Excitability changes in the motor cortex in preparation for self-paced and cue-guided movements: a transcranial magnetic stimulation study

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ABSTRACT

Reaction time tasks are characterised by two features: preparatory suppression of corticospinal excitability that precedes facilitation and movement onset; and intersensory facilitation, in which receipt of a second stimulus around the time of the imperative signal shortens reaction times. These are usually explained using a model of subthreshold accumulation of motor commands where preparatory suppression prevents premature release of "subthreshold" commands. Conversely, additional sensory inputs during intersensory facilitation speeds up processing of the imperative stimulus and reduces the time to trigger movement initiation.

Here we question that interpretation by studying movements that are self-paced or timed with predictable cues. In the subthreshold model, premovement suppression of excitability should be absent in self-paced movements. Similarly, they should not show intersensory facilitation for the same reasons. Predictable movements also could have no need for either preparatory inhibition or an onset trigger. If their preparation was started at an appropriate time, they would evolve naturally to threshold at the required time. In contrast with these predictions, the results showed that corticospinal excitability evolves over the same time course regardless of movement type. We conclude that inhibition is not a brake on release, it is an integral part of movement preparation. In addition, intersensory facilitation-like effects occur in self-paced movements, suggesting that they require a trigger event before movement is initiated. Unexpectedly, intersensory facilitation is not seen in predictive movements, suggesting they use different triggering mechanisms, or that they involve active suppression of distracting external events.

SIGNIFICANCE

Intuitive reasoning suggests that planning for a forthcoming movement should involve subthreshold preparation of motor commands. In reaction movements release of these

commands is triggered by an external input whereas in self-paced tasks, movement could start as soon as preparation is complete. Here we provide evidence in humans using TMS of motor cortex that this is incorrect. Preparation for movement appears to involve the motor cortex entering a novel state characterised by a small reduction in overall excitability. This is then triggered into execution by either an external event, or, on the case of self-paced tasks, by an equivalent internal event.

Introduction

Simple reaction time (RT) movements are usually viewed as the stimulus-triggered release of prepared, "subthreshold", movement commands (Tanji and Evarts, 1976; Prut and Fetz, 1999). But what happens if movements are made without reference to an external trigger? For example, self-paced (SP) movements, or movements that are made to coincide with an external event rather than being triggered by it ("predictive" task: PT). If a movement is self-paced or predictive, then are movement commands still stored in advance and released by an internal trigger? Or does movement occur immediately preparation is complete? Perhaps no preparation is needed at all.

The aim of the present experiments was to compare movement preparation in RT, PT and SP movements. In particular, we tested whether 2 key characteristics of RT movements differed in the three tasks.

The first characteristic was preparatory inhibition. In RT tasks, corticospinal excitability is reduced around the time of the imperative stimulus (Hasbroucq et al., 1997). This has been interpreted as preventing the premature release of the prepared "subthreshold" motor plan (Duque et al., 2017). It is compatible with animal experiments showing that there are changes in the firing of neurones in motor cortex at a time when there is no overt EMG activity in the periphery (Omrani et al., 2017). If preparatory inhibition is necessary

to prevent premature release of movement commands (Duque et al., 2010), then it may be reduced in PT movements where the time to move is known, and it should be absent in SP movements when there is no constraint on the time of execution.

The second characteristic of RT movements investigated here was inter-sensory facilitation, in which the presence of a second stimulus at the time of the imperative stimulus reduces reaction times and speeds release of the prepared movement (Nickerson, 1973). It has been postulated that the additional input shortens the time taken to identify the imperative stimulus (Pascual-Leone et al., 1992b). SP and PT movements may not require an external trigger since they could start immediately preparation was complete. If so they would not display inter-sensory facilitation.

Recent work on the relationship between movement preparation and execution leads to rather different predictions. Recordings of neural population activity in cortical motor areas of monkeys show that activity of neurons in the preparatory period may differ from that in the same neurons during execution (Churchland et al., 2010; Kaufman et al., 2014). Preparatory activity is not a "subthreshold" version of that seen during execution and therefore, preparatory suppression would seem to be unnecessary (Kaufman et al., 2014). Indeed, in human experiments, the degree of preparatory suppression was positively correlated to the RT, suggesting that, if anything, preparatory suppression was causally involved in preparation to move rather than in preventing its premature release (Hannah et al., 2018). Thus, preparatory suppression might be seen in SP and PT movements as well as in RT tasks.

