


Dynamical entrainment of corticospinal excitability during rhythmic movement observation: a Transcranial Magnetic Stimulation study

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Abstract

Spontaneous modulations of corticospinal excitability during action observation have been interpreted as evidence for the activation of internal motor representations equivalent to the observed action. Alternatively or complementary to this perspective, growing evidence shows that motor activity during observation of rhythmic movements can be modulated by direct visuomotor couplings and dynamical entrainment. In-phase and anti-phase entrainment spontaneously occur, characterized by cyclic movements proceeding simultaneously in the same (in-phase) or opposite (anti-phase) direction. Here we investigate corticospinal excitability during the observation of vertical oscillations of an index finger using Transcranial Magnetic Stimulation (TMS). Motor-evoked potentials (MEPs) were recorded from participants' flexor and extensor muscles of the right index finger, placed in either a maximal steady flexion or extension position, with stimulations delivered at maximal flexion, maximal extension or mid-trajectory of the observed finger oscillations. Consistent with the occurrence of dynamical motor entrainment, increased and decreased MEP responses – suggesting the facilitation of stable in-phase and anti-phase relations but not an unstable 90° phase relation – were found in participants' flexors. Anti-phase motor facilitation contrasts with the activation of internal motor representation as it involves activity in the motor system opposite from activity required for the execution of the observed movement. These findings demonstrate the relevance of dynamical entrainment theories and methods for understanding spontaneous motor activity in the brain during action observation and the mechanisms underpinning coordinated movements during social interaction.

Introduction

Human brain activity in motor regions is spontaneously modulated during action observation. Such activity has received a growing interest since the discovery of 'mirror neurons' in the premotor cortex (area F5) of macaque monkeys that respond in a congruent manner to both action observation and execution (Di Pellegrino *et al.*, 1992; Rizzolatti *et al.*, 1996). When a monkey passively observes someone else grasping an object, these neurons discharge as if the monkey was actively performing the action. It has been proposed that a similar action observation-execution matching system exists in the human brain (Fadiga *et al.*, 1995; Iacoboni *et al.*, 1999).

Transcranial Magnetic Stimulation (TMS) studies have revealed that the observation of an action modulates the amplitude of Motor-evoked potentials (MEPs) (Fadiga *et al.*, 1995). Increased

amplitudes have been found during the observation of an action, especially for the muscles involved in its execution, and these modulations tend to be time locked to the muscle activity exhibited if executing the movement (see Naish *et al.* (2014) for a review). These observations have led to the hypothesis that there is a direct activation of an equivalent internal motor representation during the observation of an action resulting in modulation of corticospinal excitability (direct matching hypothesis) (Iacoboni *et al.*, 1999; Blakemore & Frith, 2005; Brass & Heyes, 2005). The activation of such representations is considered to facilitate motor responses that are similar to the observed action, and more generally, the success of a wide variety of social behaviors, including behavioral imitation and interpersonal coordination (Rizzolatti & Craighero, 2004; Sebanz *et al.*, 2006; Novembre *et al.*, 2014; Hadley *et al.*, 2015).

Alternatively or complementary to this influential perspective, growing evidence in behavioral research suggests that the modulation of corticospinal excitability during action observation could result from the universal phenomenon of dynamical

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entrainment – inspired by the physical laws of mechanics – underlying the emergence of spontaneous spatiotemporal order in numerous complex biological systems (Kelso, 1997; Pikovsky *et al.*, 2003; Strogatz, 2003; Coey *et al.*, 2012). Movements coordinated in time and space with external stimuli, other people in particular, can simply emerge from direct visual perception and dynamical entrainment processes (Schmidt & Richardson, 2008). Via direct visuomotor coupling, human movements spontaneously entrain to observed rhythmic movements. Movements are attracted toward in-phase spatiotemporal relations characterized by movements proceeding simultaneously in the same direction (0° relative phase) but also, with lower occurrence, toward anti-phase relations characterized by movements proceeding simultaneously in opposite directions (180° relative phase) (Schmidt & O'Brien, 1997; Richardson *et al.*, 2007). Other phase relations have been found to act as repellers and are spontaneously avoided, with 90° phasing corresponding to a quarter cycle lead or lag on the observed movement being particularly unstable.

Modulation of corticospinal excitability favoring anti-phase and in-phase relations – as predicted by the dynamical entrainment perspective – would, however, contrast with the direct matching hypothesis as anti-phase relations involve activity in the motor system opposite from the one needed for the execution of the observed movement. To test these differing accounts, we examined the modulation of corticospinal excitability during the observation of rhythmic movements of an index finger using TMS. MEPs were recorded from flexor and extensor muscles of participants' right index finger, placed in either a maximal comfortable flexion or extension position, when watching videos of an index finger oscillating vertically on a computer screen (see Fig. 1A). Stimulations were delivered at maximal flexion, maximal extension or mid-trajectory of the observed finger oscillations, corresponding to relative phases between participants' and observed finger positions equal to 0° , 180° and 90° , depending on participants' finger position. Motor facilitation was expected to increase from 90° through 180° to 0° if corticospinal excitability is modulated by visuomotor entrainment processes. Alternatively, if corticospinal excitability is modulated by the activation of an equivalent internal motor representation, then increased responses in flexor and extensor muscles were expected only when observing finger flexion and extension, respectively.

