internal replica of the observed action (kinematic coding), the predictive activation (predictive coding), and the adaptive integration of congruent and non-congruent reactions (response coding). The findings presented here confirm and extend previous literature indicating both a low-level correspondence between the effectors of the

13

person being observed and the observer's ones (Avenanti, Bolognini, Maravita, & Aglioti, 2007; Borroni et al., 2005; Borroni & Baldissera, 2008; Fadiga et al., 1995; Gangitano et al., 2001; Montagna et al., 2005; Strafella & Paus, 2000; Urgesi et al., 2006) and an anticipatory modulation of motor activations according to the final end of the perceived movement. While the video in which the soccer player suddenly stopped after kicking the ball ('still' condition) determined a decrease in CS excitability - signaling a transition to the motor profile designated by the action seen, observing the videos in which the player continued to run extended CS activity until the final phase of the action sequence. Notably, TMS was delivered at the very same time point in all conditions, that is at the moment of maximum leg extension of the soccer player after kicking the ball, right before the second step (i.e., stopping or continuing to run). This suggests a motor activation in line with the predicted movement (Kilner et al., 2004; Knoblich & Flach, 2001), finely tuned to early cues in the observed actions (Aglioti et al., 2008; Sartori et al., 2011a; Makris & Urgesi, 2014; Stapel, Hunnius, & Bekkering, 2012). Predicting another person's behavior has immediate implications for one's own action selection system because, depending on the output of action simulation, a suitable action can be selected from a multiplicity of possible alternatives (Bekkering et al., 2009; Sartori et al., 2012c). In social interactive contexts, in particular, the initially observed motor act must be coded from the very beginning in terms of the subsequent steps required to fulfill the overall action goal. A point worth noting is that much of previous work investigating predictive processes during action observation by means of magnetic stimulation of the human primary motor cortex and electromyography recording of participants' muscles was performed while they were watching transitive (i.e., goal directed) movements (e.g., Urgesi et al., 2010). Here, we found evidence of predictive coding also with intransitive actions. A finding in accordance with behavioral (Bertenthal, Longo, & Kosobud, 2006; Liepelt et al., 2010) and neurophysiological (Press, Bird, Walsh, & Heyes, 2008) studies showing motor facilitation effects for transitive as well as intransitive actions.

14

Moreover, the present findings extends previous evidence on response preparation in hand muscles (Newman-Norlund, Noordzij, Meulenbroek, & Bekkering, 2007; Ocampo & Kritikos, 2010; Sartori, Cavallo, Bucchioni, & Castiello, 2011b, 2012b; Sartori, Betti, & Castiello, 2013a, 2013b; Sartori et al., 2013c) showing a modulation of CS excitability also when the observed action calls for a gesture involving different body parts with respect to the observed ones. In the present study, observers' upper limb muscles were activated while observing a soccer player kicking a ball straight in their direction. It should be noted that evidence for fast and automatic motor resonant responses comes from paradigms that usually required a fixed stimulus-response matching. Our data suggest that when the observed action triggers a spontaneous reaction in effectors not involved in the observed action, motor coding can be influenced by a top-down mechanism related to the observer's action intentions (Ondobaka, de Lange, Newman-Norlund, Wiemers, & Bekkering, 2012; Ondobaka, de Lange, Wittmann, Frith, & Bekkering, 2014). Consistent with that finding, Longo and colleagues (Longo, Kosobud, & Bertenthal, 2008) reported that automatic imitation is modulated by top-down influences. They demonstrated that the level of action coding can be changed (e.g., towards coding in terms of movements) depending on task requirements.

In terms of alternative coding levels, the findings outlined here suggest that different processes – providing literal copies of the observed action, predictive and non-congruent muscular activations – can coexist in a not mutually exclusive way. Prior to the present study, there has been no such direct evidence. In contrast, previous investigations argued that motor resonant plans, once primed, either proceed to completion or are suppressed if discrepancies are revealed by visual input (Gangitano, Mottaghy, & Pascual-Leone, 2004). Interestingly, recent evidence shows that action sequences are encoded separately, rather than as indivisible ensembles (Janssen et al., 2013). This might explain why variations of CS output can reflect different coding levels, depending on the interplay between actual and expected movements.

