

Direct mapping rather than motor prediction subserves modulation of corticospinal excitability during observation of actions in real time

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Gueugneau N, Mc Cabe SI, Villalta JI, Grafton ST, Della-Maggiore V. Direct mapping rather than motor prediction subserves modulation of corticospinal excitability during observation of actions in real time. *J Neurophysiol* 113: 3700–3707, 2015. First published March 25, 2015; doi:10.1152/jn.00416.2014.—Motor facilitation refers to the specific increment in corticospinal excitability (CSE) elicited by the observation of actions performed by others. To date, the precise nature of the mechanism at the basis of this phenomenon is unknown. One possibility is that motor facilitation is driven by a predictive process reminiscent of the role of forward models in motor control. Alternatively, motor facilitation may result from a model-free mechanism by which the basic elements of the observed action are directly mapped onto their cortical representations. Our study was designed to discern these alternatives. To this aim, we recorded the time course of CSE for the first dorsal interosseous (FDI) and the abductor digiti minimi (ADM) during observation of three grasping actions in real time, two of which strongly diverged in kinematics from their natural (invariant) form. Although artificially slow movements used in most action observation studies might enhance the observer's discrimination performance, the use of videos in real time is crucial to maintain the time course of CSE within the physiological range of daily actions. CSE was measured at 4 time points within a 240-ms window that best captured the kinematic divergence from the invariant form. Our results show that CSE of the FDI, not the ADM, closely follows the functional role of the muscle despite the mismatch between the natural and the divergent kinematics. We propose that motor facilitation during observation of actions performed in real time reflects the model-free coding of perceived movement following a direct mapping mechanism.

action observation; corticospinal excitability; motor facilitation; direct mapping; motor prediction; forward models

CONVERGENT EXPERIMENTAL EVIDENCE indicates that passively viewing other individuals' actions activates the motor system of the observer (Grafton 2009; Rizzolatti and Craighero 2004). In humans, action perception leads to a consistent increment in the level of corticospinal excitability (CSE) that is muscle specific (e.g., Fadiga et al. 1995; Montagna et al. 2005; Petroni et al. 2010; Strafella and Paus 2000). In this article, we refer to this phenomenon as motor facilitation. To date, the precise mechanism underlying this modulation is unknown. One possibility is that motor facilitation is driven by a predictive mechanism, reminiscent of the role of forward models in motor control (e.g., Miall 2003; Wolpert et al. 2003). Experimental

support for this view comes from two transcranial magnetic stimulation (TMS) studies (Gangitano et al. 2001, 2004) showing that motor facilitation is congruent with the observed action when the kinematics to grasp an object follow those of natural prehension, but not when they significantly diverge from it (e.g., when the hand grip suddenly closes at the time of maximal aperture and reopens thereafter to grasp the object). On the basis of these results, the authors hypothesized that a motor plan loaded entirely at movement onset drives motor facilitation as long as the visual features of the perceived action match those predicted by the corresponding motor plan. In the same vein, a more recent work has shown that inducing an explicit mismatch between actual and expected kinematics by introducing a semantic cue (e.g., an object labeled “light” or “heavy”) suppresses corticospinal modulation in the observer (Senot et al. 2011). To date, several studies have directly or indirectly alluded to a predictive mechanism as key in driving motor facilitation during action observation (Cattaneo et al. 2009; Fadiga et al. 2005; Hauk et al. 2008).

Alternatively, motor facilitation may result from a model-free mechanism by which the basic elements of the observed action are directly mapped onto their cortical representations. In this article, we refer to this as “model free” to differentiate it from its “model-based” counterpart mentioned above. This mechanism is reminiscent of “direct matching,” which is often coined in the mirror neuron literature to refer to action understanding (Gallese et al. 1996; Iacoboni et al. 2005; Rizzolatti et al. 2004, 2010). Experimental evidence supporting a model-free mechanism comes from studies reporting that CSE follows the functional role of the implied muscle even when the original motor plan to achieve the goal is changed before movement onset (Alaerts et al. 2010b; Cavallo et al. 2013; Jansen et al. 2013). Furthermore, we have recently shown that motor facilitation during observation of grasping actions performed in real time is congruent with the functional role of the muscle even when the motor plan is amended during the movement. This was the case for an action corrected online during which hand aperture, initially aimed at a large object, closed to grasp a small object during the time course of the movement (Mc Cabe et al. 2014). Although our work provides strong evidence in favor of a model-free mechanism, it cannot rule out the contribution of a predictive mechanism. This is partly because CSE was only measured before and after the correction took place, i.e., 440 and 680 ms after movement onset, leaving a 240-ms window during which two different internal models could have been used sequentially to make

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predictions. Moreover, the fact that the duration of the correction based on the change in grip aperture was relatively long makes the action corrected online suboptimal for tracking subtle changes in the activity of the functional muscle when used alone.