Material and methods

Participants

Twelve females and 14 males volunteered to participate in this study. The mean age of the participants was 28.23 (SD = 9.30), all were neurologically healthy, right-handed, and had normal or corrected-to-normal vision. None of the participants had contraindications to TMS and they all provided their written informed consent prior to the experiment. The experiment was approved by the Western Sydney University Ethics Committee. Participants were excluded if they reported that they were taking psychiatric or neuroactive medications, had consumed alcohol in the last 24 h, had insufficient or abnormal sleep the night before or had consumed more than two cups of coffee or other caffeinated drinks in the 2 h prior to the experiment, as these factors can influence corticospinal excitability.

Apparatus and stimuli

A chair with a soft support for holding the participant's right forearm was employed and two foam blocks (tall or short) were used to maintain the right index finger in a relaxed up or down position (see Fig. 1A). A 21.5-inch high definition BenQ LCD computer monitor (60 Hz refresh rate) was positioned approximately 60 cm away from the participant at eye level to present the visual stimuli. In control (baseline) conditions, a white fixation dot was displayed at the center of the screen on a black background. In movement observation conditions, a video showing the right hand of a male performing rhythmic vertical movement of the index finger (i.e. flexion and extension) at 0.5 Hz was displayed behind the fixation dot. Six different videos were recorded for the study with a metronome indicating the tempo. Videos were recorded at 25 fps and lasted 90 s. The individual in the videos trained before the recordings in order to perform smooth and continuous movements with minimum frequency and amplitude variability, flexion-extension asymmetries, and horizontal deviations.

The videos were scaled in such a way that the size of the hand on the screen corresponded to the size of the model's hand in reality. The middle of the movement trajectory of the finger of each video was positioned at the level of the fixation dot (i.e. the center of the screen). For all videos, one or two letters randomly occurred on the screen slightly above or below the fixation dot. The letters were used for a letter detection task in order to keep the participant

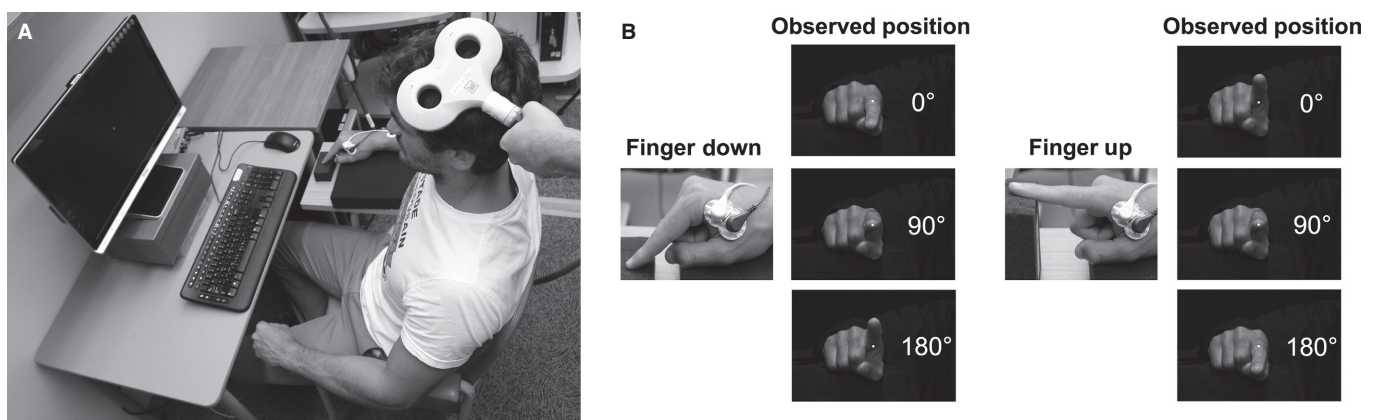


FIG. 1. Experimental setup (A) and illustration of the experimental conditions (B) in which the participant's finger was placed in either a maximal steady flexion or extension position while TMS (coil held for illustrative purpose) stimulation was delivered at maximal flexion, maximal extension or mid-trajectory of the observed finger oscillations.

focused on the displays throughout the trials. Adobe Premiere Pro CS6 (Adobe Systems Inc., San Jose, California) was used to edit all the videos and extract the times of each maximal flexion and extension (i.e. turning points) and middle position of the finger trajectory, which were used to trigger the TMS pulses during the experiment.

