Premovement suppression of corticospinal excitability may be a necessary part of movement preparation

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ABSTRACT (199/200)

In reaction time (RT) tasks corticospinal excitability (CSE) rises just prior to movement. This is preceded by a paradoxical reduction in CSE, when the time of the imperative ("GO") stimulus is relatively predictable. Because RT tasks emphasise speed of response, it is impossible to distinguish whether reduced CSE reflects a mechanism for withholding prepared actions, or whether it is an inherent part of movement preparation. To address this question, we used transcranial magnetic stimulation (TMS) to estimate CSE changes preceding a) RT movements; b) movements synchronized with a predictable signal (predictive timing or PT movements); and c) self-paced movements. Results show that CSE decreases with a similar temporal profile in all three cases, suggesting that it reflects a previously unrecognised state in the transition between rest and movement. Although TMS revealed reduced CSE in all movements, the TMS pulse itself had different effects on movement times. TMS given ~200ms before the times to move speeded the onset of RT and self-paced movements, suggesting that their initiation depends on a form of trigger that can be conditioned by external events. On the contrary, PT movements did not show this effect, suggesting the use of a different triggering strategy prioritizing internal events.

KEYWORDS

Voluntary movements; Transcranial Magnetic Stimulation; Self-paced movements; Corticospinal excitability

Introduction

In preparation for voluntary movements, there are substantial changes in the activity of neurones in primary motor cortex (M1) even though electromyographic (EMG) activity in task-related muscles remains constant (Tanji and Evarts 1976). Previous studies in

humans have used transcranial magnetic stimulation (TMS) over M1 to probe how this might occur by probing corticospinal excitability (CSE) changes at different time points relative to when movements begin. They have documented a variety of different forms of inhibition or suppression of excitability in motor cortical outputs that could potentially account for these effects (Duque et al. 2017). One of the most relevant and yet least understood is "preparatory inhibition", which describes a period of reduced CSE relative to baseline (Hasbroucq et al. 1997; Touge et al. 1998; Duque et al. 2017) that is observed prior to movement in muscles that are both involved or uninvolved in an action (Duque and Ivry 2009; Duque et al. 2010; Bestmann and Duque 2015; Greenhouse et al. 2015). For practical reasons, work focussed on preparatory inhibition has mainly been done using reaction time (RT) tasks. Under such conditions, CSE decreases in both the involved and uninvolved effectors around the time of a temporally predictable imperative ("GO") cue (Hasbroucq et al. 1997; Touge et al. 1998). Different models have been proposed to explain this effect. Competition resolution proposes that inhibition is necessary to suppress competing movements, at least in situations in which the movement to be performed is not precisely known in advance (Burle et al. 2004); a second possibility, known as impulse control theory, is that inhibition is necessary to withhold a prepared movement until the "GO" cue is detected (Duque and Ivry 2009; Duque et al. 2010); a third possibility, sometimes known as the spotlight hypothesis, is that preparatory inhibition reduces background motor activity to speed movement onset because excitatory inputs that select the chosen response stand out better against a quiescent background (Greenhouse et al. 2015; Lebon et al. 2019).

A drawback of these previous studies is that they employ cue-driven paradigms. This makes it difficult to determine whether preparatory inhibition is limited to movements involving external cues, or if it rather represents an inherent state undergone by a

population of cortical neurones when they shift from a state that maintains a constant output to a state that triggers a movement. Of relevance in this regard, recent studies have provided evidence in primate for common neural population dynamics in M1 during preparation for movements initiated in different contexts (Lara et al. 2018).

The aim of this study was to examine whether preparatory inhibition occurs in movements triggered by different types of signals: (1) RT movements (as above); (2) a predictive timing (PT) task in which movement initiation is timed to coincide with the last event in a predictable countdown-like sequence, and (3) in self-paced movements that are devoid of any external trigger. In all cases, the movement was pre-specified with no choice element, thus eliminating the possibility that CSE suppression was due to conflict resolution (see also Quoilin et al. 2019). Self-paced movements specifically require that movement is not withheld since they are instructed to be spontaneous. PT movements also do not require withholding of movement since correctly timed initiation of the preparatory process could necessarily progress to movement execution at the appropriate time. Thus we argue that if premovement suppression is present in all 3 movement types, then it likely reflects a previously unrecognised transition state in the evolution of movement.

Although the primary purpose of TMS was to provide an instantaneous probe of CSE, we could also examine its subsequent effects on movement onset. Previous experiments have shown that the noise and scalp sensation of a TMS pulse can speed the onset of RT movements because of intersensory facilitation, i.e., the speeded release of a prepared movement when a secondary stimulus (a TMS pulse in our case) is delivered at about the time of the imperative stimulus (Nickerson 1973). In movements made in a RT context, intersensory facilitation is usually explained in terms of shortening the time taken to identify the imperative stimulus (Pascual-Leone, Valls-Sole, et al. 1992). Self-paced and

PT movements may not require an external trigger to be initiated since they can start immediately once preparation is complete. If this is the case, they should not display intersensory facilitation.

Materials and Methods

Participants

In total, 33 right-handed healthy subjects participated in this study (28 ± 1 years old; age range 20-45 years; 15 females). Fifteen participants (7 females) took part in experiment 1 and 18 (8 females) took part in experiment 2. All of them reported no contraindications to TMS (Rossi et al. 2011) and had normal or corrected to normal visual acuity. The study was approved by the University College London Ethics Committee and warranted to be in accordance with the Declaration of Helsinki. All participants signed a written informed consent prior to the experimental session.

Recordings

Participants sat in a comfortable chair with both forearms resting on a pillow placed on their lap and the index finger of the right hand (Experiment 1) or the two hands (Experiment 2) resting on a keypad through which button press times were recorded. A screen was placed ~1 m in front of the participants. They also wore ear defenders to reduce the influence of loud sounds generated by the TMS discharges.

EMG signals were obtained from the right first dorsal interosseous (FDI) muscle for experiment 1 and of both hands in experiment 2. EMG activity from the right abductor digiti minimi (ADM) muscle was also recorded. Amplitudes of the motor-evoked potentials (MEPs) recorded from the right FDI were the primary dependent measure in this study. The ADM was used as a control muscle for the assessment of MEP changes: it was also targeted by the TMS but, unlike the right FDI, it was not directly involved in

the response. Therefore, in line with previous work, ADM MEP amplitudes were not expected to show an increase in CSE leading to movement but rather a reduction of excitability up until the time at which muscles showed voluntary activation (Duque et al. 2010). This inhibition of the non activated effector muscles at the time at which movements are initiated has been previously interpreted to represent a surround inhibition mechanism ensuring that effector muscles close to the activated one are not activated for the movement (Beck and Hallett 2011). Recording electrodes were placed on the muscle bellies, with reference electrodes on the closest metacarpophalangeal joint. The ground electrode was placed on the right wrist. EMG signals were amplified, band-pass filtered between 20 Hz and 2000 Hz (Digitimer D360, 2015 Digitimer Ltd, United Kingdom) and acquired at 5000 Hz sampling rate with a data acquisition board (CED-1401, Cambridge Electronic Design Ltd 2016) connected to a PC and controlled with either Signal or Spike^2 software (also by CED).

Once EMG electrodes were set, the participants' TMS hotspot was located. This was done by finding the point over M1 giving the largest MEPs in the contralateral FDI for a given stimulus intensity. A 70 mm figure-of-eight coil connected to a Magstim 200^2 magnetic stimulator (Magstim, Whitland, UK) was placed tangentially on the scalp and held at a 45^o angle to the sagittal plane with the handle pointing backwards. An electroencephalography cap was mounted on the participants' heads to provide a visual reference of the location of the M1 hand representation area. Once the hotspot was found, the 1 mV intensity was determined by adjusting the TMS output until 5 out of 10 MEPs larger than 1mV could be obtained (Rossini et al. 2015; Hannah et al. 2018; Dupont-Hadwen et al. 2019). The estimated 1 mV TMS intensity was then used to assess CSE changes in the three paradigms tested in this study.