The current study was designed to discern whether motor facilitation during observation of actions in real time is more consistent with a mechanism based on motor prediction or direct mapping. For this purpose, we recorded the time course of CSE for the first dorsal interosseous (FDI) and the abductor digiti minimi (ADM) during observation of three different videos of actions in which objects were grasped with the index and thumb fingers. One action involved reaching and grasping a large object with natural (unperturbed) kinematics (natural action, NA). The second action underwent an online correction, as in our previous study, from a large to a small object as the movement unfolded (corrected action, CA). Finally, a third action, initially directed to the large object, involved a fast sequential closure and reopening of the hand grip before grasping the large object. The latter mimicked a condition used by Gangitano et al. (2004), which was associated with a drop in CSE after the abrupt hand closure. To parallel his terminology, we refer to this as the “unnatural” action (UA). CSE was recorded at four key time points that captured the deviant kinematics of CA and UA from their invariant (natural) grasp (NA). In contrast with most previous studies, which used slow actions ranging from 2.4 to 5.6 s to track the time course of CSE (e.g., Cavallo et al. 2013; Gangitano et al. 2001, 2004; Jansen et al. 2013; Lago and Fernandez-del-Olmo 2011), in the present study actions were displayed in real time (movement duration was 1.16 s). Note that whereas artificially slow movements used in most action observation studies might enhance the observer’s discrimination performance, actions in real time are crucial to maintain the time course of CSE within the physiological range characteristic of natural actions (e.g., Barchiesi and Cattaneo 2013; Cattaneo et al. 2009). Another key difference from previous studies was the fast sampling rate used to record CSE (4 TMS pulses were applied within a 240-ms time window), which allowed us to finely track kinematic changes with high temporal resolution. This factor is critical when CSE is used as a proxy for motor facilitation, in which neural mechanisms are ultimately inferred on the basis of processing times.

The intrinsic differences in the time course of hand aperture for the UA and the CA allowed us to capture the temporal coupling between the observed kinematics and CSE. First, hand closure was much faster for the UA (and latency to FDI peak was shorter), permitting the precise tracking of changes in muscle activity. Second, the reopening phase of the hand in the UA took place during the closing phase of the CA. This dephasing in movement kinematics allowed an interpretation of CSE changes in terms of the two alternative processes. We hypothesized that if a predictive mechanism based on forward models is involved in motor facilitation, the level of CSE should drop during the closing phase of the CA and the UA, as the action diverges from the invariant form, and remain low thereafter. If, in contrast, direct mapping prevails, then CSE should follow the functional role of the muscle, with an increase during the closing phase of the CA and the UA, and a decrease thereafter as the hand opens.

MATERIALS AND METHODS

Participants. Fourteen right-handed subjects (5 men, 9 women; age 26.2 ± 4.1 yr) participated in this study after giving written, informed consent. They did not present any neurological or psychiatric disorders and had no family history of epilepsy. The experimental procedure was approved by the local Ethics Committee and carried out according to the Declaration of Helsinki.

General setup. Subjects sat on a comfortable and adjustable armchair throughout the experiment. They were placed 80 cm in front of a 17-in. liquid crystal display monitor (Samsung 732N PLUS) on which videos of three different grasping actions were displayed. Subjects were instructed to keep their hands still and relaxed throughout the experiment. The right hand was placed over the lap in a prone position underneath a table to prevent subjects from viewing it, and the elbow rested comfortably over the armrest. Four superficial cup electrodes were placed following a belly-tendon mount over the right FDI and ADM muscles. Ground electrodes for each muscle were placed over the wrist and elbow. The skin was cleaned with alcohol before the electrodes were placed. Electromyographic (EMG) activity was amplified with the use of two AC amplifiers (P5 series; Grass Instruments, Quincy, MA) with a bandwidth between 10 and 1,000 Hz. The signal was amplified 1,000 times, digitized at 5,000 Hz (National Instruments, Austin, TX), and collected on a personal computer using a program written in Lab-View (version 7.1; National Instruments).

Experimental paradigm. The visual stimuli consisted of three color video clips of an actor’s right arm performing three different types of reaching to grasp actions. In each video clip the actor had to reach and grasp either a small ($1.8 \times 0.8 \times 1$ cm) or a large ($1.8 \times 8.8 \times 1$ cm) object mounted on the same structure, attached to a vertical platform at a height of 17 cm from the tabletop (see Fig. 1).

The platform was located 22 cm from the hand’s start position. This arrangement allowed subjects to maintain similar arm kinematics across conditions and prevented subjects from anticipating the final goal of the action (i.e., the object to be grasped). The three grasping conditions were as follows: 1) natural action (NA), i.e., reaching and grasping the large object with a natural and smooth movement; 2) action corrected online (CA), during which hand aperture, initially aimed at the large object, closed to grasp the small object during the time course of the movement; and 3) unnatural action (UA) directed to the large object, involving the fast closure and reopening of the hand grip during the movement before object contact. Images displayed in Fig. 1 illustrate the three video clips.

The actor performed the movements while grasping a small cylinder, which was attached to the hand with surgical tape, using the middle, ring, and little fingers. This procedure ensured that she grasped the objects using the index and thumb fingers only. Previous EMG recordings show that this configuration activates the FDI during the closing phase of the NA and CA but produces no significant levels of activity on the ADM at any point of the movement (Mc Cabe et al. 2014).

