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The multiform motor cortical output: Kinematic, predictive and response coding

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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.

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1. 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

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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; Giorcello & 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, Buccioni, & Castiello, 2012; Strafella & Paus, 2000; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006) and to its temporal pattern (Aglioti, Cesari, Romani, & Urgesi, 2008; Borroni & Baldissera, 2008; Borroni, Montagna, Cerri, & Baldissera, 2005; Gangitano, Mottaghy, & Pascual-Leone, 2001; Janssen, Steenbergen, & Carson, 2015; Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004; Montagna, Cerri, Borroni, & Baldissera, 2005; Urgesi et al., 2010). Behavioral studies have also demonstrated motor compatibility 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, 2011). 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 et al. 2009; Sartori, Xompero, Buccioni, & Castiello, 2012).

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, Buccioni et al., 2012) and the hypothesis that the observer's motor system codes the distal goal of the observed acts, irrespective 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, Buccioni, Castiello, & Becchio, 2013; Janssen et al., 2015; 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, Buccioni, & Castiello, 2013).

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); and (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 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

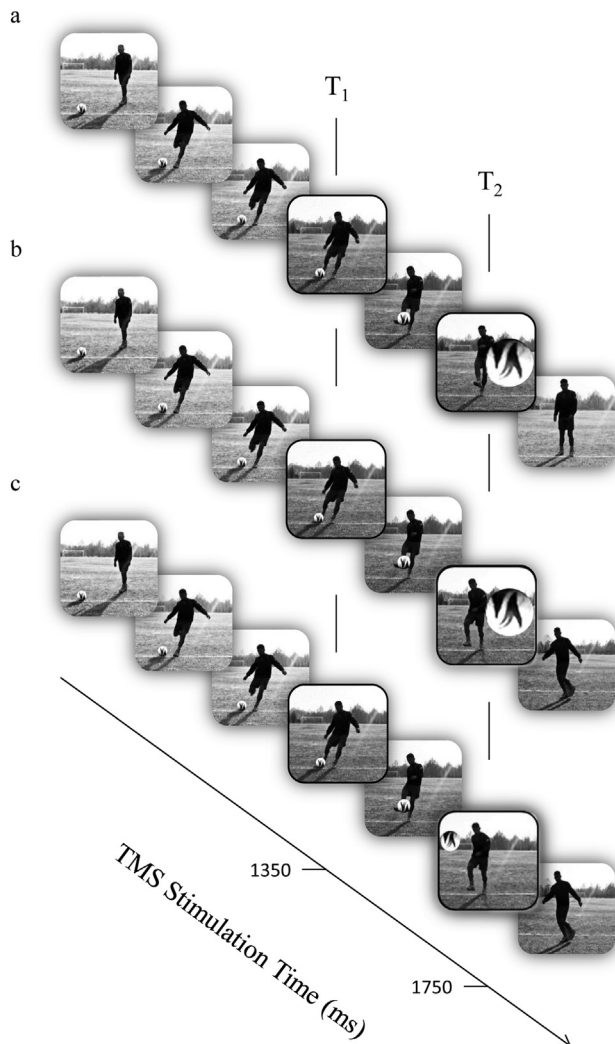


Fig. 1 – Sequence of events taking place for each condition: (a) Still, (b) Run, (c) Side. The vertical lines denote the time points when single TMS pulses were delivered: at T₁ (when the player's foot makes contact with the ball) and at T₂ (when the ball trajectory reaches its highest peak).

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 differentiates the two conditions is whether the ball is directed or not toward the observer).

2. Materials and methods

2.1. 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, Savonitto, Fabbro, & Aglioti, 2012; Weissensteiner, Abernethy, Farrow, & Müller, 2008). Notably, also observational practice may contribute to action prediction abilities (Urgesi et al., 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 et al., 1998). None were aware of the experiment's purpose and all gave their written informed consent at the time they were recruited. 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.