According to Chinellato and colleagues (Chinellato, Ognibene, Sartori, & Demiris, 2013), the switch from congruent to incongruent motor simulation would be part of a dynamic interplay

15

between the Action Observation System (AOS) and the Action Planning System (APS). The AOS is in charge of monitoring the actions of the person being observed, mainly by matching them to the observer's own motor repertoire (low-level coding). The APS is, instead, the neural system able to plan and monitor the execution of all types of actions (high-level coding). Automatic imitation (Heyes, 2011) and mirroring effects (e.g. Di Pellegrino et al., 1992, Fadiga et al., 2005), as well as the increased CS excitability for congruent motor responses highlighted in this study, seem to indicate that AOS controls by default the motor system, producing a resonant response to observed behaviors. When a complementary, incongruent action is expected or required, such as when the ball is getting in the observer direction, and the natural response is to prepare for blocking it, the AOS needs to leave the control of the motor system to the APS. Notably, when the APS takes control over the AOS, monitoring the other person's actions is still performed by the AOS, and could directly affect on-line action execution. The process of selecting the appropriate action, therefore, does not necessarily bypass low-level motor simulation, but seems to proceed in a parallel way. The fact that CS excitability seems to travel along parallel lines raises an interesting questions: If observing an action performed using a specific effector can trigger responses in different muscles, what mechanism selects the effectors and the motor pattern needing to be activated for an appropriate response? We propose it is an associative memory which by default implements a mirroring behavior, but that can adapt through experience to generate complementary responses: the existence of counter-mirror effects supports indeed this hypothesis (Catmur, Walsh, & Heyes, 2007; Barchiesi & Cattaneo, 2013; Cavallo, Heyes, Becchio, Bird, & Catmur, 2013b).

It is our contention that motor resonance's role is to maintain a functional trace of an observed action in order to facilitate selection between alternatives when there are a variety of possible responses (Sartori et al., 2012b, 2012c, 2013b). Running different coding levels while processing the observed action would be helpful to prepare an correct reaction (Bekkering et al., 2009). According to the theoretical framework proposed by Chinellato and colleagues (2013), a two level competition is at the basis of the mechanism for choosing appropriate responses to an observed

16

action. Not only it is necessary to find the motor pattern that matches the observed action (e.g. kicking the ball) and the one which constitutes the most suitable complementary response (e.g. blocking the ball), but also to decide whether either or both should be actually executed (in the above example, kicking should be inhibited to avoid affecting the quality of the blocking response). In this vein, it is worth noting that since observers cannot foveate both the player's leg and the approaching ball, attentional mechanisms could also be linked to the results outlined here. If attention is critical for motor resonance, motor resonant neural responses to observed actions should diminish whenever a participant's attention is diverted from action observation. And, in accordance with some reports concerning the link between attention and mirrored actions (Bach, Peatfield, & Tipper, 2007; Chong, Cunnington, Williams, & Mattingley, 2009), it is possible that when our soccer player kicked the ball straight in the onlooker's direction and came to a stop, MEP activation was confined to the upper limb muscles because the participants' attention was focused on the ball. When, instead, the soccer player continued running after kicking the ball, MEP activity slightly decreased in the arm muscle because the participants' attention was directed towards two aspects of the scene: the soccer player's leg and the ball. Divided attention may in this case have led to a selective reduction in processing efficiency (Castiello & Umiltà, 1990, 1992). This hypothesis is partially confirmed by the decrease in MEPs activity evident for the upper limb muscles during the observation of the soccer player running after kicking the ball (Fig. 2a,b).

In conclusion, the findings from this experiment provide for the first time neurophysiologic evidence of a dynamic interplay between three different levels of motor coding according to different predicted end-state of the same observed movement (i.e. stopping after kicking vs. continuing to run; kicking straight vs. to the side) promoting a parallel activation of different responses to action observation in different effectors.

17

ACKNOWLEDGEMENT

REFERENCES

Abernethy, B., Zawi, K., & Jackson, R.C. (2008). Expertise and attunement to kinematic constraints. *Perception,* 37(6), 931–948.

Aglioti, S. M., Cesari, P., Romani, M., & Urgesi, C. (2008). Action anticipation and motor resonance in elite basketball players. *Nature Neuroscience*, 11(9), 1109–1116.

Alaerts, K., Heremans, E., Swinnen, S. P., & Wenderoth, N. (2009). How are observed actions mapped to the observer's motor system? Influence of posture and perspective. *Neuropsychologia*, 47(2), 415–422.

Avenanti, A., Bolognini, N., Maravita, A., & Aglioti, S. M. (2007). Somatic and motor components of action simulation. *Current Biology*, 17(24), 2129–2135.

Bach, P., Peatfield, N. A., & Tipper, S. P. (2007). Focusing on body sites: the role of spatial attention in action perception. *Experimental Brain Research,* 178(4), 509–517.

Barchiesi, G., & Cattaneo, L. (2013). Early and late motor responses to action observation. *Social Cognitive and Affective Neuroscience*, 8(6), 711–719.