Figure 2 shows the kinematics (hand aperture) extracted from the 3 videos, each of which consisted of 40 frames of 40-ms duration. Each video had a total length of 1,600 ms. However, movement duration from movement onset (the instant when the finger lifted the button) to movement offset (the time of object contact) lasted 1,160 ms. This timing is consistent with that of natural prehension (for corresponding execution times, refer to Mc Cabe et al. 2014). The task was programmed using MATLAB’s Psychtoolbox (The MathWorks, Natick, MA) (Brainard 1997).

TMS and EMG recording. Corticospinal excitability measurement was based on the size of motor evoked potentials (MEPs) elicited on the FDI and ADM by TMS. Single pulses of TMS were delivered using a Magstim 200 (Magstim, Whitland, UK) through a 70-mm figure-of-eight coil positioned over the optimum scalp location corresponding to the left motor cortex, with the handle pointing backward at 45° from the midline. Earplugs were provided to the participants to reduce sound level and protect their ears. The procedure was as

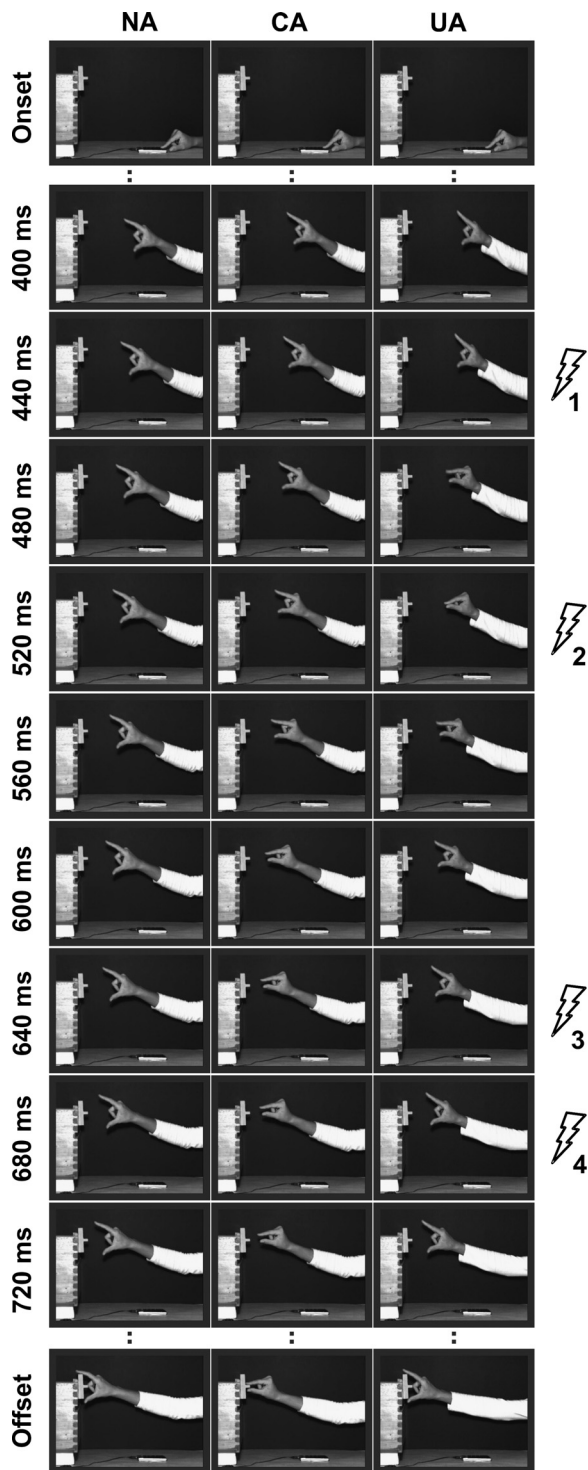


Fig. 1. Illustration of the videos corresponding to the 3 grasping actions. Shown are images of the video frames corresponding to the normal action (NA; left), corrected action (CA; middle), and unnatural action (UA; right). Each frame was 40 ms long. Movement onset corresponds to the instant when the index finger lifts the keypad, whereas movement offset corresponds to the time of object contact. Times of transcranial magnetic stimulation (TMS) are indicated by lightning bolts.

follows. First, the optimal scalp position to elicit MEPs from the FDI was identified. This location was marked on a rubber cap with a soft-tipped pen. The coil was then moved until a nearby position produced maximal MEPs from the ADM while still evoking similar or

larger MEPs from the FDI. This latter location was also marked and served as a reference point from which to determine the resting motor threshold and to deliver TMS pulses during the experiment. Resting motor threshold was determined as the intensity to produce 5 of 10 MEPs of at least $50 \mu\text{V}$ in the ADM.