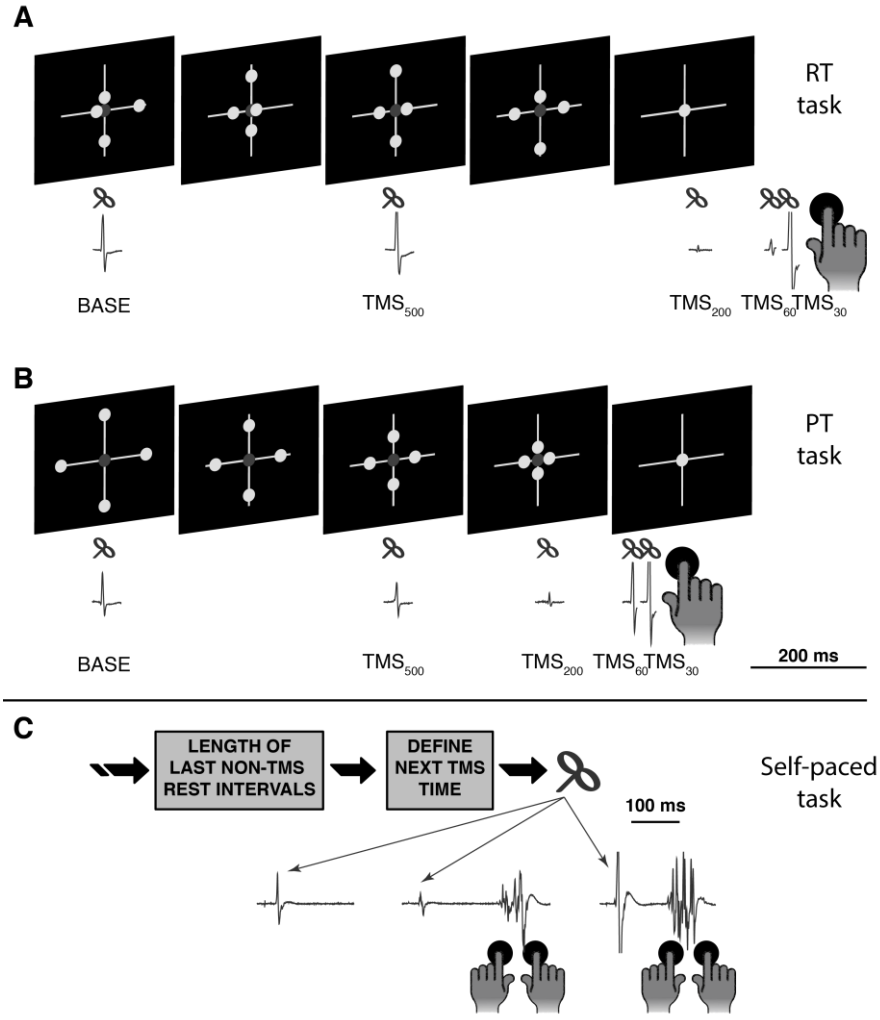
The experimental paradigms were implemented using custom-made MATLAB routines (MathWorks, MA, USA). Synchronization of TMS pulses with EMG and movement events was realised using Cogent 2000's utilities (Cogent 2000 team at the FIL and the ICN and Cogent Graphics developed by John Romaya at the Wellcome Department of Imaging Neuroscience) to control the parallel port of the computer running the experimental paradigms. Data analysis was carried out using custom-made MATLAB functions and SPSS software (IBM, NY, USA).

Experiment 1 – TMS recordings preceding movements in RT and PT tasks

For this experiment, participants performed two types of movement paradigms: 1) a RT task in which movements were initiated following an imperative stimulus (Fig. 1-A); and 2) a PT task in which movements were timed with an external countdown-like signal (Fig. 1-B). In both cases, each trial of the motor task consisted in pressing a button of a keypad with the right index finger at the times indicated by a visual cue. Unilateral movements were chosen to make experiments as similar as possible to most simple reaction time tasks used in previous TMS studies on movement preparation in which only one type of response is required (Duque et al. 2010; Greenhouse et al. 2015; Hannah et al. 2018).

Figure 1. Movement tasks and TMS recordings. (A) In each trial of the reaction time (RT) task -experiment 1- four circles move randomly along the axes of a cross for 1 s. After this delay period, all circles suddenly collapse at the intersection point of the cross and this is the "GO" signal making participants react as fast as they can, performing a button press with their right index finger. (B) In the predictive time (PT) task -experiment 1- participants have to perform the button press at the end of a 1-s period, which is informed by showing four white circles moving along the axes of a cross reaching simultaneously the intersection point from the extremes. Both in PT and RT paradigms, single-pulse TMS was either not delivered (non-TMS trials) or delivered when the four circles appeared onscreen

at the beginning of the delay period (BASE), half way through the delay period (TMS_{500}), and 200 ms (TMS_{200}), 60 ms (TMS_{60}) and 30 ms (TMS_{30}) before the average EMG onset time of each participant in each task. (C) In experiment 2, participants performed self-paced movements consisting of simultaneously pressing two keypad buttons with the index fingers of their two hands. An algorithm was run in parallel to characterize the times at which movements were performed in the non-TMS trials. This information was in turn used to distribute TMS pulses in subsequent TMS trials with different time intervals between the stimuli and the movements.



Each trial of the RT task consisted of a resting phase of 2 s followed by a delay period of 1 s during which four circles moved randomly along the four arms of a cross. After the end of the delay period, the four circles were plotted at the intersection point of the cross,

and this event was to be considered the "GO" cue. Participants were instructed to make a fast and ballistic button press with their right index finger in reaction to seeing the "GO" cue. After each button press, the trial ended by giving participants feedback about the time at which the button press event had been detected (the time of the key press relative to the time at which the "GO" cue was given was used for this). Participants were told to respond as quickly as possible to achieve positive feedback. In the set-up used in these experiments, an interval of ~90 ms separated the FDI EMG onset and the time at which the button press event was detected. The feedback was displayed for a random period of time between 1-3 s, and it consisted of the time at which the button press had been detected and a font colour code indicative of the performance. Button presses in the interval 250-300 ms resulted in feedback with green text (presses within this interval implied that the FDI activation onset had taken place with a reaction time of 160-210 ms in most cases). Yellow text was used for button presses in the intervals 200-250 ms and 300-350 ms. Finally, red text and the warning messages "too early" and "too late" were given as feedback in case button presses were performed before or after these intervals.

The PT task had the same trial structure as the RT task but, in this case, during the delay period the four circles moved from the extremes of a cross towards its centre with a velocity inversely proportional to the remaining distance to the intersection point (initial distance 4.5 cm). Unlike in the RT task, participants were now instructed to time their movements with the overlapping of the four circles at the intersection point. Since PT movements were supposed to be performed at around the time at which the circles collapsed, the feedback was different from the one used in the RT task. Green text was used for button presses done between 50-100 ms relative to the time at which circles overlapped, thus encouraging participants to aim at pressing the button within this interval (which in turn implied activating the FDI muscle at around the time at which circles

collapsed at the intersection point of the cross); yellow text was used for button presses in the intervals 0-50 ms and 100-150 ms; red text was used in any other case. Additionally, the messages "too early" and "too late" were displayed when button presses were performed before or 150 ms after circles collapsed. Note that the purpose of the feedback in both PT and RT tasks was to maintain subject performance and motivation, and also to ensure that movement times in the non-TMS blocks was approximately the same as in the TMS blocks. All participants were easily able to perform both tasks and obtain good feedback on the non-TMS trials.