Biphasic stimulations were delivered with a Magstim Rapid² system and a 70 mm figure-of-eight focal coil (Magstim, Whitland, UK). A PC computer with Presentation software (Neurobehavioral Systems Inc., Albany, CA, USA) controlled the visual displays and triggered synchronized TMS pulses. Self-adhesive disposable Ag/AgCl electrodes were used to record TMS-induced MEPs for four muscles involved in the flexion and extension of the right index finger: the flexor digitorum superficialis (FDS), involved in the flexion of the finger; the extensor indicis proprius (EIP) and extensor digitorum communis (EDC), involved in finger extension; the first dorsal interosseus (FDI), which is principally involved in the abduction of the index finger but also in flexion and extension (Cross & Iacoboni, 2014). After cleaning the skin with alcohol, electrodes were placed over the belly and the associated tendon of each muscle. A ground electrode was placed over the right lateral epicondyle of the humerus (grounding FDS and EDC muscles) and another ground electrode was placed over the right ulnar styloid process (grounding FDI and EIP muscles). Electromyography (EMG) signals were amplified and digitized at 4 kHz using ADInstruments Dual Bio amplifiers and PowerLab 16/30 recording system (ADInstruments Pty Ltd., Australia), and stored on a computer for off-line analyses.

Procedure

Upon arrival the participant was told that the study investigated letter detection performance and the distracting effects of observing movement. Instructions specified that the task consisted of reporting letters that appeared on the screen when watching a moving finger video and/or when the primary motor cortex was stimulated. These instructions ensured that the participant remained naïve to the purpose of the study and maintained attention on the visual displays throughout the trials.

The participant sat in the chair in front of the computer screen and the electrodes were placed for the EMG recording. The participant was told to keep the hand and forearm immobile and relaxed on the support. The TMS coil was then positioned over the participant's left primary motor cortex (M1) tangentially to the head surface with the handle pointing backward and laterally at 45° away from the midline. The optimal scalp position was determined by moving the coil until we induced MEPs of similar and maximal amplitude in the four muscles (see also Fadiga *et al.*, 1995; Aglioti *et al.*, 2008; Novembre *et al.*, 2012; Ticini *et al.*, 2012; Panyakaew *et al.*, 2016). This position was then marked on the scalp to ensure that the stimulated spot remained constant throughout the experiment. The resting Motor Threshold (MT) was defined as the minimal intensity required to induce MEPs of at least 0.05 mV peak-to-peak amplitude in half of ten consecutive stimulations (while the participant's muscles were all relaxed). The experimenter also checked that these MEPs occurred comparably in the four different muscles that were recorded to make sure that no particular muscle was better targeted than the others. During the experiment, the stimulator intensity was set at 120% of the MT. The average intensity during the experiment ranged from 49 to 88 ($M = 63.62$; $SD = 9.29$) of the stimulator output.

The participant then performed sixteen trials of 90 s with the task of remembering the last letter that appeared on the screen and reporting it at the end of the trial using the keyboard with the left

hand. In each trial, a random letter occurred at a random time for 200 ms slightly below or above the fixation dot, and in some trials, a second random letter occurred at a random time during the last 10 s. This simple task was intended to ensure that the participant paid attention to the visual displays until the end of each trial without heightening cognitive load, which could modulate corticospinal excitability (Rossini *et al.*, 1999).

The two-first and last trials were control trials. Only a white fixation dot on a black background was displayed. These trials were used to obtain a control baseline without movement observation. The twelve other trials between these controls trials were experimental trials in which a moving finger video was displayed on the screen. Twelve TMS pulses were delivered in each control and experimental trial. Pulses were delivered every 6 s plus a negative or positive random offset between 0 and 1 s to avoid habituation. No stimulation was delivered during the first 6 s of each trial to allow enough time for entrainment to be established, and no stimulation was delivered in the 6 s following the presentation of a letter to avoid interference induced by the letter detection task.

In the experimental trials, TMS stimulations randomly occurred either at a maximal flexion, maximal extension or middle positions of the observed finger trajectory. Four stimulations were delivered for each of these three positions in each trial. For the middle position, two stimulations were delivered when the finger moved from maximal flexion to maximal extension and two stimulations were delivered when the finger moved from maximal extension to maximal flexion. For all the observed finger positions, the stimulations were delivered 100 ms before the actual time at which the finger reached these positions to account for the tendency for muscle activity to anticipate movement (Kilner *et al.*, 2004; Borroni *et al.*, 2005). All videos started 2 s after the beginning of the trial with the index finger moving from a middle position to maximal flexion. Only a fixation dot was displayed during the first 2 s of the trial.

The position of participant's index finger was manipulated during the experiment using the two pre-selected foam blocks. Eight trials were performed with the finger in a down position and eight trials were performed with the finger in an up position. Down and up conditions were set up such that the participant's finger was comfortably positioned at maximum flexion and extension, respectively.

For the twelve experimental trials, the six different videos were played once for each finger position using the same 12 stimulation time points. Four stimulations were delivered for each 0° (in-phase), 180° (anti-phase), and 90° relative phase relations. When participant's index finger was in a down position, stimulations for the 0°, 180°, and 90° relative phase conditions occurred when the observed finger was in a down, up, and middle position, respectively. When participant's index finger was in an up position, stimulations for the 0°, 180°, and 90° relative phase conditions occurred when the observed finger was in an up, down, and middle position, respectively (see Fig. 1B).