The intensity used to stimulate was 120% of the motor threshold. The head coil was fixed in position using an articulated arm (Manfrotto, Venice, Italy). Subjects were instructed to keep the head still throughout each experimental block. In addition to the head rest, head movements were restrained by means of foam supports applied to both sides of the neck.

Procedure. Subjects were familiarized with the experimental setup and the visual material during the EMG setup phase. They were shown the three types of videos, which were introduced to the subjects as “normal action” (NA), “corrected action” (CA), and “unnatural action” (UA). Subjects used this information to perform the attentional task (see below). CSE was measured by applying single pulses of TMS at four time points that best captured the abrupt variations in the hand’s kinematics for the CA and the UA (shown as lightning bolts in Fig. 1 and as arrows in Fig. 2). TMS pulses were delivered on every single trial at one of the four time points, with only one TMS pulse applied per trial. Based on MEG evidence showing that the visual signal during the closing phase of a precision grasp takes 40 ms to reach the contralateral sensorimotor cortex (Nishitani and Hari 2000), TMS pulses were synchronized to the offset of the video frame (video frames were 40 ms long). The interval between videos was at least 7 s to avoid cumulative effects of TMS (Chen et al. 1997). Each subject carried out three experimental blocks. Each block consisted of the presentation of 25 video clips for each action in a pseudorandom order (i.e., 75 video clips per block). Thus, in each block, subjects received 5 stimulations at each of the 4 time points for each grasping action, resulting in a total of 15 trials per time point and per action across all 3 blocks.

The kinematics of the three grasping actions were very similar until the maximal aperture was reached, i.e., from movement onset to 440 ms into the movement, but differed drastically from 480 ms to movement offset (see Fig. 2). The first TMS pulse was delivered at maximal hand aperture (440 ms), before any finger modulation took place in the CA or the UA. At this point, grip aperture was indistinguishable among the three conditions. The second stimulation point was applied at the minimal aperture of the UA and at the midpoint between the maximal and minimal aperture of the CA (520 ms). The third pulse was delivered once the correction had ended for the CA (i.e., minimal hand aperture) and the UA was already in its maximal aperture approaching the large object (640 ms). In our previous study,

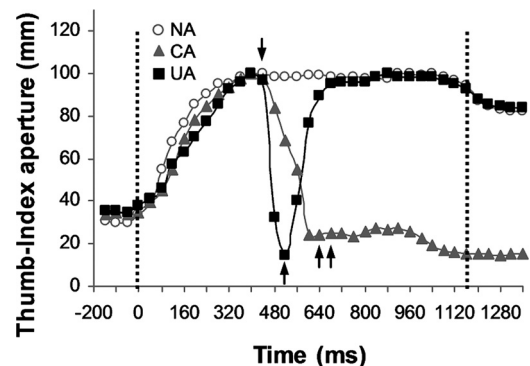


Fig. 2. Finger kinematics corresponding to the 3 grasping actions. Kinematics were computed, based on the videos, as the distance between the index finger and thumb tips. Open circles represent the NA, black squares the UA, and gray triangles the CA. The arrows indicate the stimulation times, during which single pulses of TMS were applied over the left M1. The 2 vertical dotted lines indicate movement onset (left) and offset (right), where movement onset corresponds to the instant when the index finger lifts the keypad and movement offset to the time of object contact.

this time point yielded a reliable CSE increment in the CA relative to the NA (Mc Cabe et al. 2014). The fourth TMS pulse was applied one frame later, at which point hand aperture for both the CA and the UA was identical to that in the previous frame (680 ms). This last time point was chosen to capture a possible drop in CSE following the divergence from the natural motor plan, hypothesized by the predictive mechanism. Note that hand aperture was constant through the four stimulation points for the NA.

The number of stimulation points and their temporal proximity allowed us to precisely track the time course of CSE according to modulations in hand kinematics. Each block lasted ~10 min, and the total duration of the experiment including a subject's preparation and rest was about 2 h. Two 5-min rests were included between blocks to maximize the attention level and minimize fatigue.

To ensure participants followed the actions in the videos, we designed an attentional task. In 30% of the trials, subjects were prompted to report either the type of movement or the object grasped in the action last seen by pressing a pedal with the right foot. Requests appeared in written form after the end of the trial. Participants were told to press the pedal only when the action matched the request. Subjects were told to observe the videos carefully and to follow the hand paths during each trial. Before starting the experiments, all subjects performed a couple of attentional trials to make sure they could distinguish all three conditions.

Data analysis. Peak-to-peak amplitude of the MEPs for the ADM and the FDI was measured for each action. Trials in which MEP amplitudes were larger than two standard deviations from the block mean and those where muscle activity exceeded 100 μ V during the 100 ms preceding the TMS pulse were excluded as outliers. On average, 2.2 (1.6) [mean (SE)] of the 15 MEPs were excluded for each stimulation point.

To pool the three blocks together and avoid nonspecific effects due to differences in coil position and/or hand posture across blocks, we normalized the MEPs by calculating the Z scores for each block (Aglioti et al. 2008). Statistical analyses were carried out in SPSS (IBM). Repeated-measures ANOVA were conducted when needed. Post hoc comparisons were assessed whenever the interaction of interest was significant using the Sidak test to contrast MEP values across actions for specific time points. Sphericity of the data was verified before statistical analysis was performed (Mauchly's $P > 0.05$).