In the initial part of each experiment, participants practised the RT and PT paradigms without TMS until they showed consistent response times (~30 trials per task). Thirty additional movements were then performed for each paradigm so that the subject- and task-specific average movement onset times based on the FDI EMG activity (EMG onset times) could be estimated. After the initial training phase, the TMS recordings were carried out, consisting in two blocks of 78 trials per paradigm. The blocks of the two paradigms were interleaved. In each block, six conditions were tested using a randomized order of TMS conditions. TMS conditions differed from each other with regards to the timing of the stimulus: 1) no TMS delivered (non-TMS condition); 2) TMS at the beginning of the delay period (BASE); 3) TMS halfway through the delay period (TMS_{500}); and 4-5-6) TMS 200 ms (TMS_{200}), 60 ms (TMS_{60}) and 30 ms (TMS_{30}) before the average EMG onset time, respectively. This allowed us to have matched conditions (baseline and different time points relative to EMG onset time) to compare the evolution of CSE in the RT and PT paradigms. The only exception was TMS_{500}, in which the timing is calculated with respect to the onset of the moving dots. This means that the delay between TMS_{500} pulses and EMG onset was about 500ms in the PT paradigm, when EMG onset is coincident with the collapse of the moving dots to a single point, and about 700ms

in the RT paradigm because in this case, collapse of the dots was the signal to react, leading to an EMG response about 200ms later. Feedback was omitted in TMS trials to avoid that participants tried to compensate the possible influence that TMS could have on EMG onset times (Pascual-Leone, Valls-Sole, et al. 1992; Terao et al. 1997; Ziemann et al. 1997).

Experiment 2 – TMS during the resting phases between self-paced movements

The task involved participants sitting still and comfortably, with both index fingers resting on a keypad. They were instructed to make ballistic bilateral button presses with the left and right hand index fingers every 4-8 s, whilst avoiding pre-movement muscle activation and ensuring movements were always made in a similar way (Fig. 1-C). Bilateral movements allowed us to measure EMG onset times from the left (non-stimulated) hand to estimate the intervals between the TMS pulses and subsequent movements without being affected by the TMS-induced delays of EMG voluntary activations of the right hand FDI in cases where the stimulus was given in close proximity with the intended EMG onset time (Ziemann et al. 1997). Importantly, bilateral synchronous actions present almost identical EMG onset times when no stimulus is given (Schneider et al. 2004). Participants were instructed to perform their movements spontaneously and to avoid any form of internal countdown to decide when to initiate the movements. It was stressed to participants that they must not let the TMS alter their decision to move. A resting period of time followed by a button press was considered a trial, and 12 blocks were performed by each participant with 65 trials making up a block. During blocks, EMG was monitored to ensure the hand was relaxed between button presses.

A custom-made MATLAB program was used to determine the timing of a TMS stimulus on a given trial based on the timings of the button presses in the previous 5 trials

performed by each participant without TMS (this number of trials was empirically chosen to allow the code program to quickly adapt to changes in participants' behaviours). TMS timing was distributed so that in 4% of the trials, stimuli were delivered early after the previous movement (3 s after the previous button press); 8% of the trials were non-TMS trials, which were then used to monitor inter-movement intervals in the absence of external stimuli along the experiment. Finally, in 88% of the trials, TMS pulse timings were defined based on the probability density function of inter-movement intervals considering the 5 most recent non-TMS trials. For that, a Gaussian fit was estimated and the next TMS firing time was selected according to the left-hand side of this probability density function. TMS firing times were thus programmed to be delivered at a time interval relative to the previous button press such that it was always below the average inter-movement interval estimated. In the cases when participants waited for over 10 s between button presses, participants were given an indication by the experimenter to reduce the inter-movement time intervals.

Data processing and statistical analysis

In both experiments, the onsets of the EMG were used as the reference points indicating the times of movement initiations. In order to obtain these EMG onset times in each trial, EMG was first rectified and then a moving average of 5 ms was applied to obtain a smoothed envelope of the EMG signal. EMG recordings of all trials whilst participants were at rest were analysed to obtain subject-specific resting EMG levels. Thresholds set at five times these levels were used to determine EMG onset times. These levels were also used to detect and remove trials with pre-TMS or pre-movement activation of all the muscles registered. All trials were then visually inspected and manually corrected to ensure that EMG-based movement onsets were estimated properly and that no building-up of EMG activity was apparent before the TMS. Two repeated measures ANOVA

(rmANOVA) tests were run separately with data from experiments 1 and 2 to assess that EMG peak-to-peak amplitudes in the right FDI during the 200 ms intervals preceding the TMS pulses did not differ across the TMS conditions tested, *i.e.*, five TMS times tested in experiment 1 and three TMS times in experiment 2 (see below and results section). Results of these tests showed that the background EMG activity in the right FDI was not significantly different across conditions ($P > 0.2$ in all cases). Finally, EMG onset times and peak-to-peak amplitudes of MEPs were estimated. MEP amplitudes were estimated from the acquired EMG signals without applying any additional filters. A logarithmic transformation (to the base e) of MEP amplitudes was performed before the statistical tests to ensure normality of the samples.

To select the tests to compare EMG onset times and log-transformed MEP amplitudes across paradigms, TMS conditions and muscles in experiments 1 and 2, normality was checked by assessing that z-scores of the populations' kurtosis and skewness were below a critical value of 2 (Kim 2013). All compared variables satisfied the condition of normality except for the comparison between EMG onset times in the training trials and non-TMS trials in TMS blocks in experiment 1, and for the multiple comparisons of the intervals between TMS times and EMG onset times in experiment 2 (where a subset of the paired comparisons did not satisfy normality). In these cases, Wilcoxon signed rank tests were run.

To compare EMG onset times and MEP amplitudes across conditions in experiment 1, log-transformed MEP amplitudes and times of EMG (right FDI) onsets of all trials were labelled according to the type of paradigm (PT, RT) and to the time at which TMS was delivered (BASE, TMS_{500}, TMS_{200}, TMS_{60}, TMS_{30}). MEP amplitudes were also labelled according to the muscles from which they were registered (FDI, ADM). EMG onset times were referenced to the ones in the non-TMS trials. A 2-way rmANOVA (TIME x

PARADIGM) was performed to compare EMG onset times across conditions. A 3-way rmANOVA (TIME x PARADIGM x MUSCLE) was performed to test for changes in MEPs. Post-hoc comparisons were run in the case of finding significant effects. Alpha P-levels obtained from paired comparisons between BASE and the other TMS conditions were Bonferroni-corrected by multiplying them by a factor of 4.

To assess if TMS affected movement times in experiment 2, we compared for each participant the observed histograms of the TMS-to-EMG onset intervals with simulated intervals obtained using the non-TMS trials that had been interspersed along the experiments. To obtain the simulated distributions of the lengths of the TMS-to-EMG onset intervals, the non-TMS trials were used by a simulation algorithm (running 500 iterations) that: *i*) randomly selected 5 trials; *ii*) obtained a simulated TMS time for the "next" trial as in the actual experiment; *iii*) randomly selected a new non-TMS trial; *iv*) obtained the time interval between the FDI EMG onset time and the simulated TMS time and kept it if it was positive (that is, if the simulated TMS time preceded the movement time). The resulting simulated distributions of TMS-to-EMG onset intervals were compared with the actually registered TMS-to-EMG onset intervals by comparing their histograms. This comparison was performed by running individual Wilcoxon tests between bins of 40 ms of width of the two histograms (real and simulated) from 1 s before the EMG onsets. The resulting *P*-values were corrected for multiple comparisons by multiplying them by the number of bins assessed (26 bins). This comparison was run separately for TMS-to-EMG onset intervals obtained using the right and left hand FDI muscles to assess their similarity. We also run an additional analysis to justify the need of using EMG onsets from the left hand FDI by showing that EMG onsets in the right hand FDI were delayed when TMS pulses were given in close proximity to EMG onset times (Ziemann et al. 1997). Using the TMS trials of all participants, we assessed the

difference between the EMG onsets in the left and right hand FDI muscles as a function of the interval between the TMS times and the corresponding left hand FDI EMG onset times. This assessment was done using a bootstrapping approach equivalent to the one used to look for changes in MEP amplitudes and explained in the next paragraph.