Recordings of neural activity also show that all types of movement are preceded by the same patterns of activity consisting of a preparation period during which discharge rates change without any overt EMG activity (Lara et al., 2018). This is followed by a transition to an execution phase that appears to be caused by a separate timing signal (Haith et al.,

2016; Kaufman et al., 2016). If this timing signal corresponds to an internal trigger for movement, then it may be susceptible to inter-sensory facilitation in SP and PT movements.

Our results show that SP and PT movements display preparatory suppression of corticospinal excitability consistent with the idea that it is a necessary part of movement preparation rather than preventing premature release. SP movements also display inter-sensory facilitation-like effects, suggesting that execution depends on some form of trigger. In contrast, PT movements do not display inter-sensory facilitation, which could indicate that there is active filtering of irrelevant sensory events that might distract from the internal trigger used in predictive timing.

Materials and Methods

Participants

In total, 28 right-handed, healthy participants (9 females, 28 ± 5 years old, age range 19 - 36 years) participated in this study. 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 index fingers resting on a keyboard which registered movement times. A screen was placed approximately one meter in front of them. Participants wore ear defenders during the TMS experiments to reduce the influence of loud sounds generated by the discharges of the TMS coils.

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 in the two experiments. MEPs recorded from the right FDI were the main outcome of this study. The ADM was used as a control muscle for the MEPs: it was activated by the TMS stimuli but, unlike the right FDI, it was not directly involved in the movement, so it was only supposed to show a monotonic reduction of excitability up until the time at which muscles showed voluntary activation (Duque et al., 2010). Surface recording electrodes were placed on the muscle bellies, with references 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 the Signal and Spike^2 software (also by CED).

Once EMG sensors 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. The TMS coil was always held at a 45° angle to the sagittal plane with the handle pointing backwards. Once the hotspot was found, the resting motor threshold (RMT) and 1 mV intensity were determined. The RMT was estimated by adjusting the TMS output until 5 out of 10 MEPs could be obtained over 50 μV. The 1 mV intensity was defined as that able to elicit MEPs of around 1 mV amplitude. 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[1]'s utilities to control the parallel port of the PC 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 RT and PT movements

In this experiment, participants performed two types of movement paradigms: 1) a PT task in which movements were timed with an external countdown signal (Fig. 1-A); and 2) a RT task in which movements were initiated following and imperative stimulus (Fig. 1-B). During task execution, TMS pulses were delivered at predetermined time points relative to the average task- and subject-specific movement times to probe motor cortical excitability. The PT task consisted of a resting phase of 2 s followed by a delay period of 1 s during which four circles moved from the extremes of a cross towards its centre with a velocity inversely proportional to the remaining distance to the centre of the cross (initial distance 4.5 cm). Participants were instructed to time their movements with the merging of the circles so that button presses with the FDI were performed as soon as the circles fully overlapped. After each movement, the trial ended by giving participants feedback about the button press times. This 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, expressed in ms, and a font colour code indicative of the performance: 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; yellow text was used for button presses in the intervals 0-50 ms and 100-150 ms; finally, red text was used in any other case. Additionally, participants were given "too early" and "too late" messages when button presses were performed during the delay

[1] By Cogent 2000 team at the FIL and the ICN and Cogent Graphics developed by John Romaya at the LON at the Wellcome Department of Imaging Neuroscience

period or more than 200 ms after its end. The RT task had the same states with matched durations as the PT task but, in this case, the movement of the circles during the delay period followed a random and uninformative path along the four arms of the cross (Fig. 1). In this case participants were instructed to wait until the circles suddenly appeared in the centre of the cross, which was considered the "GO" cue. Participants were specifically asked to avoid any predictions of when the imperative cues were appearing. For that, they were told to specifically use the sudden overlapping of the circles in the centre of the screen to make a fast and reactive movement.

At the start of each task, participants practised the two paradigms until they showed consistent movement times. Thirty additional movements were then performed with each paradigm so that the subject- and task-specific average movement onset times (based on the FDI EMG activity) could be estimated. Then, the actual recording took place, consisting in two blocks per paradigm (interleaving the blocks of the two paradigms). In each block, six conditions were tested for 10 trials each, using a randomized order of conditions. These conditions differed from each other with regards to the timing of the TMS pulse: 1) no TMS delivered (control condition); 2) TMS at the beginning of the delay period (baseline condition); 3) TMS halfway through the delay period; 4) TMS 200 ms before the average movement onset time; 5) TMS 60 ms before the average movement onset time; and 6) TMS 30 ms before the average movement onset time.