Barchiesi, G., Wache, S., & Cattaneo, L. (2012). The frames of reference of the motor-visual aftereffect. *PloS one*, 7(7), e40892.

Bekkering, H., De Bruijn, E. R., Cuijpers, R. H., Newman-Norlund, R., Van Schie, H. T., & Meulenbroek, R. (2009). Joint action: Neurocognitive mechanisms supporting human interaction. *Topics in Cognitive Science*, 1(2), 340–352.

Bertenthal, B. I., Longo, M. R., & Kosobud, A. (2006). Imitative response tendencies following observation of intransitive actions. *Journal of Experimental Psychology: Human Perception and Performance,* 32(2), 210–225.

Borroni, P., & Baldissera, F. (2008). Activation of motor pathways during observation and execution of hand movements. *Social Neuroscience*, 3(3-4), 276–288.

Borroni, P., Montagna, M., Cerri, G., & Baldissera, F. (2005). Cyclic time course of motor excitability modulation during the observation of a cyclic hand movement. *Brain research*, 1065(1), 115–124.

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *Journal of Clinical Neurophysiology*, 9(1), 132–136.

Brass, M., & Heyes, C. M. (2005). Imitation: Is cognitive neuroscience solving the correspondence problem? *Trends in Cognitive Science,* 9(10), 489–495.

Briggs, G. G., & Nebes, R. D. (1975). Patterns of hand preference in a student population. *Cortex*, 11(3), 230–238.

Candidi, M., Vicario C. M., Abreu, A. M., & Aglioti, S. M. (2010). Competing mechanisms for mapping action-related categorical knowledge and observed actions. *Cerebral Cortex,* 20, 2832–2841.

Castiello, U., & Umiltà, C. (1990). Size of the attentional focus and efficiency of processing. *Acta psychologica,* 73(3), 195–209.

Castiello, U., & Umiltà, C. (1992). Splitting focal attention. *Journal of Experimental Psychology: Human Perception and Performance*, 18 (3), 837.

Catmur, C., Walsh, V., & Heyes, C. (2007). Sensorimotor learning configures the human mirror system. *Current Biology*, 17(17), 1527–1531.

Cattaneo, L., Maule, F., Barchiesi, G., & Rizzolatti, G. (2013). The motor system resonates to the distal goal of observed actions: testing the inverse pliers paradigm in an ecological setting. *Experimental Brain Research*, 231(1), 37–49.

Cavallo, A., Bucchioni, G., Castiello, U., & Becchio, C. (2013a). Goal or movement? Action representation within the primary motor cortex. *European Journal of Neuroscience*, 38(10), 3507–3512.

Cavallo, A., Heyes, C., Becchio, C., Bird, G., & Catmur, C. (2013b). Timecourse of mirror and counter-mirror effects measured with transcranial magnetic stimulation. *Social Cognitive and Affective Neuroscience*, 9(8), 1082–1088.

Cavallo, A., Sartori, L., & Castiello, U. (2011). Corticospinal excitability modulation to hand muscles during the observation of appropriate versus inappropriate actions. *Cognitive neuroscience*, 2(2), 83–90.

20

Chinellato, E., Ognibene, D., Sartori, L., & Demiris, Y. (2013). Time to change: deciding when to switch action plans during a social interaction. In Lepora, N., Mura, A., Krapp, H., Verschure, P., & Prescott, T. (Eds.), *Biomimetic and Biohybrid Systems* (pp. 47–58). Springer Berlin Heidelberg.

Chong, T. T., Cunnington, R., Williams, M. A., & Mattingley, J.B. (2009). The role of selective attention in matching observed and executed actions. *Neuropsychologia,* 47(3), 786–795.

Di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. *Experimental Brain Research*, 91(1), 176–180.

Fabbri-Destro, M., & Rizzolatti G. (2008). Mirror neurons and mirror systems in monkeys and humans. *Physiology,* 23(3), 171–179.

Fadiga, L., Craighero, L., & Olivier, E. (2005). Human motor cortex excitability during the perception of others' action. *Current Opinion in Neurobiology*, 15(2), 213–218.

Fadiga, L., Fogassi, L., Pavesi, G., & Rizzolatti, G. (1995). Motor facilitation during action observation: a magnetic stimulation study. *Journal of Neurophysiology*, 73(6), 2608–2611.

Gallese, V., & Goldman, A. (1998). Mirror neurons and the simulation theory of mind-reading. *Trends in Cognitive Science,* 2(12), 493–501.