RESULTS

MEPs from the right FDI and ADM were measured while subjects passively watched the three grasping actions. The results from the attentional task showed that subjects responded correctly 95.01 (0.97)% of the time for the NA, 92.63 (1.59)% of the time for the CA, and 92.5 (1.55)% of the time for the UA. This suggests that they paid attention to the actions displayed in the videos. A two-way ANOVA with action and experimental block as factors indicates that the level of attention did not differ across type of action or block [main effect of action: $F_{(2,26)} = 2.47$, $P = 0.104$; main effect of block: $F_{(2,26)} = 2.95$, $P = 0.07$; action by block interaction: $F_{(4,52)} = 1.94$; $P = 0.118$].

Table 1 depicts the corticospinal excitability before normalization of MEP values (raw values). The time course of CSE post-MEP normalization is shown in graphic form in Fig. 3. Note that whereas the MEP amplitude for the FDI remained low and unchanged during the NA across all stimulation points, it was temporally modulated during the CA and the UA (Fig. 3A). In contrast, CSE for the ADM showed little or no modulation (Fig. 3C). A three-way repeated-measures ANOVA with muscle, action, and time point as factors yielded

Table 1. *Nonnormalized corticospinal excitability*

	Stimulation Time Point			
	1	2	3	4
FDI MEP, mV				
NA	1.41 \pm 0.75	1.40 \pm 0.82	1.33 \pm 0.80	1.39 \pm 0.77
CA	1.36 \pm 0.74	1.39 \pm 0.80	1.55 \pm 0.89	1.57 \pm 0.73
UA	1.39 \pm 0.71	1.59 \pm 0.91	1.31 \pm 0.76	1.37 \pm 0.81
ADM MEP, mV				
NA	0.66 \pm 0.43	0.65 \pm 0.44	0.64 \pm 0.42	0.65 \pm 0.47
CA	0.65 \pm 0.47	0.66 \pm 0.46	0.68 \pm 0.46	0.64 \pm 0.45
UA	0.64 \pm 0.50	0.67 \pm 0.51	0.67 \pm 0.49	0.63 \pm 0.52

Values are means \pm SD corresponding to the raw motor evoked potentials (MEPs) of the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles for each of 4 stimulation points and each experimental condition (NA, natural action; CA, corrected action; UA, unnatural action).

a significant triple interaction [$F_{(6,78)} = 2.32$; $P = 0.04$]. No main effects reached significance [$F_{(1,13)} = 2.41$, $P = 0.14$; $F_{(2,26)} = 1.34$, $P = 0.27$; and $F_{(3,39)} = 0.85$, $P = 0.47$ for muscle, action and time point, respectively]. To facilitate the interpretation of the results, we split the interaction into a two-way ANOVA for each muscle. The ANOVA for the FDI identified a temporal modulation of the MEPs that varied with the type of action as revealed by the action by time point interaction [$F_{(6,78)} = 3.76$, $P = 0.002$]. Main effects did not reach significance [$F_{(2,26)} = 2.84$, $P = 0.76$ and $F_{(3,39)} = 0.85$, $P = 0.47$ for action and time point, respectively]. Post hoc comparisons conducted at each stimulation point allowed quantifying differences in the level of modulation across actions. Not surprisingly, no significant differences were found for the first time point ($P > 0.6$ for all comparisons), when hand aperture was maximal for all three conditions (see corresponding video frame in Fig. 1). In contrast, the second TMS pulse yielded significantly larger MEPs for the UA than for the other two actions (UA vs. NA: $P = 0.017$; UA vs. CA: $P = 0.013$). This is consistent with the functional role of the implied muscle in hand closure (Cole and Abbs 1987; Collins et al. 1999). At this point, hand aperture was minimal for the UA, intermediate for the CA, and nearly maximal for the NA. Finally, during the third and fourth stimulation time points, the MEPs for the CA were significantly larger than those for the other two actions (CA vs. UA: $P = 0.003$ and CA vs. NA: $P = 0.022$ for the 3rd point; CA vs. UA: $P = 0.009$ and CA vs. NA: $P = 0.003$ for the 4th point). Note that at these time points, hand aperture was minimal for the CA but nearly maximal for the NA and the UA.

Post hoc comparisons conducted for each action as a function of time also allowed us to characterize precisely the time course of motor facilitation. Although no differences were detected across the four time points for the NA ($P > 0.3$ for all comparisons), the MEPs elicited at the second time point of the UA were larger than those evoked at the other three time points ($P < 0.05$ for all 3 comparisons). On the other hand, the MEPs measured at the third and fourth time points of the CA were larger than those elicited at the first two time points ($P < 0.05$ for all comparisons) but were no different from each other ($P = 0.33$).