Experiment 2 – TMS during the resting phases between SP movements

The task involved participants sitting still and comfortably, with both index fingers resting on buttons on a keyboard. They were instructed to make ballistic bilateral button presses every 4-8 seconds, whilst avoiding pre-movement muscle activation and ensuring movements were made in a similar way along the whole experiment (Fig. 1-C). Movements of the left index finger (non-targeted by the TMS) allowed accurate estimates

of the onset of voluntary movements (Schneider, 2004) without being affected by the TMS induced delays of voluntary actions in cases where the stimulus was given in close proximity with the intended movement onset time (Ziemann et al., 1997). 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 pulses 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 in a relaxed condition between movements.

A custom-made Matlab program was used to determine the timing of a TMS stimulus on a given trial based on the button press times registered 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 pulse timing were 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 82% 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 movement such that it was always below the average inter-movement interval estimated. It was important to ensure that time intervals left by participants between button presses were not too long, as this would result in reduced chances of delivering TMS pulses at points in time close to the button presses. In the

cases when participants waited for over 10 s between movements for 3-4 trials in a row, participants were given an indication by the experimenter to reduce the inter-movement time intervals.

Analysis of results and statistics

All the analyses were done using the onsets of the EMG as the reference points indicating the times of movement initiations. In order to obtain EMG references in each trial, the absolute value of the EMG recordings was estimated and then a moving average of 5 ms and a low pass filter (fc < 5Hz) were applied to obtain a smoothed envelope of the EMG signal. EMG recordings of all trials whilst the participant was at rest were analysed to obtain a subject-specific baseline level. A threshold set at five times the baseline value was used to determine EMG onset times. This level was 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 pulses. Once trials were corrected, 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 of MEP amplitudes was performed before the statistical tests to ensure normality in the distributions of amplitudes.

In Experiment 1, MEP amplitudes and times of EMG onsets of all trials were labelled according to the type of paradigm (PT v RT) and to the time at which TMS was delivered. Movement onset times were referenced to the ones obtained with each participant in the control trials without TMS (subtracting the average movement onset times). A two-way repeated measures ANOVA (rmANOVA) with factors TIME and PARADIGM was performed to test for changes in movement onset times. A three-way rmANOVA (TIME

x PARADIGM x MUSCLE) was performed to test for changes in MEPs. Post-hoc comparisons were done between the baseline and 200 ms before movement TMS conditions.

In Experiment 2, two types of trials were extracted per session: TMS trials in which a TMS stimulus was delivered before the movement, and non-TMS trials in which no TMS pulse was scheduled so they could be used as control trials to define the correct times of subsequent TMS pulses. To check if TMS pulses were inadvertently used by the participants as cues to start the movements in TMS trials, we compared inter-movement intervals in TMS and non-TMS trials using a paired t-test. Additionally, to analyse the effect of TMS pulses on the times at which participants decided to move, non-TMS trials were used to simulate the length of the TMS-movement intervals had participants not been biased by TMS stimuli. To do this, all non-TMS trials obtained from each session were used by a simulation algorithm (5000 iterations) that: *i*) randomly selected 5 trials in each iteration; *ii*) obtained a simulated TMS time for the "next" trial in the same way as in the actual experiment; *iii*) randomly selected a new trial of the same participant; *iv*) obtained the time interval between the movement onset time and the simulated TMS time and kept it if it was positive (*i.e.*, if the TMS was delivered before the movement).

Regarding the TMS recordings in experiment 2, since the times of the MEPs relative to the posterior movements could not be well controlled (due to the free nature of SP movements), standard bootstrap statistics were applied to all participants' MEPs recordings in order to identify, in an unbiased way, intervals of interest, *i.e.*, intervals that contained significant increases or decreases in MEP amplitudes. To do this, the following steps were repeated for 100 iterations: 1) 200 TMS-trials per participant were chosen at random. Since the number of TMS trials obtained with each participant was different, this step balanced the influence of each participant's data when combining data from all

participants. 2) MEPs selected from each participant were referenced to MEPs in the interval [-1500 : -500] ms relative to the onset of movements, *i.e.* MEPs were subtracted the mean and divided by the standard deviation of the MEPs in the defined interval. 3) MEP 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 (standard bootstrapping procedure) and used to calculate a mean. This was repeated 1000 times, thus generating 1000 means for every window. These values were arranged in ascending order and the 5th and 995th values were taken as confidence intervals. This whole process was repeated 100 times (using different sets of 200 trials per participant each time), and an average of all estimated confidence intervals was taken to produce the definitive confidence intervals of MEP changes across the time in preparation for the movements. Subsequently, a two-way rmANOVA was performed in SPSS with factors TIME and MUSCLE to test for changes in MEP amplitudes. Here, the TIME factor included the baseline period [-1500:-500] ms, and the periods of time in which significant decreases or increases of the right FDI MEPs were observed (*i.e.*, time intervals when both confident interval lines obtained from the previous bootstrap analysis were either positive or negative).