Therefore, we had a 2 (Finger Position: Down and Up) × 3 (Relative Phase: 0°, 180° and 90°) experimental design with 24 stimulations for each condition. Together with 48 stimulations for the control (baseline) condition (i.e. 24 stimulations for the two required finger positions), a total of 192 stimulations were delivered during the experiment. The order of the participant's index finger position in the control trials was counterbalanced and the order of finger position and the videos was randomized across experimental trials. However, participant's finger position was never the same for more than two consecutive trials to avoid habituation.

Before each trial, the finger position of the participant was changed according to the instructions displayed on the screen and a

reminder was given to remember the last letter that appeared on the screen and to keep the right hand still and relaxed. After completing all sixteen trials, a funnel debriefing procedure was performed to determine whether the participant guessed the true purpose of the study. All participants reported that they thought that the purpose of the study was the investigation of letter detection performance and none guessed the actual purpose of the investigation.

Data analysis

EMG signals were filtered using a third-order Butterworth band-pass filter between 10 and 1000 Hz and a 50 Hz notch filter to remove electrical power contamination. Peak-to-peak amplitudes of the MEPs for FDS, EIP, EDC and FDI muscles were calculated as the absolute distance between the minimum and maximum values between 10 and 80 ms after the TMS pulse. MEPs that visual inspection revealed to be contaminated by overt movement were removed (approximately 0.6%).

MEPs were then normalized for each participant's finger position as a percentage of the average amplitude in the control (baseline) condition. MEPs in the *up* condition were normalized using control data from the *up* condition and MEPs in the *down* condition were normalized using control data from the *down* condition. MEPs were then submitted to a 4 (Muscles: FDS, EIP, EDC and FDI) \times 2 (Finger Position: Down and Up) \times 2 (Observed Position: Max Flexion and Max Extension) repeated-measures ANOVA, which, in a second step, was broken down into 2 (Finger Position: Down and Up) \times 3 (Relative Phase: 0°, 180° and 90°) repeated-measures ANOVAs for each muscle (due to a significant 3-way interaction). For each muscle, MEPs obtained in the middle finger position condition when the observed finger moved from a maximal flexion to a maximal extension were compared with those obtained when the observed finger performed the inverse trajectory using paired Student's *t*-tests before averaging them to yield a single 90° condition.

These comparisons also allowed us to test the direct matching prediction that responses in participant's finger flexor and extensor should increase when observing a flexion and an extension, respectively. To further test this prediction, a 2 (Finger Position: Down and Up) \times 2 (Observed Position: Max Flexion and Max Extension) repeated-measures ANOVA was performed on MEPs of each muscle induced by TMS at maximal flexion and extension positions of the observed finger. Bonferroni *post hoc* tests were used to determine the nature of the effects when necessary.

Results

The 4 (Muscles: FDS, EIP, EDC and FDI) \times 2 (Finger Position: Down and Up) \times 2 (Observed Position: Max Flexion and Max Extension) repeated-measures ANOVA on MEP amplitude yielded a significant 3-way interaction $F_{6,150} = 2.20$, $P = 0.046$, $\eta_p^2 = 0.081$. Separate analyses on the four muscles revealed modulations of corticospinal excitability for participants' index finger flexor muscles (FDS) consistent with the occurrence of dynamical entrainment rather than direct matching. No significant modulation of corticospinal excitability was found for other muscles.

Occurrence of entrainment in FDS

T-tests comparing MEPs induced in the middle finger position condition for FDS, involved in index finger flexion, did not show any significant differences $t_{25} = 2.028$, $P = 0.053$ and $t_{25} = 0.074$, $P = 0.942$ when participants' index fingers in down and up

positions, respectively). This indicates that responses in participants' index flexors were not greater when observing a flexion than an extension. Confirming this result, the 2 (Finger Position: Down and Up) \times 2 (Observed Position: Max Flexion and Max Extension) ANOVA did not reveal significant main effects of Finger Position $F_{1,25} = 3.384$, $P = 0.078$, $\eta_p^2 = 0.119$, Observed Position $F_{1,25} = 4.118$, $P = 0.053$, $\eta_p^2 = 0.141$ or the interaction between these two factors $F_{1,25} = 0.927$, $P = 0.345$, $\eta_p^2 = 0.036$.

The 2 (Finger Position: Down and Up) \times 3 (Relative Phase: 0°, 180° and 90°) ANOVA performed on FDS MEPs did not show significant main effects of Finger Position $F_{1,25} = 2.553$, $P = 0.123$, $\eta_p^2 = 0.093$ and Relative Phase $F_{2,50} = 1.242$, $P = 0.297$, $\eta_p^2 = 0.047$, but yielded a significant interaction between these two factors $F_{2,50} = 5.369$, $P = 0.008$, $\eta_p^2 = 0.177$. As shown in figures 2 and 3, for the down condition there was an increase in MEP amplitude from 90° through 180 to 0°, while for the up condition there was a decrease from 90° through 180 to 0°. Bonferroni *post hoc* comparisons revealed significant differences between up and down conditions for 0° ($P < 0.000001$) and 180° ($P < 0.005$) but not for 90° ($P > 0.10$). These results indicate that activity in FDS was modulated to facilitate the occurrence of in-phase and anti-phase relations – increased activity in FDS in the *down* condition facilitating maximal flexion of participant's index finger towards in-phase and anti-phase relations and decreased activity in FDS in the *up* condition indirectly facilitating (due to inhibition of the antagonist) maximal extension of participant's index finger towards in-phase and anti-phase relations.