Gallese, V., Fadiga, L., Fogassi, L., & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain,* 119(2), 593–609.

Gangitano, M., Mottaghy, F. M., & Pascual-Leone, A. (2001). Phase-specific modulation of cortical motor output during movement observation. *Neuroreport*, 12(7), 1489–1492.

Gangitano, M., Mottaghy, F. M., & Pascual-Leone, A. (2004). Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements. *European Journal of Neuroscience*, 20(8), 2193–2202.

Giorello, G., & Sinigaglia, C. (2007). Perception in action. *Acta Biomedica,* 78(1), 49–57.

Grezes, J., & Decety, J. (2001). Functional anatomy of execution, mental simulation, observation, and verb generation of actions: a meta-analysis. *Human Brain Mapping*, 12(1), 1–19.

Hamilton, A. F. (2013). The mirror neuron system contributes to social responding. *Cortex*, 49(10), 2957–2959.

Heyes, C. (2011). Automatic imitation. *Psychological Bulletin*, 137(3), 463–483.

21

Hunnius, S., & Bekkering, H. (2010). The early development of object knowledge: A study of infants' visual anticipations during action observation. *Developmental Psychology,* 406(2), 446.

Janssen, L., Steenbergen, B., & Carson, R. G. (2013). Anticipatory planning reveals segmentation of cortical motor output during action observation. *Cerebral Cortex*, bht220.

Keysers, C. (2009). Mirror neurons. *Current Biology,* 19(21), R971–973.

Kilner, J. M., Vargas, C., Duval, S., Blakemore, S. J., & Sirigu, A. (2004). Motor activation prior to observation of a predicted movement. *Nature Neuroscience*, 7(12), 1299–1301.

Knoblich, G., & Flach, R. (2001). Predicting the effects of actions: Interactions of perception and action. *Psychological Science*, 12(6), 467–472.

Lago, A., & Fernandez-del-Olmo, M. (2011). Movement observation specifies motor programs activated by the action observed objective. *Neuroscience Letters*, 493(3), 102–106.

Liepelt, R., Prinz, W., & Brass, M. (2010). When do we simulate non-human agents? Dissociating communicative and non-communicative actions. *Cognition,* 115(3), 426–434.

Longo, M. R., Kosobud, A., & Bertenthal, B. I. (2008). Automatic imitation of biomechanically possible and impossible actions: Effects of priming movements versus goals. *Journal of Experimental Psychology: Human Perception and Performance*, 34(2), 489–501.

Makris, S., & Urgesi, C. (2014). Neural underpinnings of superior action prediction abilities in soccer players. *Social Cognitive and Affective Neuroscience*. Advance online publication. doi: 10.1093/scan/nsu052

Mc Cabe, S. I., Villalta, J. I., Saunier, G., Grafton, S. T., & Della-Maggiore, V. (2014). The Relative Influence of Goal and Kinematics on Corticospinal Excitability Depends on the Information Provided to the Observer. *Cerebral Cortex.* Advance online publication. doi: 10.1093/cercor/bhu029

Mills, K. R., Boniface, S. J., & Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 85(1), 17–21.

Molenberghs, P., Cunnington, R., & Mattingley, J. B. (2012). Brain regions with mirror properties: A meta-analysis of 125 human fMRI studies. *Neuroscience & Biobehavioral Reviews,* 36(1), 341–349.

22

Montagna, M., Cerri, G., Borroni, P., & Baldissera, F. (2005). Excitability changes in human corticospinal projections to muscles moving hand and fingers while viewing a reaching and grasping action. *European Journal of Neuroscience*, 22(6), 1513–1520.

Mukamel, R., Ekstrom, A.D., Kaplan, J., Iacoboni, M., & Fried, I. (2010). Single-neuron responses in humans during execution and observation of actions. *Current Biology,* 20(8), 750–756.

Newman-Norlund, R. D., Noordzij, M. L., Meulenbroek, R. G., & Bekkering, H. (2007). Exploring the brain basis of joint action: co-ordination of actions, goals and intentions. *Social Neuroscience*, 2(1), 48–65.

Ocampo, B., & Kritikos, A. (2010). Placing actions in context: motor facilitation following observation of identical and non-identical manual acts. *Experimental Brain Research*, 201(4), 743–751.

Ondobaka, S., de Lange, F. P., Newman-Norlund, R. D., Wiemers, M., & Bekkering, H. (2012). Interplay between action and movement intentions during social interaction. *Psychological Science*, *23*(1), 30–35.