Overall, all results are reported as group mean ± standard error of the mean (SEM). Bonferroni *post-hoc* corrections are applied in the cases of multiple comparisons. P values < 0.05 are considered to be significant. The Greenhouse–Geisser procedure was applied where necessary to correct for violations of sphericity in rmANOVAs.

Results

Experiment 1

The average movement onset times (based on EMG) obtained in the baseline trials without TMS were -35 ± 6 ms and 200 ± 9 ms for the PT and RT paradigms, respectively. The average time intervals between the estimated EMG onset times and the button press events were 93 ± 3 ms (PT) and 91 ± 3 ms (RT). Fig. 2 shows the observed average movement times in the two paradigms for different TMS firing times. There appear to be two main effects. First, if the TMS pulses are given 30 or 60 ms prior to average movement times, then reactions are delayed. Previous work has ascribed this to the effect to the silent period following the TMS-evoked MEP (Ziemann et al., 1997). The second effect is that reaction times are reduced in the RT task when TMS pulses are given 200ms prior to average movement times at around the time of the imperative stimulus. Previous work refers to this as a form of intersensory facilitation caused by the auditory click and scalp stimulation from the TMS pulse (Terao et al., 1997).

These conclusions are borne out in the statistical analysis. rmANOVA showed a significant main effect of TIME ($F_{[4,6]} = 18.154$; $P < 0.001$), reflecting delayed responses when TMS pulses were delivered in proximity to the average movement times (*i.e.*, higher movement times were found when TMS pulses where delivered 60 ms and 30 ms before the average movement times). In addition, there was a significant interaction of PARADIGM x TIME ($F_{[4,6]} = 11.943$; $P = 0.003$). Post-hoc comparison between paradigms for trials in which TMS pulses were delivered 200 ms before the average movement time revealed a significant reduction of the movement times in the RT movements relative to the PT (mean difference of -29.8 ± 8.8 ms; $P = 0.008$). No significant difference was found between paradigms for baseline TMS trials (mean difference -3.8 ± 5.0 ms; $P = 0.473$).

Resting motor threshold and 1mV levels were 53 ± 3 % and 63 ± 3 % of the maximum stimulator output, respectively. Fig. 3 shows average MEP results. rmANOVA showed a

main effect of factors MUSCLE ($F_{[1,9]}$ = 8.327; P = 0.018; difference between FDI and ADM: 0.7 ± 0.2 log(μV)) and TIME ($F_{[4,6]}$ = 5.731; P = 0.010). The post-hoc comparison between MEPs obtained from TMS pulses delivered either at the baseline point or 200 ms before the average movement times revealed a significant reduction of MEPs at the latter point (mean difference: -0.5 ± 0.1 log(μV); P = 0.009). There was a significant interaction of MUSCLE x TIME ($F_{[4,6]}$ = 11.943; P < 0.001), due to the diverging directions of MEP changes in the FDI and ADM at the time of movement initiation (Duque et al., 2010). Post-hoc comparisons between baseline trials and trials with TMS delivered 200 ms before the average movement time revealed significant reductions of MEPs both in the FDI (mean difference -0.5 ± 0.2 log(μV); $P = 0.018$) and in the task-irrelevant ADM (mean difference -0.4 ± 0.1 log(μV); $P = 0.040$). No significant differences were found in the reductions (TMS 200 ms before movements) of the FDI MEPs between the RT and PT paradigms (P = 0.590). MEPs obtained with TMS pulses delivered 200 ms before average movement times relative to baseline MEPs showed an average reduction of 31 % and 35 % for PT and RT tasks, respectively.