2.2. 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 the video so that the different position of the foot at T₂ 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; Sartori, Becchio et al., 2011; Sebanz & Shiffrar, 2009). Moreover, all the videos implied the same amount of perceived movement and the leg at T₂ 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 msec 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 msec after the video began and the ball trajectory reached its highest peak approximately 400 msec later (1750 msec after onset of the video). The ball was travelling at a velocity of approximately 10 m/sec during its trajectory. An animation effect was obtained by presenting a series of single frames each lasting 25 msec (resolution 720 x 576 pixels, color depth 24 bits, frame rate 30 fps) following the first frame which lasted 500 msec. 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,

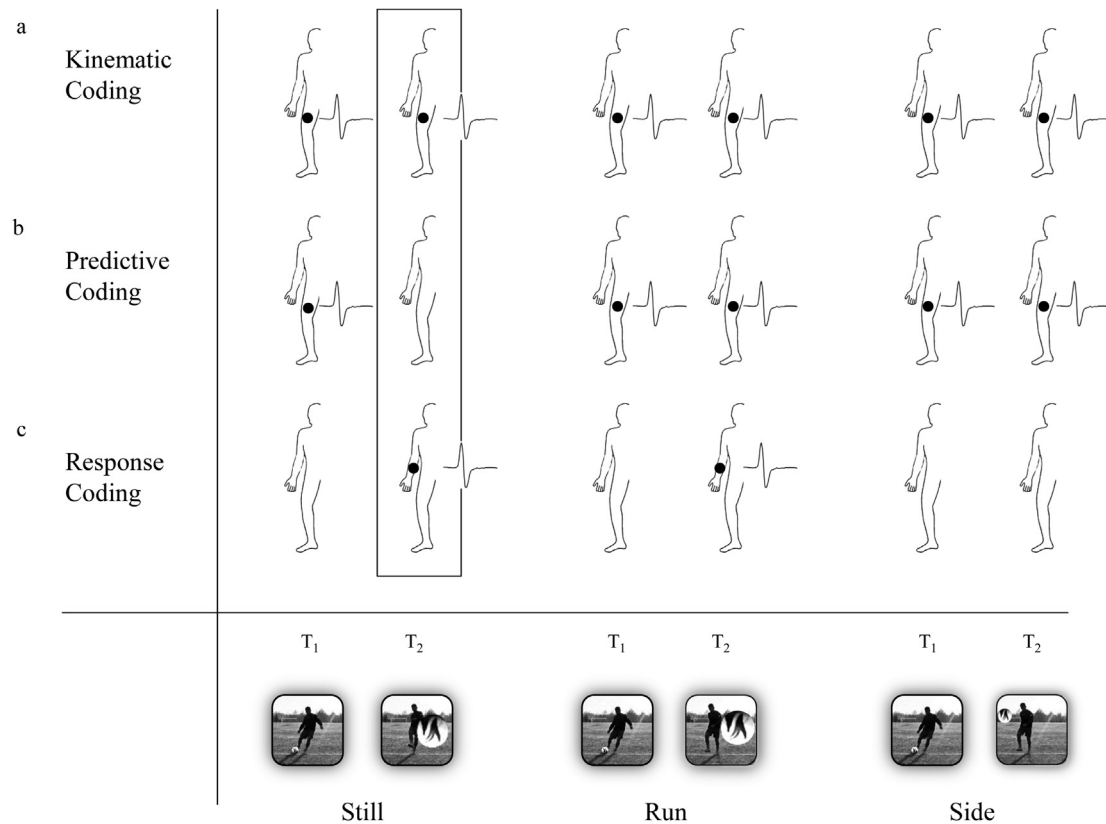


Fig. 2 – Graphical representation of the three levels of motor coding (Kinematic, Predictive, Response) and corresponding expected muscular dissociations. We hypothesize that while at T₁ the CS excitability should be equal in all muscles through all conditions, it should clearly dissociate at T₂ (e.g., in the Still condition), depending on motor coding.

confirmed that only observing the approaching ball led in the onlooker an impulse to react with the upper limbs (97% of positive responses).