Ondobaka, S., de Lange, F. P., Wittmann, M., Frith, C. D., & Bekkering, H. (2014). Interplay Between Conceptual Expectations and Movement Predictions Underlies Action Understanding. *Cerebral Cortex*. Advance online publication. doi: 10.1093/cercor/bhu056

Press, C., Bird, G., Walsh, E., & Heyes, C. M. (2008). Automatic imitation of intransitive actions. *Brain & Cognition,* 67(1), 44–50.

Rizzolatti, G., & Craighero, L. (2004). The mirror-neuron system. *Annual Review of Neuroscience, 27*, 169–192.

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, 120(12), 2008–2039.

Sartori, L., Becchio, C., & Castiello, U. (2011a). Cues to intention: the role of movement information. *Cognition*, 119(2), 242–252.

Sartori, L., Betti, S., & Castiello, U. (2013a). Corticospinal Excitability Modulation During Action Observation. *Journal of Visualized Experiments*, 82, e51001–e51001.

23

Sartori, L., Betti, S., & Castiello, U. (2013b). When mirroring is not enough: that is, when only a complementary action will do (the trick). *NeuroReport*, 24(11), 601–604.

Sartori, L., Bucchioni, G., & Castiello, U. (2012a). Motor cortex excitability is tightly coupled to observed movements. *Neuropsychologia*, 50(9), 2341–2347.

Sartori, L., Bucchioni, G., & Castiello, U. (2013c). When emulation becomes reciprocity. *Social Cognitive and Affective Neuroscience*, 8(6), 662–669.

Sartori, L., Cavallo, A., Bucchioni, G., & Castiello, U. (2011b). Corticospinal excitability is specifically modulated by the social dimension of observed actions. *Experimental Brain Research*, 211(3-4), 557–568.

Sartori, L., Cavallo, A., Bucchioni, G., & Castiello, U. (2012b). From simulation to reciprocity: the case of complementary actions. *Social Neuroscience*, 7(2), 146–158.

Sartori, L., Xompero, F., Bucchioni, G., & Castiello, U. (2012c). The transfer of motor functional strategies via action observation. *Biology Letters*, 8(2), 193–196.

Sebanz, N., & Shiffrar, M. (2009). Detecting deception in a bluffing body: The role of expertise. *Psychonomic Bulletin & Review*, 16(1), 170–175.

Stapel, J. C., Hunnius, S., & Bekkering, H. (2012). Online prediction of others' actions: the contribution of the target object, action context and movement kinematics. *Psychological Research*, 76(4), 434–445.

Strafella, A. P., & Paus, T. (2000). Modulation of cortical excitability during action observation: a transcranial magnetic stimulation study. *Neuroreport*, 11(10), 2289–2292.

Tomeo, E., Cesari, P., Aglioti, S. M., & Urgesi, C. (2012). Fooling the kickers but not the goalkeepers: behavioral and neurophysiological correlates of fake action detection in soccer. *Cerebral Cortex*, 23(11), 2765–2778.

Turella, L., Tubaldi, F., Erb, M., Grodd, W., & Castiello, U. (2012). Object presence modulates activity within the somatosensory component of the action observation network. *Cerebral Cortex,* 22(3), 668–679.

Urgesi, C., Candidi, M., Fabbro, F., Romani, M., & Aglioti, S. M. (2006). Motor facilitation during action observation: topographic mapping of the target muscle and influence of the onlooker's posture. *European Journal of Neuroscience*, 23(9), 2522–2530.

24

Urgesi, C., Maieron, M., Avenanti, A., Tidoni, E., Fabbro, F, & Aglioti, S. M. (2010). Simulating the future of actions in the human corticospinal system. *Cerebral Cortex,* 20(11), 2511–2521.

Urgesi, C., Savonitto, M. M., Fabbro, F., & Aglioti, S. M. (2012). Long-and short-term plastic modeling of action prediction abilities in volleyball. *Psychological Research*, 76(4), 542–560.

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 108(1), 1–16.

Weissensteiner, J., Abernethy, B., Farrow, D., & Müller, S. (2008). The development of anticipation: A cross-sectional examination of the practice experiences contributing to skill in cricket batting. *Journal of Sport & Exercise Psychology*, 30(6), 663–684.

Figure Captions

Figure 1 Sequence of events taking place for each condition: (a) Still, (b) Run, (c) Side. The vertical lines denote the time points when single TMS pulses were delivered: at T_1 (when the player's foot makes contact with the ball) and at T_2 (when the ball trajectory reaches its highest peak).

Figure 2 Illustrative representation of the three levels of motor coding (Kinematic, Predictive, Response) and corresponding expected muscular dissociations. We hypothesize that while at T_1 the CS excitability should be equal in all muscles through all conditions, it should clearly dissociate at T_2 (e.g., in the Still condition), depending on motor coding.