Finally, for the MEP analysis in the self-paced movements task in experiment 2, we wanted to find TMS-to-EMG onset intervals within which significant differences in MEP amplitudes compared to basal conditions could be observed. In this case, due to the free nature of the self-paced movements studied, the times of the TMS pulses relative to the subsequent movements could not be well controlled and neither could it be predicted when possible changes in MEPs, if any, should take place based on prior knowledge. To overcome these limitations, we used a two-steps analysis. In the first step, bootstrap statistics were applied using all participants' MEP amplitudes in order to identify, in an unbiased way, TMS-to-movement intervals of interest, *i.e.*, intervals between TMS pulses and FDI EMG onsets within which significant increases or decreases in MEP amplitudes were observed. Then, MEPs contained in these estimated intervals were compared using an analysis that was equivalent to the one described before for MEPs registered in experiment 1. For the bootstrapping analysis, the left FDI EMG onsets were considered since right FDI EMG onsets could be biased by the TMS-induced delays of voluntary actions in the trials where the stimulus was given in close proximity with the subsequent movement (Ziemann et al. 1997). To look for intervals of interest, the following steps were repeated for 100 iterations: 1) 200 TMS-trials per participant were chosen at random; 2) MEPs selected from each participant were referenced to baseline (BASE) MEPs obtained more than 500 ms before the estimated left FDI EMG onset times by computing the z-scores, *i.e.* MEPs were subtracted the mean and divided by the standard deviation of the BASE MEPs; 3) MEP amplitude values from all participants were

merged; 4) a sliding window of 40 ms in steps of 20 ms was applied from -1 s to the movement onset. For each window, 40 MEPs were picked at random with replacement and used to calculate a mean (Graimann et al. 2002). This was repeated 1000 times, thus generating 1000 means for every window. The 5^{th} and 995^{th} ranked values were taken as confidence intervals. After this process, an average of all estimated confidence intervals was taken to produce the definitive confidence intervals of MEP changes across the time in preparation for movements. This allowed us to estimate time intervals of interest in which MEPs changed. Since our bootstrap analysis was based on merging the data from all subjects, the estimated time intervals of interest were used in the second step of this analysis to run a standard 2-way rmANOVA with factors TIME and MUSCLE (FDI and ADM) to study the changes in MEP amplitudes on a group level. This allowed us to obtain results comparable to those in Experiment 1. The TIME factor included the BASE period (more than 500 ms before movements), and the periods of time in which significant decreases or increases in the right FDI MEPs were observed (referred to as TMS_{DEC} and TMS_{FAC} conditions in the results section). Alpha P-levels obtained from paired comparisons between BASE and the other TMS conditions were Bonferroni-corrected by multiplying by a factor of 2.

Throughout the manuscript, results are reported as group mean ± SD and P values < 0.05 are considered to be significant, unless indicated otherwise. The Greenhouse–Geisser procedure was applied where necessary to correct for violations of sphericity in rmANOVAs.

Results

Experiment 1

Movement times

The average movement onset times (based on EMG) obtained in the non-TMS trials during the initial training phase of the experiments were -7 ± 23 ms in the PT (indicating that participants successfully synchronised EMG activity to the onset of the trigger) and 211 ± 19 ms in the RT paradigm. The average EMG onset times in non-TMS trials of the experimental blocks were not significantly different (-19 ± 19 ms, $P = 0.135$ for PT; 206 ± 12 ms, $P = 0.277$ for RT), indicating a similar level of performance throughout the experiments.

Turning to the experimental blocks, Fig. 2 plots the difference in the average time of movement onset (measured to EMG onset) in TMS trials compared with non-TMS trials; positive values indicate EMG onset is delayed. If the TMS pulse was given at the onset of the trial (i.e. when the moving balls appeared on the screen: BASE) or 500 ms into the delay period (TMS_{500}) it had no effect on the time of movement onset compared with non-TMS trials. However, movement onset was delayed if TMS pulses were given 30 ms or 60 ms prior to the expected time of EMG onset as determined in non-TMS trials (TMS_{30} and TMS_{60}). The delay was the same in PT and RT blocks and has been previously ascribed to the silent period that follows the MEP which delays EMG onset (Day et al. 1989). Finally, if TMS was applied 200 ms prior to expected EMG onset (TMS_{200}), it speeded up movement onset in RT blocks. Since the mean reaction time was 211 ms (see above), this means that reactions are speeded when the TMS pulse is applied at around the time of the imperative stimulus (a stationary single ball in RT blocks). Previous work refers to this effect on reaction times as a form of intersensory facilitation (Terao et al. 1997). Interestingly, TMS pulses applied at a similar time prior to EMG onset in the PT task had no effect on movement time.

Figure 2. Movement onset times (relative to non-TMS trials; mean ± SEs) for the different TMS conditions in the reaction time (RT, white) and predictive blocks of trials (PT, black) tasks. The presence of the TMS stimulus delays movements in both in RT and PT blocks when TMS is given just prior to the expected EMG onset time (TMS_{60} and TMS_{30}). Regarding the comparison between paradigms, the most striking effect seen here is at 200ms prior to expected EMG onset (TMS_{200}), when TMS speeds up movement in RT blocks but has no effect in the PT blocks. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with BASE time point within each movement task. +++$P < 0.001$, RT vs PT.

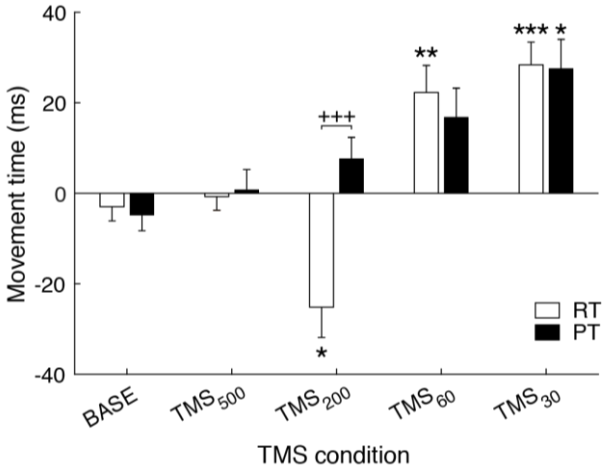


Table 1 summarises the main results obtained from the statistical analysis of movement onset times. There was a significant main effect of TIME ($F_{[4,56]} = 12.035$; $P < 0.001$) with post-hoc paired comparisons showing significantly delayed EMG onset times for TMS_{60} and TMS_{30} conditions compared to BASE ($P < 0.001$; $d = 3.878$ for TMS_{60}; $d = 5.688$ for TMS_{30}). In addition, there was a significant effect of the PARADIGM x TIME interaction ($F_{[4,56]} = 8.652$; $P < 0.001$). Post-hoc paired comparisons showed a significant difference between RT and PT conditions when the TMS pulse was given 200 ms before mean EMG onset (TMS_{200}) ($P < 0.001$; $d = 5.542$). The EMG onset times were significantly reduced in the RT task for TMS_{200} condition compared to BASE ($P = 0.028$; $d = 3.626$). No other significant differences were found between paradigms for EMG onset times ($P > 0.1$ in all cases). Comparisons between BASE and the other TMS

conditions run separately for each paradigm also revealed significantly longer onset times with condition TMS_{30} (P = 0.016; d = 3.903) for the PT paradigm and with conditions TMS_{60} (P = 0.007; d = 4.309) and TMS_{30} (P < 0.001; d = 6.385) for the RT paradigm.

One factor could potentially have confounded these measurements. When we measured TMS trials, movements with very fast onset times that started before the TMS pulse occurred were removed from the analysis because it is impossible to interpret the amplitude of the MEP when contaminated by ongoing volitional EMG activity. This would have happened most frequently then TMS was given 30ms prior to expected movement onset (TMS_{30}). However, the equivalent movements would have been included in non-TMS trials, and therefore could bias the estimate of reaction time. To rule this out, we repeated the analysis after removing from each participant's dataset the 'N' fastest (*i.e.* with the earliest onset times) trials in the BASE condition, with 'N' being the number of trials which were rejected in the TMS_{30} condition because of early EMG onset. This way, we could ensure that significantly different onset times between BASE and TMS_{60} and TMS_{30} conditions were mainly caused by the TMS induced silent period. When we did this, we obtained similar results: onset times were delayed in TMS_{30} relative to BASE in the PT paradigm (P = 0.0315; d = 3.097) and in the RT paradigm (P = 0.003; d = 4.221). In this case, the comparison between EMG onset times obtained with BASE and TMS_{60} did not survive the post-hoc correction (P = 0.058).