No modulation in extensor muscles

T-tests performed on MEPs for EIP and EDC, both involved in the index finger extension, did not reveal significant differences ($t_{25} = -0.531$, $P = 0.600$ and $t_{25} = -0.337$, $P = 0.739$ for EIP; and $t_{25} = 0.137$, $P = 0.892$ and $t_{25} = 0.691$, $P = 0.496$ for EDC, for participants' index fingers in down and up positions, respectively), indicating that responses in the index extensors were not greater when observing an extension compared to a flexion. This result was corroborated by a lack of significant differences in the 2 (Finger Position) \times 2 (Observed Position) ANOVAs for EIP (Finger Position, $F_{1,25} = 0.004$, $P = 0.953$, $\eta_p^2 = 0.000$; Observed Position, $F_{1,25} = 2.850$, $P = 0.104$, $\eta_p^2 = 0.102$; Finger Position \times Observed Position $F_{1,25} = 3.233$, $P = 0.084$, $\eta_p^2 = 0.115$) and for EDC (Finger Position, $F_{1,25} = 1.229$, $P = 0.278$, $\eta_p^2 = 0.047$; Observed Position, $F_{1,25} = 0.279$, $P = 0.602$, $\eta_p^2 = 0.011$; Finger Position \times Observed Position $F_{1,25} = 0.151$, $P = 0.701$, $\eta_p^2 = 0.006$).

The 2 (Finger Position) \times 3 (Relative Phase) ANOVAs performed on MEPs for EIP and EDC also did not reveal any significant difference (EIP: Finger Position, $F_{1,25} = 0.049$, $P = 0.826$, $\eta_p^2 = 0.002$; Relative Phase, $F_{2,50} = 1.432$, $P = 0.248$, $\eta_p^2 = 0.054$; Finger Position \times Relative Phase $F_{2,50} = 3.103$, $P = 0.054$, $\eta_p^2 = 0.110$; EDC: Finger Position, $F_{1,25} = 0.737$, $P = 0.399$, $\eta_p^2 = 0.029$; Relative Phase, $F_{2,50} = 0.752$, $P = 0.477$, $\eta_p^2 = 0.029$; Finger Position \times Relative Phase $F_{2,50} = 1.614$, $P = 0.209$, $\eta_p^2 = 0.061$), indicating that motor entrainment did not occur in participants' extensors.

No modulation in FDI

No significant differences between flexion and extension observation were found for FDI, involved in both flexion and extension of the index finger, in *t*-tests ($t_{25} = 1.591$, $P = 0.124$ and $t_{25} = -1.099$, $P = 0.282$ for down and up participant finger positions, respectively) or in the 2 (Finger Position) \times 2 (Observed Position) ANOVA, Finger

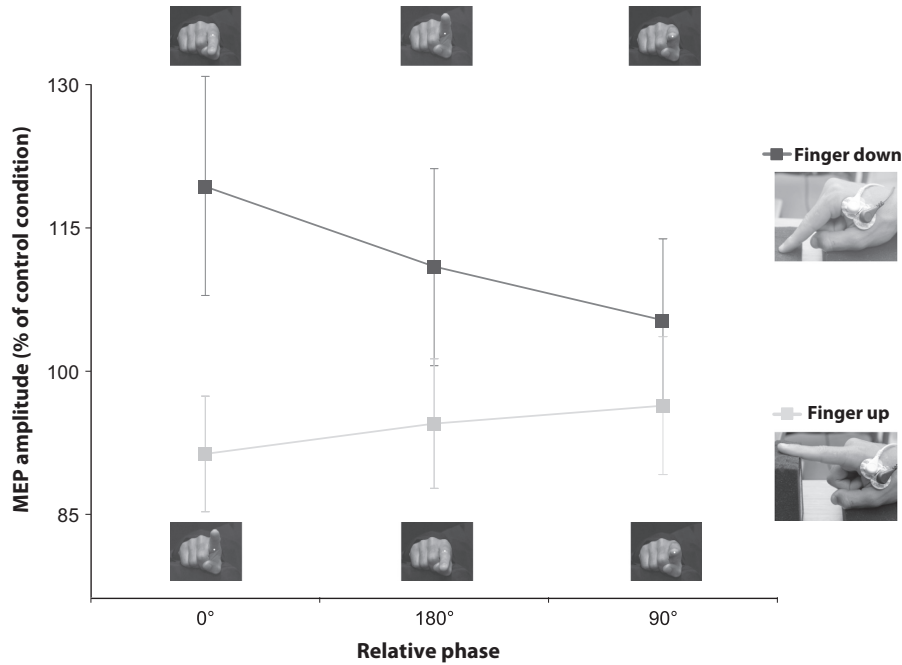


FIG. 2. Amplitude (% of the Control condition) of MEPs induced in the FDS muscle, involved in the flexion of the index finger, for each relative phase condition as a function of participants' finger position (up or down). In-phase and anti-phase relations were associated with increased MEP amplitude in the finger down condition and decreased MEP amplitude in the finger up condition. Error bars represent the standard error of the mean.