Experiment 2

The average inter-movement intervals in TMS and non-TMS trials was 4.94 ± 0.16 s and 4.99 ± 0.18 s. A paired t-test on the inter-movement intervals of TMS and non-TMS trials revealed no significant difference between both conditions (P = 0.415). Fig. 4 shows a comparison between real and simulated distributions (across all participants) of time intervals between TMS firing times and consecutive movement onset times. The experimental design was such that distribution of TMS times relative to the time of movement should reflect the left-hand side of a normal distribution (before movement), with the peak occurring close to the onset of movement. Instead, the obtained distributions show a trough around 300 ms followed by a peak at ~100 ms before the

onset of the muscle activation. This suggests that, in trials in which TMS is delivered less than 300 ms before participants were about to move, movements were speeded, thus modifying the normal distribution expected to result from the design of the paradigm. The paired sample t-test returned significant differences between the simulated and the real distributions 300 ms before the onsets of the movements and in the interval [-150 ms : -50 ms] also relative to the movement ($p < 0.001$ in both intervals). We refer to this speeding up as a form of intersensory facilitation.

Resting motor threshold and 1 mV levels were 56 ± 3 % and 66 ± 4 % of the maximum stimulator output, respectively. 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 returned a significant ($P < 0.01$) interval of MEP reduction that applied both to the FDI and the ADM muscles. For the FDI, this period with reduced MEPs peaked at -120 ms (relative to the estimated muscle activation onsets) and it was followed by an (also significant) increase of excitability towards the time of initiation of the movement. According to these results, the period of reduced excitability was defined to be at the time interval [-160 ms : -100 ms] relative to the movement (*i.e.*, the two consecutive 40 ms windows showing the strongest MEP reduction effects in the bootstrap analysis). Due to the lack of a dense enough population of MEPs at the late phase before movements were initiated, we used the MEPs located within the final 80 ms interval before starting the movements as representative of the movement initiation phase (Pascual-Leone et al., 1992b; Chen and Hallett, 1999; Chen et al., 1999). rmANOVA showed main effects of MUSCLE ($F_{[1,17]} = 26.246$; $P < 0.001$; mean difference between FDI and ADM: 1.0 ± 0.2 log(μV)) and TIME ($F_{[2,16]} = 9.046$; $P = 0.01$). The post-hoc comparison of MEPs within the baseline and excitability reduction intervals revealed a significant reduction of MEPs at the latter time

point (mean difference: 0.3 ± 0.1 log(μV); P = 0.006). There was also a significant interaction of MUSCLE x TIME ($F_{[2,16]}$ = 13.041; P < 0.001). Post-hoc comparisons between MEPs for baseline and reduced excitability conditions and for each muscle separately revealed a significant effect in both cases (P = 0.019 for FDI; P = 0.020 for ADM).

Discussion

In the present experiments, we probed the temporal evolution of M1 excitability 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. In addition, we found that TMS had distinct biasing effects on movement times across the different paradigms. While the initiation of a predictive movement was unaffected by TMS, RT and SP movements were speeded in a way resembling intersensory facilitation effects commonly reported in RT tasks (Nickerson, 1973). Overall, the results support the notion that preparatory cortical excitability changes in M1 do not have a direct functional role in preventing premature movement releases and they raise the question about the possible similarities of the mechanisms involved in triggering planned voluntary movements in the presence or absence of specific external cues.

Different movement preparation paradigms exhibit similar patterns of preparatory M1 excitability

A comparison of the results obtained in the three paradigms tested here suggests a common temporal evolution of corticospinal excitability before movements, regardless of the nature of the trigger that initiates them. It has previously been hypothesized that M1 excitability reduction before movements reflects proactive control of motor cortical

outputs preventing premature responses (Bestmann and Duque, 2015; Duque et al., 2017). However, this does not explain why we see the same premovement suppression prior to SP movements, in which there is explicitly no temporal constraint on initiation time. Strictly speaking, suppression is also unnecessary in the PT movements since if preparation was initiated at the correct time it would automatically evolve to reach threshold and initiate a movement to coincide with the external event. Only if suppression was used to reduce the variability of onset times would it serve a function.

Instead, our results appear a better fit for the alternative hypothesis that smaller responses reflect the dynamics 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). Recent studies in primates show that the time spent in this preparatory state can be compressed or extended depending on task demands (Lara et al., 2018). This observation might explain why we observed a peak in MEP suppression in SP movements which, relative to RT and PT movements, was shifted towards time points closer to the EMG onsets. It may also be relevant to the longer RTs in paradigms with unpredictable imperative stimuli (Alink et al., 2010). These could be explained by the need to make a transition through reduced excitability states after the imperative stimulus. Indeed, previous work has shown that, in reaction time tasks without prior warning cues, MEPs remain unchanged before the imperative signal but show a marked suppression right after the onset of the imperative cue (Duque et al., 2014). Altogether, our results support the notion that MEP changes in preparation for movements can largely be explained as being a result of action- and state-specific M1 evolutions towards movement rather than being a reflection of proactive control mechanisms over M1.