Concerning the ADM, the two-way repeated-measures ANOVA yielded no significant main effect of action or time and no action by time interaction, confirming a lack of modulation during observation of all three actions [main effect of

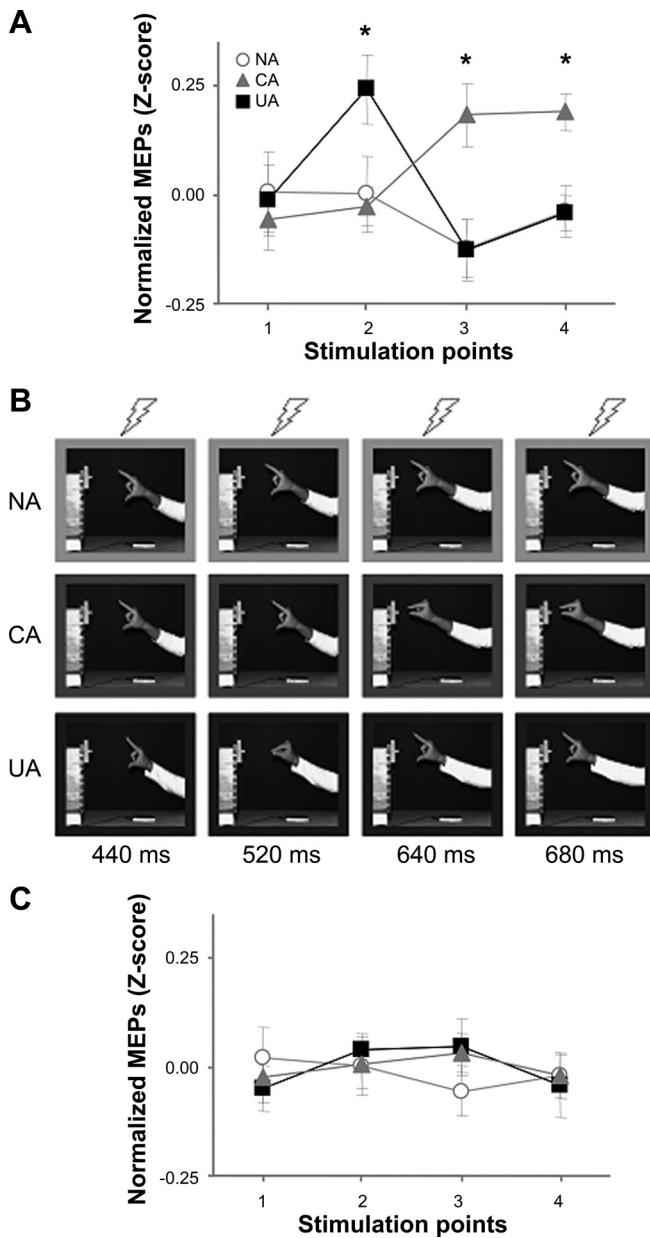


Fig. 3. Time course of corticospinal excitability (CSE) during observation of the 3 grasping actions. *A* and *C*: means \pm SE of the MEPs evoked from the first dorsal interosseous (FDI; *A*) and the abductor digiti minimi (ADM; *C*) muscles. Open circles represent the NA, black squares the UA, and gray triangles the CA. Note that black and open symbols overlap during time points 3 and 4. *B*: images depict the corresponding frames of the videos where a TMS pulse was applied, with the *top* row illustrating the NA, the *middle* row illustrating the CA, and the *bottom* row illustrating the UA.

time: $F_{(3,39)} = 0.33$, $P = 0.79$; main effect of action: $F_{(2,26)} = 0.40$, $P = 0.96$; action by time interaction: $F_{(6,78)} = 0.49$; $P = 0.81$]. In sum, our results indicate that CSE measured during observation of the UA and the CA closely followed the functional role of the FDI despite the significant divergence in kinematics from their invariant (natural) form.

Another piece of information that can be extracted from the data is the time elapsed between the observation of an action frame and the evoked change in CSE, a good indicator of the processing time involved in motor facilitation during action observation. Specifically, the time elapsed between the first

TMS pulse (440 ms), when hand aperture was nearly maximal and CSE levels were low, and the second TMS pulse (520 ms), when hand aperture was minimal and CSE levels were large, was two video frames. Given that TMS was applied at the offset of the video frame, processing times associated with motor facilitation during grasp observation likely required between 40 and 80 ms. CA provided no relevant information in this sense because it was much slower than the UA: 160 (velocity: 0.46 m/s) and 80 ms (velocity: 1.03 m/s) were necessary to achieve minimal hand aperture for the CA and the UA, respectively.

DISCUSSION

The main goal of this study was to explore whether motor facilitation elicited by observation of actions in real time is driven by a predictive mechanism based on forward models or by a direct, model-free mechanism mapping individual action elements onto their motor representations. Toward this aim, we characterized the time course of CSE for the FDI and the ADM during the observation of three distinct grasping actions: a natural, unperturbed action and two perturbed actions in which the motor plan was unexpectedly modified. CSE was measured at 4 critical time points that covered a window of 240 ms during which kinematics of the two perturbed actions mostly differed from their invariant (natural) form. Our results show that CSE of the FDI closely follows the functional role of the muscle involved in grasping, despite the mismatch between the natural and the divergent kinematics. This tight coupling of CSE and function was not observed in a nonessential muscle, the ADM.