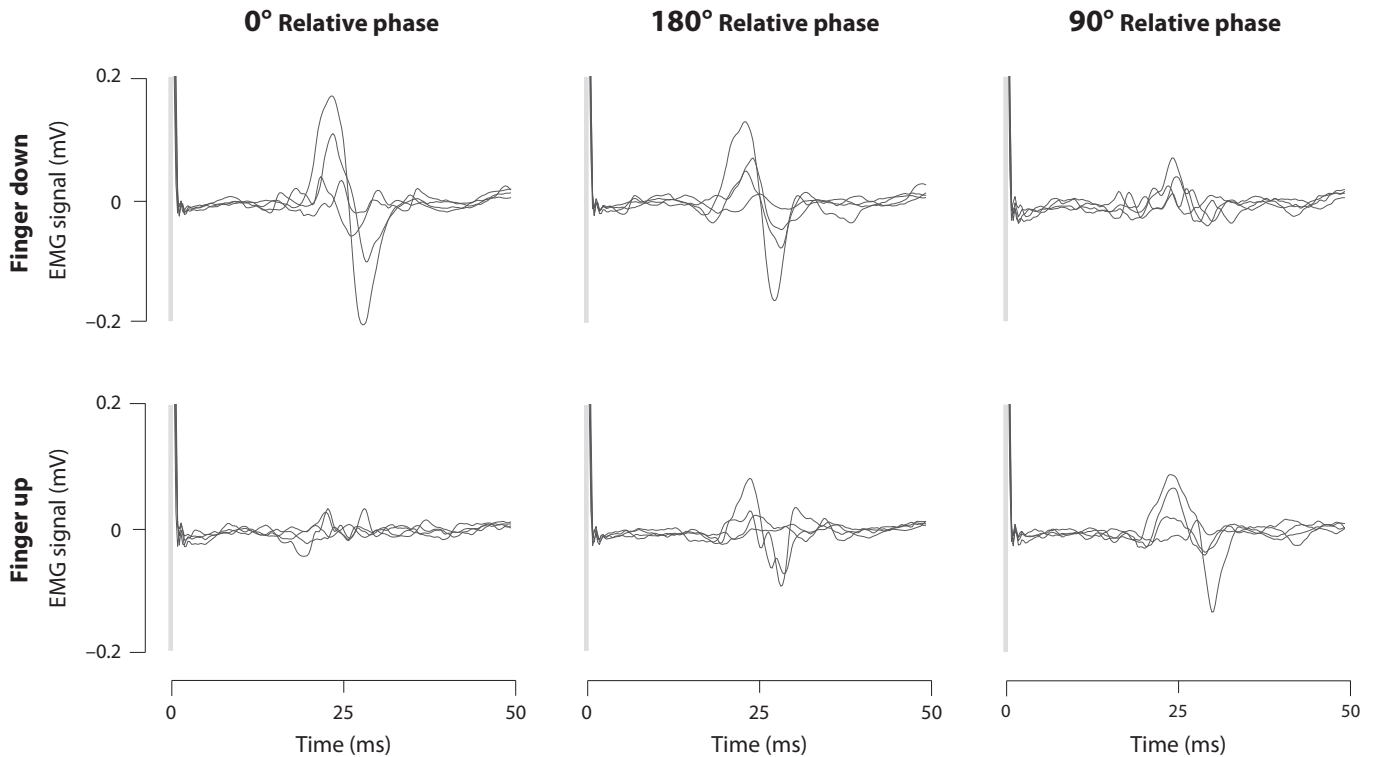


FIG. 3. MEPs induced in FDS of a representative participant for a trial with the finger in a down position (top row) and a trial with the finger in an up position (bottom row).

Position, $F_{1,25} = 1.403$, $P = 0.247$, $\eta_p^2 = 0.053$; Observed Position, $F_{1,25} = 0.495$, $P = 0.488$, $\eta_p^2 = 0.019$; and Finger Position \times Observed Position $F_{1,25} = 0.090$, $P = 0.766$, $\eta_p^2 = 0.004$). The 2 (Finger Position) \times 3 (Relative Phase) ANOVA performed on FDI

also did not reveal any significant effects, Finger Position, $F_{1,25} = 2.398$, $P = 0.134$, $\eta_p^2 = 0.088$; Relative Phase, $F_{2,50} = 0.136$, $P = 0.873$, $\eta_p^2 = 0.005$; Finger Position \times Relative Phase $F_{2,50} = 0.838$, $P = 0.439$, $\eta_p^2 = 0.032$.