Figure 3 Corticospinal activations during observation of a soccer player: (a) kicking the ball straight and then coming to a full stop (Still), (b) kicking the ball straight and then continuing to run (Run), (c) kicking the ball to the side and then continuing to run (Side). Note that the following contrasts were significant: the normalized mean MEP amplitudes in the lower limb muscle (QF) were lower at T_2 compared to T_1 in the 'Still' condition ($p < 0.05$) and were higher at T_2 both in the 'Run' ($p < 0.001$) and the 'Side' conditions ($p < 0.05$) compared to the 'Still' condition. The normalized mean MEP amplitudes recorded from the flexor carpi ulnaris (FCU) muscle were higher at T_2 compared to T_1 both in the 'Still' ($p < 0.05$) and the 'Run' ($p < 0.05$) conditions, but not for the 'Side' condition.
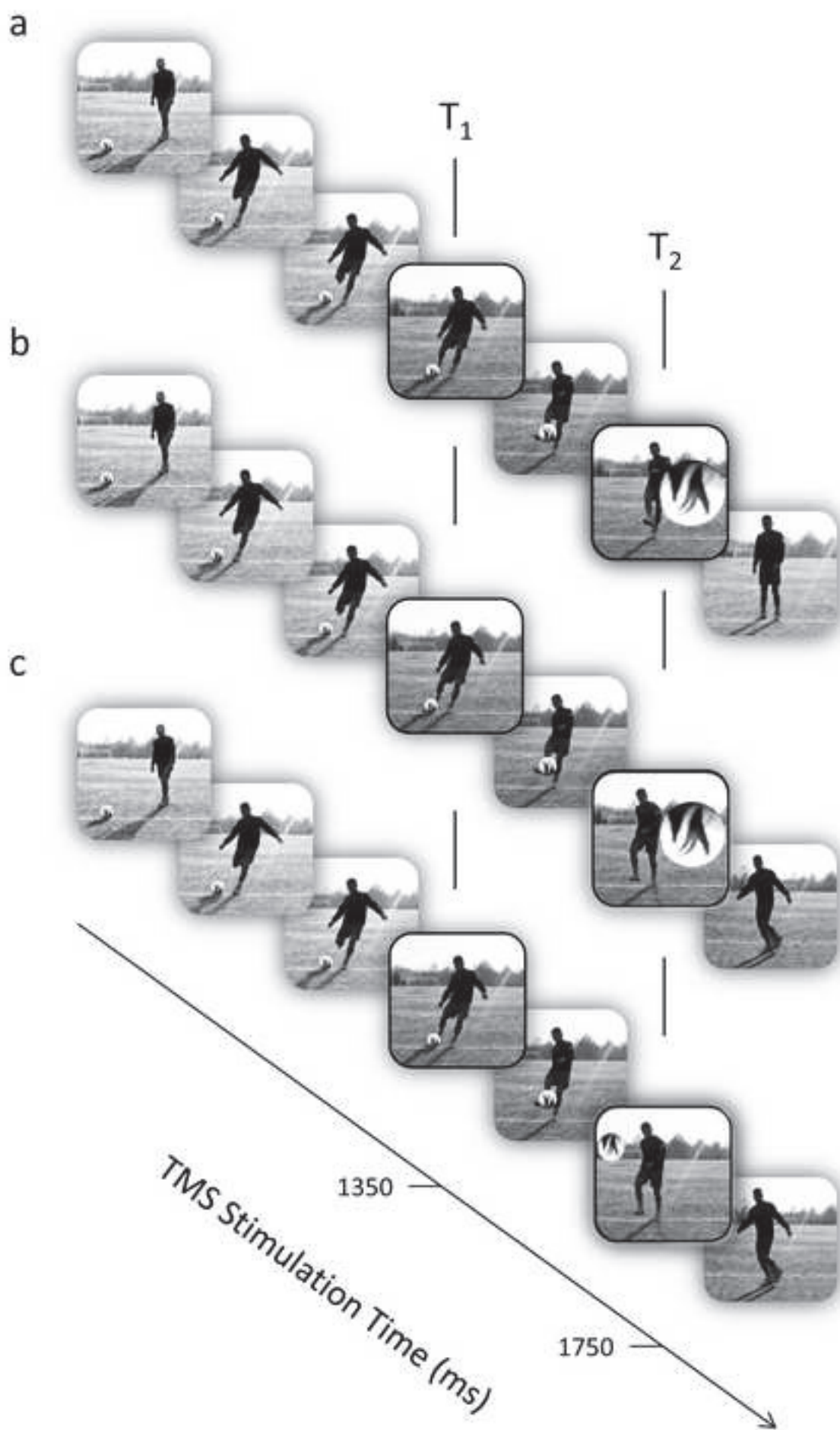
26

Table 1. Normalized mean (± SEM) peak to peak amplitude of MEPs recorded from the FCU and the QF muscles during the three experimental conditions at each time point.