2.3. Procedure

The participants were tested individually in a sound-attenuated Faraday room during a single experimental session lasting approximately 40 min 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 .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 (T₁; 1350 msec) and (ii) during the frame showing the highest peak of the ball's trajectory (T₂; 1750 msec). The same timing was applied to both the conditions. The first time point (T₁) 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 (T₂) was set at the highest peak of the ball's trajectory 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 × 3 conditions × 10 repetitions × 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 (10 x 10 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 sec 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 sec 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.

2.4. Data recording

2.4.1. Transcranial magnetic stimulation

Single-pulse TMS (pulse characteristics: 100 μ s rise time, 1 msec duration) was delivered using a 70 mm figure-of-eight coil (Magstim polyurethane-coated coil) connected to a Magstim BiStim² 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 .5 cm steps around the target area until a position was reached at which a 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 theBrainsight 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 (≥ 50 μ V 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.

2.4.2. Electromyography

MEPs were recorded from the FCU muscle of the right arm and from the 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 msec interval. The interval was time-locked to the delivery of the magnetic stimulation pulse and began 100 msec prior to the onset of stimulation and ended 200 msec 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.

2.5. 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 = MEP_{obtained}/MEP_{baseline}). 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* > .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* < .05 was set for all statistical analyses.

3. 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* > .05) or the QF (*t*₂₈ = −.037, *p* > .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 \times condition (*F*(2,56) = 6.415, *p* < .05, η^2_p = .186), a significant 2-way interaction of muscle \times time (*F*(1,28) = 9.669, *p* < .05, η^2_p = .257), and a significant 3-way interaction of muscle \times time \times condition (*F*(2,56) = 5.193, *p* < .05, η^2_p = .156). The results obtained from the post-hoc contrasts exploring the source of the significant 3-way interaction are outlined as follows.

3.1. 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* < .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' ($p = .941$) and the 'side' ($p = .771$) conditions (Table 1).

3.2. 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' ($p < .001$) and the 'side' conditions ($p < .05$) compared to the 'still' condition (Table 1). The same muscular activity was found across time points (T_1 , T_2) for the 'run' condition ($p = .604$; Fig. 2b) and the 'side' condition ($p = .381$; 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 seems 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).

3.3. 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 ($p_s < .05$; Fig. 1a,b), but not for the 'side' condition, in which the ball was kicked to the left ($p = .561$; 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 ($p_s < .05$), while at T_1 the same low MEP amplitude was found across all conditions ($p_s > .05$; Fig. 3).

4. 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 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; Makris & Urgesi, 2014; Sartori, Becchio et al., 2011; 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, Xompero et al., 2012). 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

Table 1 – Normalized mean (\pm 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 (\pm .048)	1.119 (\pm .047)	1.026 (\pm .043)	1.102 (\pm .044)	1.045 (\pm .050)	1.017 (\pm .024)
QF	1.102 (\pm .069)	.884 (\pm .042)	1.097 (\pm .060)	1.067 (\pm .036)	1.116 (\pm .066)	1.086 (\pm .060)