Corticospinal excitability

Resting motor threshold and 1mV intensity were 49 ± 12 % and 60 ± 12 % of the maximum stimulator output, respectively. On average, 21 ± 19 MEPs (mean ± SD) were averaged for each TMS condition (averages per TMS condition are shown in Fig. 1 in the supplementary files). The number of averaged MEPs was comparable across TMS conditions except for TMS_{30} in which a smaller number was used since TMS at this

timing was more likely to occur after the onset of the EMG, contaminating measurements of the MEP.

Fig. 3 shows the average amplitude of MEPs evoked in FDI (agonist muscle) and ADM (task-irrelevant muscle) at different times in both RT and PT tasks. MEPs at the time of the warning signal (BASE) were larger in FDI than ADM, but the same in both RT and PT blocks. This difference between MEPs in the two muscles is because the site of stimulation was over the FDI "hotspot" and also because the task-relevant muscle typically shows larger responses than the task-irrelevant one in this type of paradigm (Quoilin et al. 2016, 2019). The clearest effect is the reduction of MEP amplitude in both muscles at TMS_{200} in both blocks. This has been described many times previously in reaction time tasks ("preparatory inhibition": (Duque et al. 2010; Lebon et al. 2016; Hannah et al. 2018)). However this is the first time it has been noted in movements in a PT context. In FDI, the suppression also appears to be present when MEPs were given 60 ms prior to expected movement onset (MEP_{60}), but this was not significant. Finally, in both RT and PT blocks, MEPs continued to be suppressed in the (task-irrelevant) ADM 60 and 30 ms prior to the expected EMG onset. This has been interpreted as a form of "surround inhibition" in a muscle uninvolved in the prime movement (Chen and Hallett 1999; Mackinnon and Rothwell 2000).

Figure 3. MEP amplitudes in the FDI (A) and ADM (B) before movements made in the reaction time (RT) and predictive timing (PT) tasks. Note that MEPs at BASE were larger in FDI than ADM but that in each muscle they were of equal amplitude in both RT and PT blocks. The most significant effect is the reduction in amplitude in both muscles at TMS_{200} without a significant difference between paradigms. Note that MEP amplitudes are given in logarithmic scale. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with BASE; mean ± SEM values plotted.

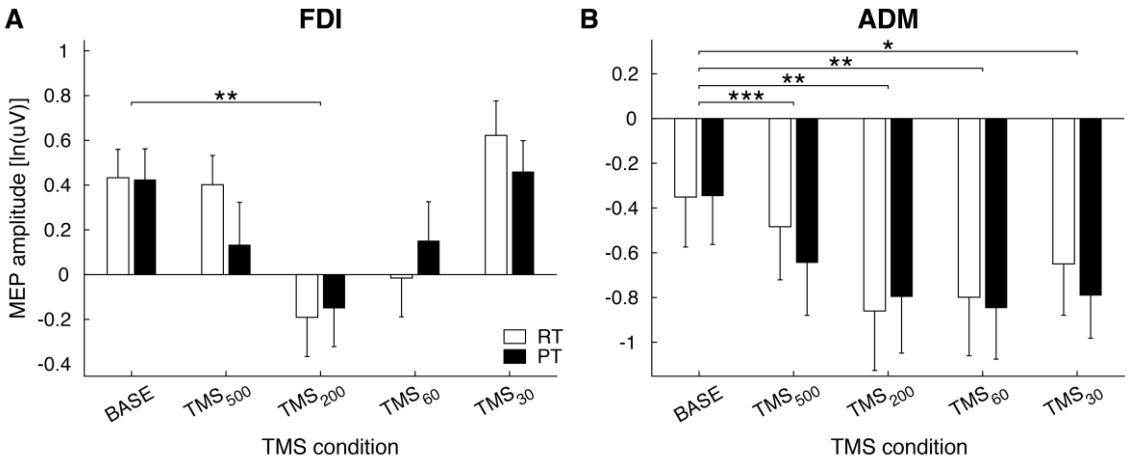


Table 1 summarises the statistical results. There were no significant differences between BASE MEP amplitudes in the RT and PT tasks (P = 0.467). A three way rmANOVA with main factors of TIME (BASE, TMS_{500}, TMS_{200}, TMS_{60}, TMS_{30}), PARADIGM (RT, PT) and MUSCLE (FDI, ADM) revealed significant main effects of TIME ($F_{[4,56]}$ = 6.407; P = 0.006) and MUSCLE ($F_{[1,14]}$ = 20.511; P = 0.006). Post-hoc paired comparisons revealed that BASE MEP amplitudes were significantly higher than for TMS_{500} (P = 0.016; d = 0.197), TMS_{200} (P = 0.001; d = 4.655) and TMS_{60} (P = 0.014; d = 3.504) conditions. There was also a significant interaction of MUSCLE x TIME ($F_{[4,56]}$ = 7.624; P = 0.003). Post-hoc comparisons revealed significant reductions of MEP amplitudes for condition TMS_{200} compared to BASE in the FDI (P = 0.003; d = 4.341), whereas in the ADM, MEP amplitudes were significantly reduced at many more timings relative to BASE MEPs: TMS_{500} (P < 0.001; d = 5.268), TMS_{200} (P = 0.004; d = 4.138), TMS_{60} (P = 0.006; d = 3.885) and TMS_{30} (P = 0.040; d = 3.000). Finally, there was a significant interaction of PARADIGM x TIME ($F_{[4,11]}$ = 3.455; P = 0.033). Post-hoc comparisons revealed significant differences between MEPs in the PT and RT tasks for TMS_{500} (P = 0.038; d = 2.287). This is probably because movements in PT blocks are

performed earlier (relative to the onset of the moving balls) than movements in RT blocks (see Fig. 1) , and therefore, MEP suppression is likely to start earlier as well. Note that because there was no significant 3-way interaction, this conclusion refers to the overall effect in both FDI and ADM, and not to each muscle separately. Importantly, there was no significant difference between the RT and PT blocks in the amount of MEP suppression in FDI for the TMS_{200} condition ($P = 0.630$). At this time point, FDI MEPs were 41 % (RT task) and 40 % (PT task) of their size compared with MEPs evoked at the start of each trial (BASE).

Table 1. Results of rmANOVAs on movement times (experiment 1) and MEP amplitudes (experiments 1 and 2)

	$F_{[df,error]}$	*P*	*ηp2*
Experiment 1 - Movement Times			
PARADIGM	$3.097_{[1,14]}$	0.100	0.181
TIME	$12.035_{[4,56]}$	<0.001	0.599
PARADIGM x TIME	$8.652_{[4,56]}$	<0.001	0.454
Experiment 1 - MEP Amplitudes			
PARADIGM	$0.560_{[1,14]}$	0.467	0.038
MUSCLE	$20.511_{[1,14]}$	<0.001	0.594
TIME	$6.407_{[4,56]}$	<0.001	0.442
PARADIGM x MUSCLE	$0.016_{[1,14]}$	0.900	0.001
PARADIGM x TIME	$3.455_{[4,56]}$	0.024	0.207
MUSCLE x TIME	$7.624_{[4,56]}$	<0.001	0.440
PARAD. x MUSCLE x TIME	$1.468_{[4,56]}$	0.065	0.144
Experiment 2 - MEP amplitudes			
MUSCLE	$53.193_{[1,17]}$	<0.001	0.758
TIME	$11.952_{[2,34]}$	<0.001	0.413
MUSCLE x TIME	$7.657_{[2,34]}$	0.002	0.311