There are at least two possible mechanisms that can explain the time course of CSE reported in our study. One of them relies on a computational theory of motor control that involves motor prediction, i.e., the prediction of future sensorimotor states based on a copy of the motor command (Wolpert et al. 1995). Prediction in motor control is thought to be essential to maintain movement precision despite long delays in sensory feedback (Desmurget and Grafton 2000). The complexity inherent in predicting the consequence of a motor command during multijoint movements, such as grasping or reaching, has led to the proposal of internal models, neural processes that can simulate the dynamics of an effector in a given environment. In this sense, a forward model is an internal representation of the body dynamics that relates an action with its sensory consequences (Wolpert et al. 1995). Forward models are thought to be key in mediating online corrections (e.g., Desmurget et al. 1999; Miall et al. 2007) and motor learning (e.g., Tseng et al. 2007) and have been hypothesized to subserve action observation, social interaction, and reading the intentions of others (Flanagan et al. 2003; Kilner et al. 2007; Miall 2003; Wolpert and Flanagan 2001; Wolpert et al. 2003). How would action observation be achieved? According to Wolpert and Flanagan (2001), contextual information from the experimental setting would trigger the parallel activation of forward models compatible with the observed action, which might be used to make predictions about their sensory consequences. Modulation of the observers' motor system would occur when the prediction made by one of the models matches the sensorimotor features of the observed action. In terms of motor facilitation, this conceptual framework would be consistent with a muscle-

specific increase in CSE when the predicted and the observed kinematics match, and a lack of facilitation when they mismatch.

In agreement with this view, Gangitano and colleagues (2001, 2004) have shown that observation of unnatural grasping actions, such as the UA included in our study, is associated with a drop in CSE as soon as the kinematic profile diverges from the invariant (natural) grasp. An abolishment in the level of CSE has also been reported during lifting, when explicit information regarding the observed object and the associated kinematics is in conflict (Senot et al. 2011). In contrast with these studies, our findings show no evidence for a drop in the level of CSE following the divergence in movement kinematics from the invariant form. Rather, the sudden hand closure observed in the UA halfway into the movement elicited a peak in the level of CSE specific to the FDI. This is in line with the functional role of this muscle (Cole and Abbs 1987; Collins et al. 1999). Interestingly, a drop in the amplitude of the MEPs was evident 120 ms later, as the hand approached maximal aperture. In accordance, hand closure for the CA at 640 ms into the movement was accompanied by an increment in the level of CSE specific to the FDI. Crucially, the level of motor facilitation persisted 40 ms later even though hand aperture remained unchanged, further suggesting that information regarding the actual movement and not the predicted movement drove the time course of corticospinal excitability.

One could claim that the unexpected change in kinematics observed in the CA and the UA may have triggered the loading of a new motor plan to replace the one corresponding to the invariant form. The narrow time window examined in our study established that motor facilitation in the UA took place sometime between 40 and 80 ms after hand closure, which is in line with previous work (Lepage et al. 2010; Nishitani and Hari 2000). Given that at least 40 ms are required to process basic aspects of visual stimuli such as orientation (Bacon-Macé et al. 2005; Berens et al. 2012), it is unlikely that the observer may have both detected the hand closure and loaded a different motor plan that better matched the observed kinematics within a 40-ms window. Alternatively, the observer could have built an internal model for the CA and the UA through observation and relied on motor prediction to achieve the same results. Two pieces of evidence, however, are against this hypothesis. First, as mentioned above, forward models require a copy of the motor command generated by the motor plan as input to make sensory predictions (Wolpert et al. 1995; Wolpert and Flanagan 2001). Yet, no motor plans are thought to exist for actions requiring a correction online, such as the CA (Desmurget et al. 1999; Desmurget and Grafton 2000; Prablanc and Martin 1992; Prablanc et al. 2003), or for unnatural actions, such as the UA, that strongly diverge from the canonical action (Gangitano et al. 2001, 2004). This is, in fact, why we chose these actions to discern between motor prediction and direct mapping. Second, we found no empirical evidence from our data suggesting that subjects learned an internal model for the UA and CA throughout the experiment. Specifically, the time course of CSE corresponding to the mean of the first three MEPs and that of the last three MEPs for the three actions did not differ [time of assessment (early vs late) \times action (NA, CA, UA) \times stimulation time point (1, 2, 3, 4) interaction: $F_{(6,78)} = 0.264$, $P = 0.952$], indicating a lack of learning throughout the experiment.