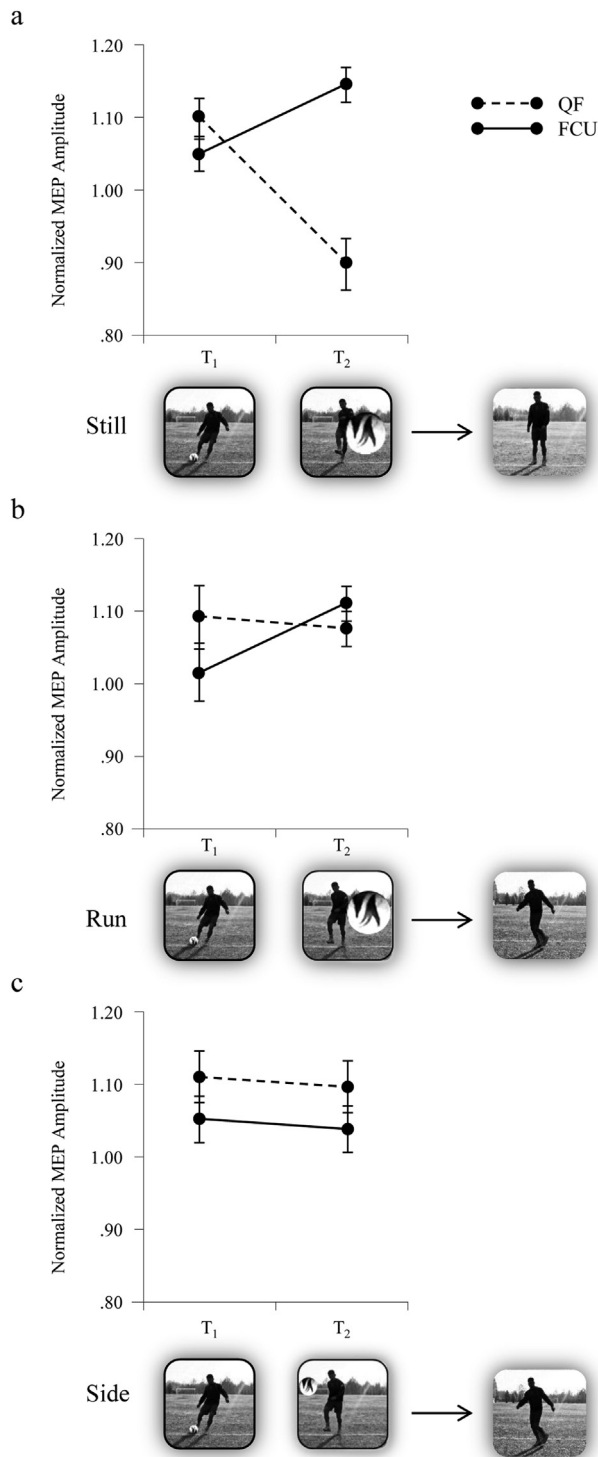


Fig. 3 – Corticospinal activations during observation of a soccer player: (a) kicking the ball straight and then coming to a full stop (Still), (b) kicking the ball straight and then continuing to run (Run), (c) kicking the ball to the side and then continuing to run (Side). Note that the following contrasts were significant: the normalized mean MEP amplitudes in the lower limb muscle (QF) were lower at T2 compared to T1 in the ‘Still’ condition ($p < .05$) and were higher at T2 both in the ‘Run’ ($p < .001$) and the ‘Side’ conditions ($p < .05$) compared to the ‘Still’ condition. The normalized mean MEP amplitudes recorded from the flexor

of predictive coding also with intransitive actions. This finding is 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.

Moreover, the present findings extends previous evidence on response preparation in hand muscles (Newman-Norlund, Noordzij, Meulenbroek, & Bekkering, 2007; Ocampo & Kritikos, 2010; Sartori, Betti, & Castiello, 2013a, 2013b; Sartori, Buccioni et al., 2013; Sartori, Cavallo, Buccioni, & Castiello, 2011; Sartori, Cavallo, Buccioni, & Castiello, 2012) 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, Kosobud, and 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., 2015). 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, Ognibene, Sartori, and Demiris (2013), the switch from congruent to incongruent motor simulation would be part of a dynamic interplay 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

carpi ulnaris (FCU) muscle were higher at T2 compared to T1 both in the ‘Still’ ($p < .05$) and the ‘Run’ ($p < .05$) conditions, but not for the ‘Side’ condition.

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 question: 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 (Barchiesi & Cattaneo, 2013; Catmur, Walsh, & Heyes, 2007; Cavallo, Heyes, Becchio, Bird, & Catmur, 2013).

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, Cavallo et al., 2012; Sartori, Xompero et al., 2012, 2013b). Running different coding levels while processing the observed action would be helpful to prepare a correct reaction (Bekkering et al., 2009). According to the theoretical framework proposed by Chinellato et al. (2013), a two level competition is at the basis of the mechanism for choosing appropriate responses to an observed 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. 3b,c).

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 versus continuing to run; kicking straight versus to the side) promoting a parallel activation of different responses to action observation in different effectors.

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