Experiment 2

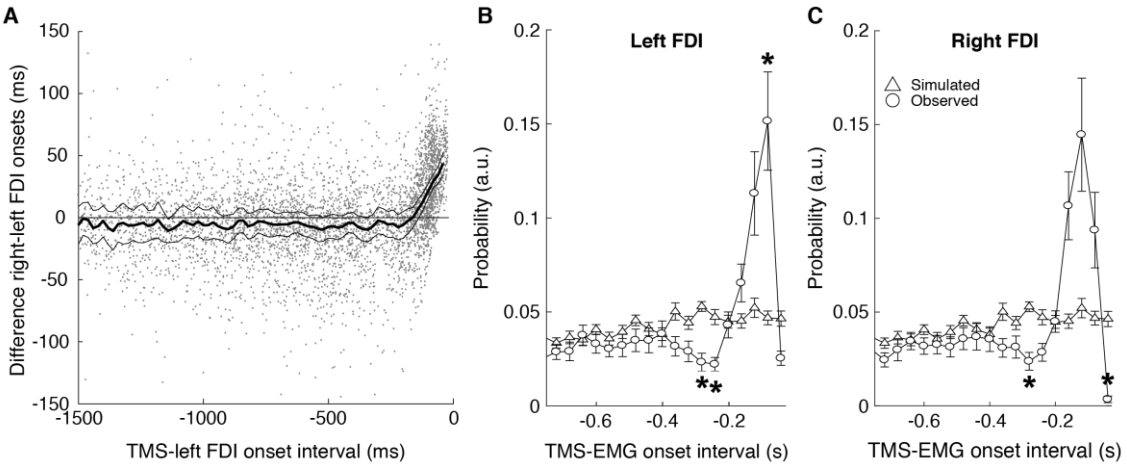
Movement times

On average, participants tended to initiate movements about every 5 s (4.94 ± 0.78 s and 4.99 ± 0.88 s in TMS and non-TMS trials respectively). The individual average inter-movement intervals (considering only non-TMS trials) are presented in Supplementary Table 1. Participants had no difficulties in performing bilaterally synchronous movements: the average (across paricipants) difference in non-TMS trials between the onset of EMG in the right and left FDI was 2 ± 4 ms (individual differences ranged from -17 ms to +14 ms). Fig. 4-A shows how the interval between the onset of right and left FDI EMG varies as a function of the time of the TMS pulse measured relative to the onset of the left EMG. The figure shows that in most trials the hands move synchronously (*i.e.*, a mean difference close to zero). However, when the TMS pulse (to the left hemisphere) occurs closer than 200 ms to EMG onset in the left ("unstimulated") FDI, the onset of EMG in the right FDI is delayed by up to 50 ms. As in experiment 1, this is because the TMS pulse evokes an MEP in the right hand that is followed by a silent period that delays onset of EMG on that side (Ziemann et al. 1997).

Figs. 4-B and 4-C show the probability of a TMS pulse being delivered at different times prior to EMG onset on the left and right sides. The two sets of symbols plot the observed distribution and the distribution calculated by assuming that the time of the TMS pulse is independent of the onset of EMG. Compared to the simulated distribution, the observed distribution of TMS-to-EMG onset probabilities has a trough around 280-260 ms: Wilcoxon tests result in P = 0. 011 at 280 ms and P = 0.015 at 260 ms for the left hand FDI and P = 0.018 at 280 ms for the right hand FDIs. This trough is then followed by a peak at 80 ms before the EMG onset (P = 0.029 for the left hand FDI). In other words, there are fewer trials than expected in which a movement starts around 280-260 ms after a TMS pulse. Conversely there are more trials than expected in which EMG onset occurs

around 80 ms after a TMS pulse. This suggests that, in trials in which TMS was delivered around 280-260 ms before participants were about to move, button presses were performed earlier than they would have been, with the result that EMG onsets are not independent of the time of the TMS pulse. Effectively, a TMS pulse given 280-260 ms before an intended movement advances movement onset so that we rarely observe TMS at this interval. Unfortunately, it is difficult to estimate by how much the onset is advanced. For example, if the EMG onset is advanced by 80 ms (Smith et al. 2019), we might have expected to see an increase in the number of trials at 200 ms interval. However, TMS pulses at 200 ms might also advance movement onset, in which case the additional counts from speeded trials at -280 ms would not be observed. Note that this effect is seen in both left and right FDI muscles, and we suggest in the discussion that it is related to the phenomenon known as intersensory facilitation, which is typically studied using RT movements.

Figure 4. EMG onset times relative to TMS in the self-paced movements task. (A) Intervals between left and right FDI EMG onsets as a function of the interval existing between the TMS pulses and the movement time estimated using the left hand FDI EMG onset in the self-paced movements task in experiment 2. Dots represent individual trials of all participants. The black traces indicate the average and upper and lower limits ($P < 0.01$) of the estimates of the mean differences between the left and right FDI EMG onsets computed in sliding windows of 40 ms. The traces show an effect of the TMS on right FDI onsets when stimuli are delivered less than 200 ms before the estimated EMG onset. (B-C) Real (circles) and simulated (triangles) intervals (mean ± SE) between the TMS pulses and posterior movements in TMS trials. Traces represent the observed distributions of TMS-to-EMG onset intervals for the left (B) and right (C) FDI muscles. Graphs are obtained by combining data from all subjects. *$P < 0.05$, TMS-to-EMG onset intervals where a significant difference across participants exists between the simulated and observed data.



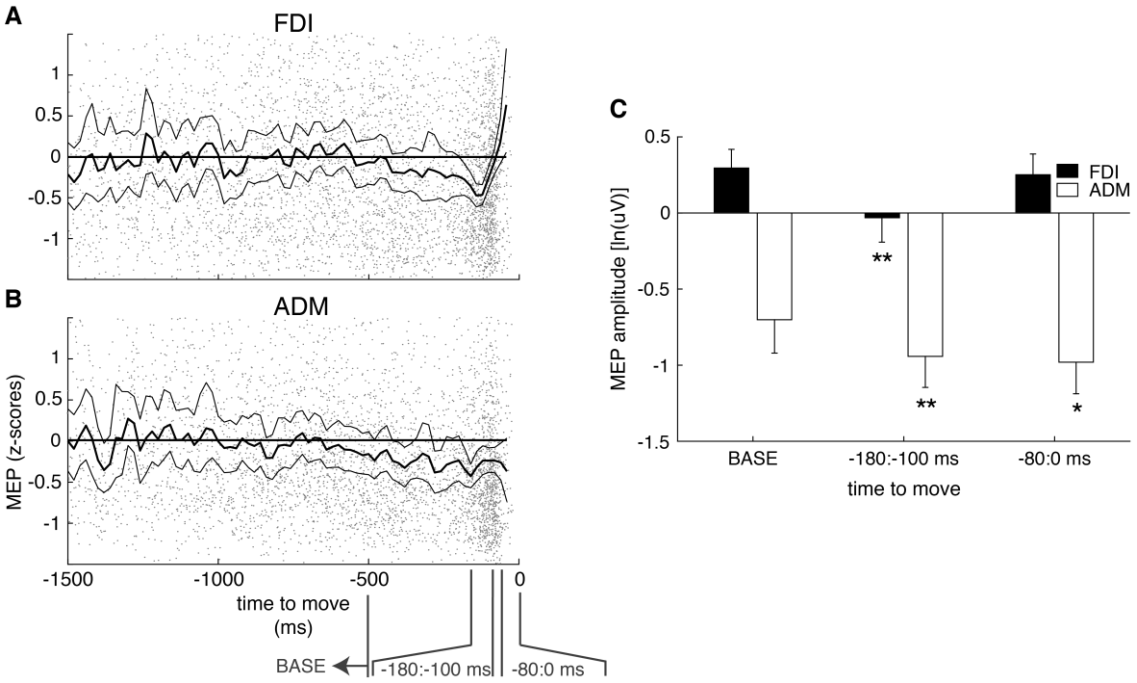
Corticospinal excitability

Resting motor threshold and 1 mV levels were 56 ± 13 % and 66 ± 17 % of the maximum stimulator output. Supplementary Fig. 1 (right) summarizes the average number of FDI and ADM MEPs used to characterize excitability states in the three TMS conditions considered.

Fig. 5 shows the summary of the MEP results obtained both using bootstrap statistics on the grouped (z-scored) data and from the posterior comparison between the intervals of interest. The bootstrap analysis in fig. 5A-B indicates that in the FDI (agonist muscle) there is a period from -180 ms to -100 ms with respect to the EMG onset during which the MEP was significantly smaller than at baseline (both confident intervals below 0). This disappears at around -80 ms, after which it is followed by a final period of facilitation. The reduction is also seen in the (non-involved) ADM over a similar time period. We defined a period of reduced excitability (TMS_{DEC}) from -180 ms to -100 ms. A late premovement phase was defined as the final 80 ms interval before EMG onset to have large enough set of samples of valid MEP amplitudes to average for that condition (TMS_{FAC}) (Pascual-Leone, Valls-Sole, et al. 1992; Chen and Hallett 1999; Chen et al.