We are not certain why our results differed so strikingly from those reported by Gangitano and colleagues (2001, 2004). It is important to note that in those studies, the maximum CSE for the FDI was found at the time of maximal hand aperture, whereas in our study and other studies (e.g., Borroni and Baldissera 2008; Mc Cabe et al. 2014; Montagna et al., 2005), it took place as the hand closed to grasp the object, which is consistent with the functional role of the muscle (Cole and Abbs 1987; Collins et al. 1999). One key difference across studies was movement duration, which was 1.16 s in our study and 2.8 s in that of Gangitano and colleagues. Long videos that depart from real movement time may hinder the correspondence between the onset of the TMS pulse and the peak of activity in the observers' M1, altering the phase between these measures. In addition, our attentional task, requiring the identification of the perceived action, forced participants to track the portion of kinematic divergence closely with their eyes. These factors may explain why the peak in CSE for the FDI reported by Gangitano et al. (2001) during observation of the natural grasping action was found at the maximal grip aperture, rather than at the minimal grip aperture, when the muscle is normally active. Urgesi et al. (2010) have interpreted this dephasing in terms of predictive coding (Kilner et al. 2007), according to which the peak in CSE would reflect the anticipation of the final action phase (in this case, hand closure). This view is based on a recent study showing that CSE increases during observation of static images with implied motion depicting incomplete action phases but not final action phases (Urgesi et al. 2010). Thus it is possible that unlike movements performed in real time, slow movements are processed more like static images. This opens the possibility that observing a picture as an isolated snapshot or in the context of an artificially slow movement may not recruit the same neural processes that are involved in decoding natural actions that unfold in real time. This hypothesis is amenable for testing.

A parsimonious explanation for our results is based on a direct, frame-by-frame mechanism in which the basic elements of the observed action are directly mapped onto their cortical representations. In the context of mirror neurons, Rizzolatti et al. (2002) have proposed the existence of two hierarchical levels for coding observed actions in the nervous system: a low level of processing, relying on brain regions where movements are coded, and a high level of processing, involving brain areas where actions are coded. Whereas the former would explain behavioral phenomena such as response facilitation, i.e., the automatic elicitation of a response in the observer whenever the action is present in the motor repertoire, the latter may explain more complex behaviors such as action understanding and imitation. Our findings suggest that motor facilitation during observation of real-time movements also emerges from a low-level processing network.

Previous studies have emphasized a preponderant role of movement during observation of intransitive (e.g., Borroni and Baldissera 2008; Fadiga et al. 1995; Strafella and Paus 2000) and goal-directed actions such as grasping and lifting (Alaerts et al. 2010a, 2010b; Cavallo et al. 2013; Sartori et al. 2012). Recently, we showed that when the type and goal of the action to be observed are specified in advance, the pattern of CSE measured while the hand is still at rest does not reflect the final action's goal but rather the next movement phase (Mc Cabe et al. 2014). Remarkably, this effect appears to vanish with time

as the perceived action unfolds and CSE follows the actual kinematics. Thus, although knowing the action's goal influences the motor system of the observer, this effect appears to be preparatory in nature, suggesting that motor facilitation during observation of actions in real time reflects coding at the movement level and not at the action level. In line with our previous work, Janssen et al. (2013) have reported that providing invalid cues regarding the action to be observed before movement onset (e.g., presenting a cue for a precision grip but showing a video of a whole grasp) elicits a pattern of CSE that is initially compatible with the specified action but later reflects the kinematics of the perceived action. In contrast with the work of Gangitano et al. (2004), the authors suggest that when a mismatch occurs between the specified and the observed action, motor facilitation ends up following that of the actual action.

Our current work takes these studies a step forward by attempting to determine the mechanism at the basis of motor facilitation. To our knowledge, this is the first TMS study that directly addresses this question within current frameworks of motor control. In contrast with the studies mentioned above, in the present study we used actions in real time and recorded MEPs within a narrow time window during which kinematics mostly diverged from the invariant action. These features were key to distinguish between the different frameworks. Furthermore, the choice of actions that diverged from the invariant form during the movement ensured that the pattern of CSE identified was not confounded by higher order modulation induced by explicit cues. We propose that motor facilitation measured during observation of actions in real time reflects the coding of the observed movement following a direct mapping mechanism that prescinds internal models. It is likely, however, that a predictive mechanism may drive motor facilitation during observation of artificially slow movements and static images and during higher order behaviors such as imitation and social interaction, involving the recruitment of successively higher levels of processing in a putative perceptual-motor hierarchy (Grafton and Hamilton 2007; Hamilton and Grafton 2008). Model-based frameworks such as predictive coding (Kilner et al. 2007) or HMOSAIC (hierarchical modular selection and identification for control; Wolpert et al. 2003) could account for motor facilitation in these contexts.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.G., S.I.M., S.T.G., and V.D.-M. conception and design of research; N.G. and S.I.M. performed experiments; N.G., S.I.M., and J.I.V. analyzed data; N.G., J.I.V., S.T.G., and V.D.-M. interpreted results of experiments; N.G. and J.I.V. prepared figures; N.G. and V.D.-M. drafted manuscript; N.G., J.I.V., S.T.G., and V.D.-M. edited and revised manuscript; N.G., S.I.M., J.I.V., S.T.G., and V.D.-M. approved final version of manuscript.

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