Discussion

This study investigated the modulation of corticospinal excitability during the passive observation of human rhythmic movements. Specifically, we tested whether changes in corticospinal excitability when observing the oscillations of an index finger are underpinned by dynamical visuomotor entrainment or internal motor representations (direct matching hypothesis) by examining TMS-induced MEPs in participants' index finger muscles. Our finding of responses facilitating both in-phase and anti-phase relations with the observed finger favors the dynamical entrainment hypothesis over the direct matching hypothesis, which predicts facilitation only for the in-phase relation (i.e. facilitation of flexion or extension when observing flexion or extension, respectively).

The dynamical entrainment hypothesis was supported by the responses induced in the flexor of participants' index fingers. MEPs in FDS increased, facilitating the *flexion* of the participant's finger towards anti-phase and then in-phase relations compared to a 90° phase relation. Interestingly, opposite results were found in FDS when participant's finger was in the up position. MEPs decreased, indirectly facilitating the *extension* of the participant's finger towards anti-phase and then in-phase relations compared to a 90° phase relation. Although the inhibition of FDS can indirectly facilitate finger extension, it is important to note that no modulation in the extensor muscles was found. The facilitation of both in-phase and anti-phase relations indicates that these increased and decreased FDS responses support the dynamical entrainment theory (Kelso, 1997; Schmidt & Richardson, 2008; Richardson *et al.*, 2009; Coey *et al.*, 2012). In line with previous behavioral research, responses increased and decreased for both in-phase and anti-phase compared with 90° relations, which are known to act as a repeller, and the magnitude of these increased and decreased responses was greater for in-phase than anti-phase (Schmidt *et al.*, 2007; Richardson *et al.*, 2007; Tognoli *et al.*, 2007; Schmidt & Richardson, 2008).

MEP responses induced in the four muscles recorded did not provide evidence for the direct matching hypothesis and thus for a continuous modulation of corticospinal excitability according to internal motor representations that match the observed rhythmic finger movements (Iacoboni *et al.*, 1999; Brass *et al.*, 2000; Kilner *et al.*, 2003; Blakemore & Frith, 2005; Brass & Heyes, 2005). Although one could consider the stronger 0° facilitation to corroborate the direct matching hypothesis, the fact that the dynamical entrainment approach also predicts this stronger 0° facilitation additionally to the responses exhibited in the other conditions, suggests that this 0° facilitation may be due to entrainment rather than, or complementary to, internal motor representation.

It is possible, however, that the nature of the movement employed in this experiment favored dynamical motor entrainment and limited the involvement of internal motor representations. The movement observed was not goal-directed, unlike movements that are often used in studies supporting the direct-matching hypothesis, such as reaching or grasping movements (Fadiga *et al.*, 1995; Baldissera *et al.*, 2001; Gangitano *et al.*, 2001; Aziz-Zadeh *et al.*, 2002; Aglioti *et al.*, 2008). Although debated in the literature, there is evidence that the goal of an action might influence modulations of corticospinal excitability related to internal motor representations (Bekkering *et al.*, 2000; Cattaneo *et al.*, 2009; Cavallo *et al.*, 2012; Mc Cabe *et al.*, 2014). Furthermore, the highly predictable nature of the continuously repeating movement observed in the current study, with full availability of visual information related to its kinematics, might have reduced reliance upon internal motor representations. Indeed, changes in corticospinal excitability reflecting the

involvement of internal representations have been typically found during the observation of brief actions (e.g. reaching, grasping and single flexion or extension), which might require predictive mechanisms. Previous research has highlighted the role of internal motor representations especially in understanding and predicting observed actions (Blakemore & Decety, 2001; Aglioti *et al.*, 2008; Sebanz & Knoblich, 2009).

In work related to the current study, Borroni and collaborators investigated the time course of corticospinal excitability during the observation of rhythmic movement and found modulations of motor responses in line with the direct-matching hypothesis (Borroni *et al.*, 2005; Borroni & Baldissera, 2008). They examined responses in forearm muscles of participants observing rhythmic flexion-extension of the wrist and found enhanced motor responses in the flexors when observing a flexion and enhanced motor responses in the extensors when observing an extension. However, direct comparisons with our study are questionable given that these earlier studies did not manipulate the position of participant's wrist, which was kept in a neutral position, making difficult to determine if anti-phase facilitation could have occurred. Moreover, Borroni *et al.* used frequencies (1 and 1.6 Hz) that were faster than the 0.5 Hz used here, and it is widely documented that the strength of anti-phase attraction decreases and even vanishes at these faster frequencies (Wimmers *et al.*, 1992; Peper *et al.*, 1995). Furthermore, the trial duration in the Borroni *et al.* studies was relatively short, with only 4–5 flexion-extension cycles compared to the 45 cycles presented in our study, which as discussed before, might have favored the involvement of internal motor representations in their study because of the observation of less predictable actions.