1999). The main results of the rmANOVA test comparing BASE, TMS_{DEC} and TMS_{FAC} are summarized in Table 1. In line with the results obtained in experiment 1, there was a significant main effect of TIME ($F_{[2,34]} = 11.952$; $P < 0.001$). The post-hoc comparison between MEPs for BASE and TMS_{DEC} conditions revealed a significant reduction of MEP amplitudes at the latter time point ($P < 0.001$; $d = 4.780$). There was also a significant interaction of MUSCLE x TIME ($F_{[2,34]} = 7.657$; $P = 0.002$). Post-hoc comparisons revealed significant differences between the FDI MEPs recorded in the intervals BASE and TMS_{DEC} ($P = 0.002$; $d = 4.101$). In the ADM, comparisons revealed significant reductions (relative to BASE) of MEPs at TMS_{DEC} ($P = 0.004$; $d = 3.597$) and TMS_{FAC} ($P = 0.012$; $d = 3.134$).

Figure 5. Changes in the FDI (A) and ADM (B) MEPs across time before movements in the self-paced movements task. Dots (in grey) show z-scores of MEP amplitudes (all participants and trials). Solid traces represent the upper and lower confidence limits (thin traces) and the mean of the observations at each point in time (thick trace), both obtained using a bootstrap analysis with all data points ($P < 0.01$). Marks at the bottom identify three intervals of interest used to extract MEPs for a subsequent group level rmANOVA test. These are aimed to contain BASE MEPs, and MEPs during the periods of reduced excitability (TMS_{DEC}) and movement initiation phase (TMS_{FAC}). (C) Normalized group means and SEs of FDI and ADM MEP amplitudes (in logarithmic scale) in the three intervals of interest and TMS conditions where significant changes in MEPs are found. *$P < 0.05$; ** $P < 0.01$.



Discussion

In the present experiments we probed the temporal evolution of CSE in paradigms with different constraints on movement initiation times. The results showed that reduced excitability can be observed prior to a voluntary movement whether it is self-paced, predictive or reactive. It therefore seems unlikely, particularly in the self-paced task, that preparatory corticospinal inhibition is necessary to prevent premature release of movement ("impulse control"). The results are more consistent with hypotheses that view corticospinal inhibition as an essential part of movement preparation. Unexpectedly, our data also revealed that the TMS pulse had distinct biasing effects on movement onset times across the different paradigms. While the initiation of predictive timing movements was unaffected by TMS given 200 ms before the average onset time, RT and self-paced movements were speeded in a way resembling intersensory facilitation commonly reported in RT tasks (Nickerson 1973). We propose that this reveals similarities in the

way planned voluntary movements are triggered in the presence or absence of specific external cues.

A period of reduced corticospinal excitability precedes EMG onset in all three types of movement.

CSE was reduced for a short period about 200 ms prior to EMG onset in RT and PT tasks, and about 140 ms prior to EMG onset in the self-paced task. In the RT task this corresponded to the approximate time of the imperative signal. Overall, the size and spatio-temporal patterns of changes in CSE were very similar to earlier work. On average, MEP amplitudes decreased by 35%, in line with previous data (Duque et al. 2014; Quoilin et al. 2016), and MEP suppression was only maintained in the task-unrelated muscle (the ADM in our case).

The three hypotheses (see Introduction) put forwards to account for preparatory CSE suppression were developed during the study of RT movements. One of these ("competition resolution") is not relevant to the present study since the same movement was performed on each trial, such that there was no need to suppress possible competing responses. As such we confine the discussion to the "impulse control" and "spotlight" hypotheses.

The "impulse control" theory proposes that in RT tasks, reduced CSE is a mechanism that reduces the probability of premature release of the prepared movement (Duque and Ivry 2009; Duque et al. 2010, 2012). However, this does not seem to be a satisfactory explanation for the very similar effect in the self-paced task since there is explicitly no temporal constraint on initiation time, and no chance of premature release. It could be argued that in both the RT and the self-paced task there are changes in

electroencephalographic (EEG) activity that start about the same time prior to movement onset. In the present version of the RT task there is effectively a warning followed, after a 1 s interval, by an imperative signal, which would give rise to the contingent negative variation (CNV) starting at the time of the warning, whereas the self-paced task would be accompanied by the more medially located Bereitschftspotential, again starting ~1 s prior to movement onset (Tecce 1972; Shibasaki and Hallett 2006). However, we argue that the crucial difference between these tasks is that even if they are both prepared (in some way) over the same time period, the RT movement is constrained to start by the imperative signal, and since it is not possible to estimate perfectly the time that the imperative signal will appear, then the theory of "impulse control" suggests that there needs to be a period of suppression to prevent the movement from starting before the imperative signal appears. In contrast, in the self-paced task, although movement may occur roughly at the same time after onset of preparatory EEG activity, there is no absolute constraint on when the movement should begin (in fact this is part of the instruction to the participant). Thus, according to the theory, preparatory suppression should not be needed. It is not a problem if movement begins with variable onset after the start of EEG preparation.

This line of argument might also explain why preparatory inhibition occurred in the PT task. At first sight it might be thought that impulse suppression in this task is unnecessary since, if preparation was started at the correct time, it would automatically evolve to reach threshold and initiate a movement coinciding with the external event. However, by analogy with preparation of the self-paced task, it is possible that the duration of the process cannot be timed perfectly, and thus early suppression of corticospinal activity may be a useful "impulse control". Finally, the idea that MEPs during movement preparation reflect a process of proactive inhibition is also at odds with the observation

that, although we observed similar suppression of CSE in both RT and PT tasks, there were marked differences in the way the TMS pulses interfered with movement onset times (i.e. intersensory facilitation was only seen in RT). Although it is possible that reduced CSE has different functions in different types of movement, it seems more likely that it reflects a process common to preparation of all movement types.

An alternative interpretation for suppression of CSE that could apply equally to all three movement types is the "spotlight" hypothesis that movement selection is facilitated during corticospinal suppression because detection of incoming excitatory input is easier against a quiescent background (Hasbroucq et al. 1997; Greenhouse et al. 2015; Duque et al. 2017). However, although this could account for our present results it does not readily explain the observations of Hannah and colleagues (Hannah et al. 2018) who found that premovement suppression of CSE in RT movements was not caused by direct inhibition of corticospinal output neurones but instead reflected reduced excitability in one of the excitatory input pathways to corticospinal neurones that are preferentially activated by TMS. Instead, our results appear a better fit for the alternative hypothesis that changes in CSE reflect properties of neural populations evolving towards more stable states from which to initiate movement (Churchland et al. 2010; Shenoy et al. 2013; Kaufman et al. 2014, 2016; Hannah et al. 2018). In fact, recent studies in primates show that movements initiated by different types of triggers are all preceded by the same patterns of activity during which discharge rates change without any overt EMG activity (Lara et al. 2018). The time spent in this preparatory state can be compressed or extended depending on task demands (Lebon et al. 2016; Lara et al. 2018). This observation might explain why MEP suppression can be modulated by the duration of the preparatory period (Lebon et al. 2016). It may also be relevant to choice reaction tasks (with different possible movements required) without prior warning cues and with long (>2 s) inter-

movement intervals in which MEPs remain unchanged before the imperative signal but show a suppression right after the onset of the imperative cue (Duque et al. 2014). Finally, it may account for the later timing of suppression in self-paced movements: the lack of constraint on precise movement onset in self-paced movements may allow a faster transition through preparatory states.

Interestingly, CSE in the non-involved ADM muscle also showed inhibition during the preparatory period although this was sustained even during the onset of the focal movement, as expected from prior descriptions of "surround inhibition" between muscle representations in the hand area of M1 (Sohn and Hallett 2004; Beck and Hallett 2011). Such surround inhibition appears to be equally important to isolate activate muscles involved in the motor task in all tested conditions regardless the temporal requirements imposed on the movements to be performed.