	Still		Run		Side	
	T_1	T_2	T_1	T_2	T_1	T_2
FCU	1.043 (± 0.048)	1.119 (± 0.047)	1.026 (± 0.043)	1.102 (± 0.044)	1.045 (± 0.050)	1.017 (± 0.024)
QF	1.102 (± 0.069)	0.884 (± 0.042)	1.097 (± 0.060)	1.067 (± 0.036)	1.116 (± 0.066)	1.086 (± 0.060)

Highlights

- Kinematic, predictive, and response coding can coexist in M1 output.

- Motor coding is influenced both by top-down and bottom-up mechanisms.

- An associative memory would implement both mirroring and complementary responses.

- These three levels can be unified into an integrated view of cortical motor coding.
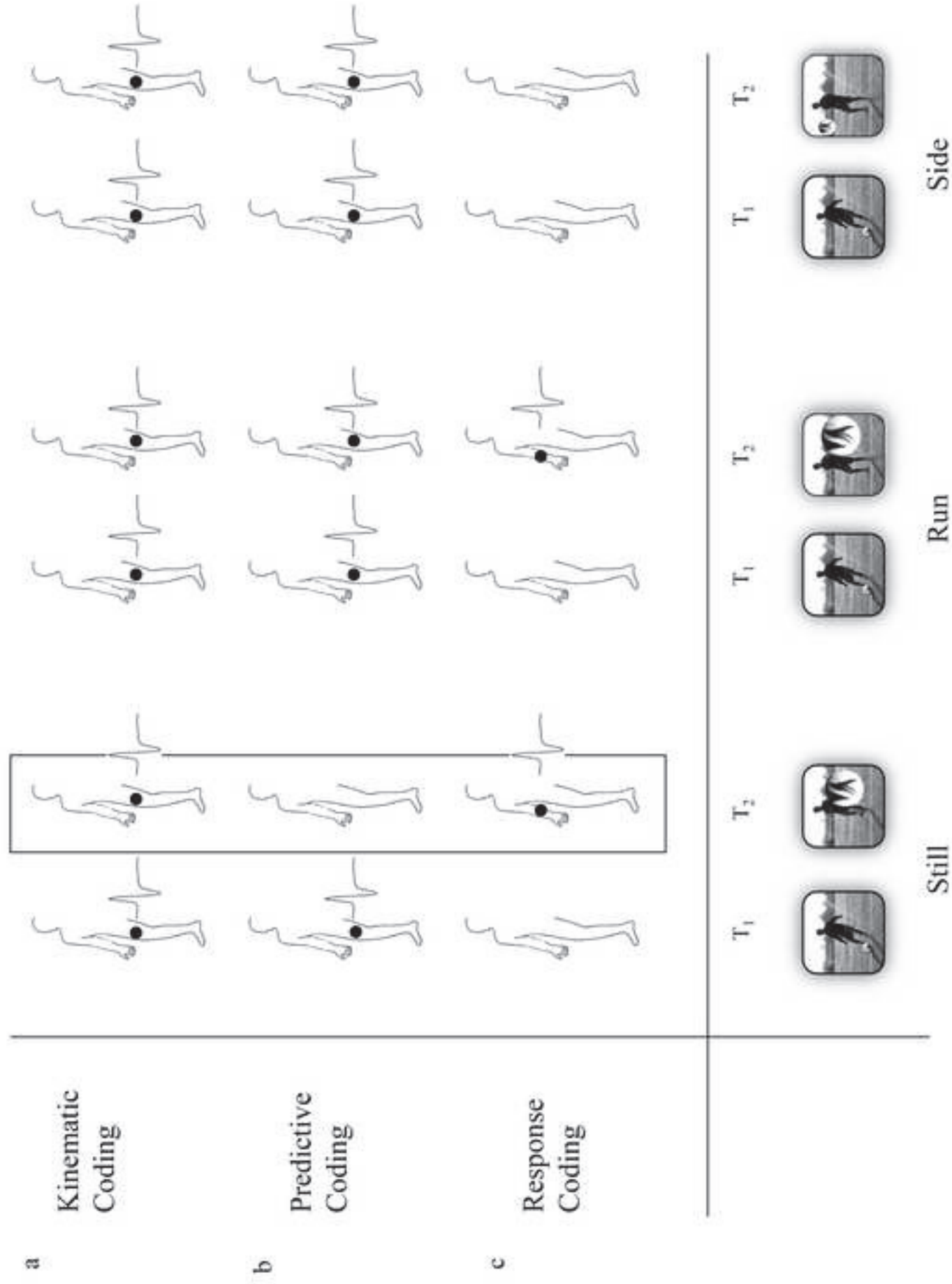
Figure 1
Click here to download high resolution image

a

b

c

T_1

T_2

TMS Stimulation Time (ms)