This last possibility suggests that the two types of processes – dynamical visuomotor entrainment and internal motor representation – may coexist but their relative involvement depends on the properties of the observed movement. Short, goal-directed and discrete actions would favor the involvement of internal motor representations to allow action understanding and prediction in the context of reduced dynamical visual information whereas visuomotor entrainment would preferentially occur when actions are extended in time, more predictable and the continuous flow of visual information is available in the environment. Although in need of further investigation, this distinction between the two processes during action observation is in line with recent proposals that the two approaches are complementary instead of alternatives (Sebanz & Knoblich, 2009; Colling & Kellie, 2014; Novembre *et al.*, 2016; MacRitchie *et al.*, in press). By revealing entrainment during action observation using TMS, the current study supports this possibility and encourages further research aimed at understanding how these two types of processes could together facilitate our understanding and adaptation to others' movements.

Also important for future research will be to investigate the social or biological specificity of the entrainment processes revealed here. Indeed, previous behavioral research demonstrating dynamical visuomotor entrainment with both human movements and simple computer-generated moving stimuli (Schmidt *et al.*, 2007; Varlet *et al.*, 2012, 2014), has so far failed to show significant differences between the two (Coey *et al.*, 2011; Ouweland & Peper, 2015). In contrast, several studies have shown that internal motor representations and direct matching preferentially occur with human or biological movements (Fadiga *et al.*, 1995; Kilner *et al.*, 2003; Borroni *et al.*, 2005). Evidence for motor representations is absent or weaker when observing computer-generated stimulus or robotic arm movements, including, for example, in the studies of (Borroni *et al.*,

2005; Borroni & Baldissera, 2008), which did not find motor facilitation when observing an oscillating metal platform.

Furthermore, a radical prediction of the dynamical entrainment hypothesis would be that entrainment occurs independently of the effector. In contrast, although the subject of debate, the direct matching hypothesis predicts that motor representations are preferentially activated for the effector corresponding to the observed one (Fadiga *et al.*, 1995; Aziz-Zadeh *et al.*, 2002; Borroni *et al.*, 2005; Borroni & Baldissera, 2008; Sartori *et al.*, 2013). In other words, motor representations of the right or left hand become activated when observing a moving right or left hand, respectively (Aziz-Zadeh *et al.*, 2002). These questions encourage future explorations of whether visuomotor entrainment occurs irrespective of the type of observed movement and effector or whether it is modulated by selective control processes that make it specific to the observed movement. Recording MEPs induced in the muscles of the little finger, such as the flexor digiti minimi brevis and abductor digiti minimi (ADM), which are not involved in the observed movement, could be a promising way to address this issue in future investigations.

An additional question raised by the current results is why entrainment, and modulations of corticospinal excitability more generally, were not observed in the extensor muscles. This may be due to a higher level of control in the flexor than the extensor muscles of the index finger. Optimal control of the flexion is required in everyday life when tapping on a computer keyboard for example or when holding an object with enough pressure to avoid it falling but not too much to avoid damaging it. Previous research showing better control for flexion than the extension in sensorimotor synchronization tasks is consistent with this possibility (Roerdink *et al.*, 2008, 2013; Miyata & Kudo, 2014). Another possibility is that, in contrast to finger flexion, which was prevented by the foam block, responses of the extensors could have led to finger movements even though participants were instructed to remain still. It is thus possible that some inhibition occurred in the extensors to avoid finger movements and to follow instructions. Further study will be necessary to differentiate these alternative accounts.

A noteworthy issue in research involving TMS modulations of specific muscle activity relates to spatial precision in the localization of brain regions of interest. Despite overlap of cortical motor representations and care taken by the experimenter to ensure that all muscles were equally stimulated, it remains possible that the stimulation site and intensity was not always optimal for each of the different muscles. This is especially pertinent given possible localization differences between distal and proximal muscle representations (Penfield & Boldrey, 1937; Sanes *et al.*, 1995; Meier *et al.*, 2008). Furthermore, the use of a neuronavigation system to better target and keep the correct stimulation site throughout the experiment is also relevant for future research, as it could help in decreasing MEP variability and revealing effects in the other muscles (Julkunen *et al.*, 2009; Cincotta *et al.*, 2010). It is finally possible that different, or more specific, effects may occur if monophasic (rather than biphasic) stimulation was used, given that monophasic stimulation can induce different neural activation of the motor system (Di Lazzaro *et al.*, 2001; Di Lazzaro & Rothwell, 2014).

To conclude, the current study used TMS to reveal dynamical visuomotor entrainment during action observation, showing that attraction and adaption to other people's movements are not only the result of the activation of internal motor representations. These findings further highlight the relevance of different theories in motor control, and dynamical systems perspective in particular, to better understand the complexity of brain responses during action observation (Kelso, 1997; Coey *et al.*, 2012; D'Ausilio *et al.*, 2015; Swinnen & Alaerts, 2015).

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Conflict of interest

The authors have declared that no competing interests exist.

Author contributions

MV, GN and PK conceived and designed the experiment, analyzed the data and wrote the paper. MV performed the experiment.

Data accessibility

Data supporting the results in the paper are available upon request.

Abbreviations

EDC, Extensor Digitorum Communis; EIP, Extensor Indicis Proprius; EMG, Electromyography; FDI, First Dorsal Interosseus; FDS, Flexor Digitorum Superficialis; MEP, Motor-Evoked Potential; MT, Motor Threshold; TMS, Transcranial Magnetic Stimulation.

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