Parallels between the effects on CSE and the activity of cortical neurones in primate experiments depend on the assumption that changes in CSE measured with TMS depend to some extent on changes in excitability of motor cortex neurones. However, the EMG response to TMS that was measured in the experiments also depends on excitability of spinal circuits, so that changes here could also contribute to the effects (Duque et al. 2010). Given the limits of interpreting TMS data we limit discussion of mechanism to cortex even though it seems likely that inputs to cortex from basal ganglia and other structures likely contribute to patterns of neural firing in cortex (Mink 1996).

Intersensory facilitation is absent in predictive tasks

In addition to evoking MEPs, the TMS pulse could also affect the timing of the volitional motor response: in both RT and PT movements, pulses timed within about 50 ms of the expected EMG onset delayed movement. Previous workers have ascribed this to the silent period following the MEP, which suppresses EMG onset (Ziemann et al. 1997). Interestingly, lower, sub-motor threshold pulses do not have this effect. Coxon et al (2006) found that low intensity pulses could speed up movement by 10 – 15 ms in a PT, presumably because the initial facilitation of CSE is not followed by the longer period of suppression that occurs after high intensity TMS (Coxon et al. 2006). In addition to this delaying effect, in RT only, pulses that occurred earlier, around the time of the imperative stimulus to move, speeded up EMG onset. The effect is thought to be due to the sensory input produced by the TMS pulse (the "click" of the coil plus stimulation of the skin and muscle of the scalp) and has been interpreted as intersensory facilitation (Nickerson 1973; Terao et al. 1997). The temporal conjunction of the imperative stimulus with the additional sensory input from the TMS pulse is thought to shorten the time for identification of the go-signal and speed up the onset of EMG (Pascual-Leone, Valls-Sole, et al. 1992).

This speeding effect was not present in the PT task, even when the TMS pulse occurred at a similar time with respect to the onset of EMG. The implication is that predictive timing movements are triggered differently to RT movements. In eye movement studies, saccades triggered by predictably timed imperative stimuli are thought to employ internal mechanisms that anticipate the timing of the external stimulus (Janssen and Shadlen 2005; Badler and Heinen 2006). It may be that similar internal timing mechanisms are used in our PT task, and these trigger the release of movement so that it coincides with timing of the "GO" cue. In this case, external signals during the delay period of PT

movements may be either downregulated (Alink et al. 2010) or simply disregarded (Rohenkohl et al. 2012), with the result that intersensory facilitation is absent.

TMS biases movement onset in self-paced movements

Analysing brain responses to external stimuli before self-paced movements with a degree of temporal precision is technically challenging. To address this difficulty we have proposed a methodology based on comparing the observed TMS-EMG onset times with simulations derived from the non-TMS trials during our self-paced experiments. Indeed, addressing the question of how external stimuli can affect the timings of self-paced movements has only been attempted in a few studies in the past. The work most relevant to the present results is that of Castellote and colleagues who showed that StartReact responses (*i.e.*, speeded responses caused by a startling stimulus) prior to self-initiated movements are comparable to StartReact responses in RT paradigms (Valls-Sole et al. 1999; Castellote et al. 2013). Interestingly, Castellote's experiment showed a biasing effect of startling stimuli on movement times that closely resembles that obtained here (Fig. 4), *i.e.*, if delivered approximately 300 ms before the forthcoming movement, startling stimuli speed-up movement onset. The features of the responses matched those obtained in StartReact paradigms using RT tasks, which allowed the authors to suggest that mechanisms engaged in the preparation for self-paced and cue-driven actions shared common elements (Castellote et al. 2013). In our case, the intensity of the applied stimuli (1 mV TMS and participants using ear defenders, which lessen the likelihood of startle response) suggests that the observed effects are closer to intersensory facilitation (Pascual-Leone, Brasil-neto, et al. 1992; Pascual-Leone, Valls-Sole, et al. 1992). Quantifying precisely how much movements are sped up would help verify this idea (Valls-Sole et al. 2008), but doing so is challenging because of the lack of a more precise

knowledge about when movements would be performed in the absence of external stimuli. Based on the fact that the stimuli used in RT and self-paced paradigms were equal and responses to TMS comparable, it is conceivable that effects observed in both cases reflect the use of a similar neural strategy to trigger actions that is not shared by PT movements.

Technical considerations and future work

We made a number of assumptions in designing these experiments that limit our interpretations. The first is to recognise the limitations of our measurement of CSE. The MEP amplitude depends on a combination of the amount of corticospinal activity evoked by TMS as well as the excitability of spinal motoneurons and interneurons. But it is important to note that the level of CSE has no direct relationship to the amount of activity in the corticospinal pathway. As a general example, the resting potential of a non-discharging neurone may lie near or far from firing threshold. The excitability of the neurone would be high in the former state and low in the latter even though the discharge rate of the neurone (zero) is the same. Similarly, a neurone may receive a large number of active synaptic inputs and discharge at a high rate. However, the total membrane resistance of the neurone will be reduced by all this synaptic activity so that it response to any additional synaptic input may be quite small. The implication is that a reduction in CSE does not necessarily mean that corticospinal activity has decreased from some premovement level of anticipatory discharge. It may be unchanged. All we can conclude is that the system is in a different state, and that this state is common to all three types of movement that we studied. Finally we recognise that the "state" refers to the combined neural state of cortical and spinal circuits. Previous studies which have tried to dissociate the contributions of cortical and spinal mechanism have been contradictory (Duque et al. 2010; Lebon et al. 2016; Hannah et al. 2018) and there is little information on spinal

excitability prior to cue-driven and self-paced movements Further experiments are required to address this question in detail.

A second limitation occurs because of our use of bilateral reaction time tasks. As noted in the methods, this was done to try to overcome the difficulties in measuring reaction time in trials in which TMS was applied close to the time of EMG onset. To eliminate the possible effect of the silent period that follows a TMS pulse (Ziemann et al. 1997), participants performed bimanual movements, so that muscle activity in the non-stimulated hand could be used to estimate the EMG onset times, uncontaminated by TMS evoked activity. The approach is likely to be valid for measurement of MEP amplitudes since previous research found comparable MEP suppression in unimanual and bimanual movements (Duque and Ivry 2009). However, whether this is true also for estimates of movement onset times is unclear. The effects of intersensory facilitation may have been biased by the TMS in a different way than the stimulated side. Therefore, precise estimations of the excitability reduction peak time in this case are not definitive.

A third assumption was that we sampled the time course of CSE in a satisfactory way. We delivered TMS at discrete time points locked to visual cues and premovement CSE, and it could be that CSE was changing at faster speeds than the resolution of this temporal sampling. If as suggested above, pre-movement CSE changes are linked to neural population changes in M1, then it seems likely that our sampling rate was reasonable given the relatively slow neural population dynamics (~5 Hz) before planned movements (Kaufman et al. 2016; Lara et al. 2018).

Finally, we did not use catch trials to make the RT and PT tasks as similar as possible (we could not use catch trials in the PT task because otherwise movements would have been initiated in a reactive way after knowing whether a movement was required in each trial). Participants were instructed to use two well differentiated strategies to trigger their

movements. In the PT task, participants had to learn how to synchronize their movement with that of the circles. In the RT task, participants were specifically instructed to react to the "GO" instruction given by the overlap of the four circles at the intersection point of the cross. None of our participants reported having difficulties in avoiding the prediction of the "GO" instruction in the RT task. The posterior analysis of the obtained results corroborates this statement: reaction times are in the range of what the literature reports and, when TMS was applied, RT movements (and not PT movements) showed a clear intersensory facilitation effect, which is a distinctive feature of preparatory states in RT tasks extensively described in the literature (Nickerson 1973; Terao et al. 1997; Hannah et al. 2018).

Conclusions

CSE, as assessed using TMS, is transiently suppressed prior to initiation of RT, PT and self-paced movements. This may indicate that it represents a common state through which neural activity must evolve in the transition from rest to movement. It may be related to mechanisms that maintain constant corticospinal output at a time when preparatory neural activity in the cortex undergoes rapid change. In addition to probing CSE, the TMS pulse also produces a strong sensory input. In RT movements, this results in intersensory facilitation that speeds up onset of movement. A similar effect is seen in self-paced movements, suggesting the existence of common neural mechanisms to trigger movement onset. This effect is not present in PT tasks, implying they are triggered differently, perhaps because priority is given to internal signals that predict the time of movement onset.

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