1350

1750

Figure 2
Click here to download high resolution image

Figure 3
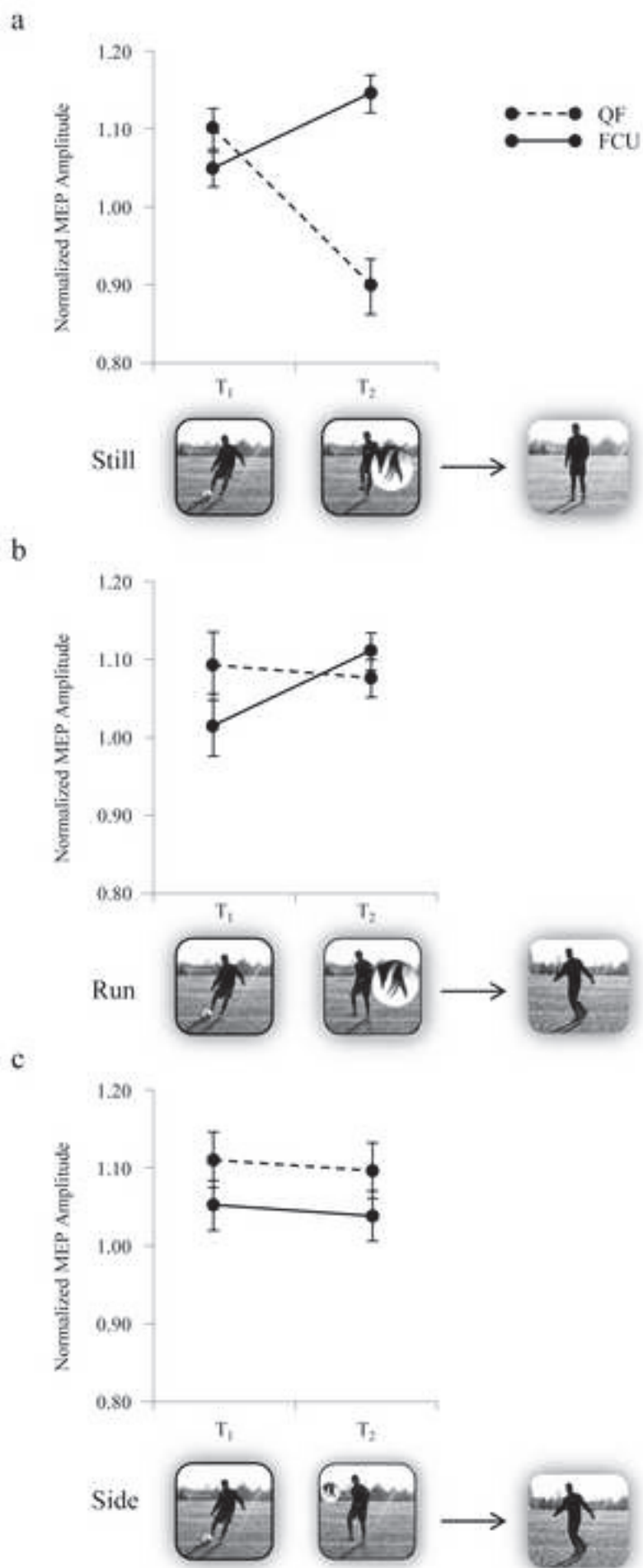Click here to download high resolution image

Table

Table 1. Normalized mean (± SEM) peak to peak amplitude of MEPs recorded from the FCU and the QF muscles during the three experimental conditions at each time point.

	Still		Run		Side	
	T_1	T_2	T_1	T_2	T_1	T_2
FCU	1.043 (± 0.048)	1.119 (± 0.047)	1.026 (± 0.043)	1.102 (± 0.044)	1.045 (± 0.050)	1.017 (± 0.024)
QF	1.102 (± 0.069)	0.884 (± 0.042)	1.097 (± 0.060)	1.067 (± 0.036)	1.116 (± 0.066)	1.086 (± 0.060)