The size and spatio-temporal patterns of the observed excitability reduction in preparation for movements, which was comparable across conditions, matched the results of previous

TMS studies based on RT paradigms. On average, MEP amplitudes decreased by 30%, in line with changes reported before (Duque et al., 2014; Quoilin et al., 2016). The FDI muscle (task-relevant) presented deeper decreases of MEP amplitudes than the control ADM muscle across conditions, in agreement with previous research suggesting a somatotopic gradient in excitability changes related to the location, certainty and specificity of forthcoming actions (Greenhouse et al., 2015).

Intersensory facilitation depends on the predictability of the imperative stimulus

Different TMS biasing effects found in PT and RT movement times indicate that these paradigms differ with respect to how motor commands are withheld and/or released. Movement initiation in RT movements is contingent upon external cues and the link between sensory processing and release of motor programs is presumed to be direct (Pascual-Leone et al., 1992b). Speeded responses in RT movements induced by TMS have been interpreted as intersensory facilitation (Nickerson, 1973; Terao et al., 1997), and have been suggested to be due to a shortening of the time for identification of external stimuli as the go-signal (Pascual-Leone et al., 1992b). In eye movement paradigms with predictable onsets of imperative cues, previous studies have proposed that inner mechanisms aimed at anticipating the timing of the environmental cues need to be deployed and used to trigger motor commands (Janssen and Shadlen, 2005; Badler and Heinen, 2006). Based on this, in the PT task tested here the lack of speeded responses can be attributed to the usage of an alternative mechanism to trigger movements, which is internally driven, once the timing of external stimuli is learned. 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).

Biasing effects of TMS in SP movements

Analysing brain responses to external stimuli before SP movements with a degree of temporal precision is technically challenging and only few studies have attempted it. Wasaka and colleagues used a SP paradigm to show that sensory suppression processes in these actions partially resembled those seen in preparation for RT movements (Shimazu et al., 1999; Wasaka et al., 2003). Castellote and colleagues showed that StartReact responses during the resting periods before self-initiated movements are closely similar to StartReac 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 tightly resembles that obtained here (Fig. 3), *i.e.*, if delivered with a certain anticipation (~300 ms) before the forthcoming movements, the startling stimuli speed-up the release of the required movements. In that work, the features of the responses matched those obtained in StartReact paradigms using RT tasks, which allowed authors to suggest that the mechanisms engaged in the preparation for SP and cue-driven actions could share 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 et al., 1992b, 1992a). Precisely quantifying 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 stimuli used in the RT and SP 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

Studying corticospinal changes before SP movements with TMS has inherent limitations. We were able to obtain movement times and MEPs in our SP paradigm despite the apparent difficulties in accurately probing excitability at specific, well-defined times relative to movement onset. However, unlike with cue-guided movements, the estimations of TMS times (relative to the movements) in the SP paradigm were done based on the times of the subsequent movements of the non-stimulated hand, which also presents intersensory facilitation effects (Hannah et al., 2018), is not affected by cortical silent period-related delaying effects (Ziemann et al., 1997), but may still have been biased by the TMS pulse in a different way than the stimulated side. Therefore, precise estimations of the excitability reduction peak time in this case are not definitive.

Previous studies testing the possible contribution of spinal mechanisms to the decrease seen in MEP amplitudes in preparation for movements in reaction time tasks have led to contradictory results (Duque et al., 2010; Lebon et al., 2016; Hannah et al., 2018). The techniques used here to probe corticospinal excitability do not allow measuring whether spinal inhibitory processes are taking place in preparation for movements performed in the three paradigms tested. Knowing if and how spinal inhibition applies to cue-driven and SP movements could help further proving the hypothesis that observed preparatory changes are not driven by proactive control mechanisms. Such analysis should be addressed in future work.

Conclusions

Taken together, results from the three compared paradigms suggest that TMS recordings of excitability changes in preparation for planned movements share a common temporal profile, likely reflecting aspects of neural population-level changes to generate the desired actions. Results also suggest possible shared neural mechanisms involved in triggering

reaction time and self-paced movements that are different from those in actions timed with fully predictable cues.

References

Alink A, Schwiedrzik CM, Kohler A, Singer W, Muckli L (2010) Stimulus Predictability Reduces Responses in Primary Visual Cortex. J Neurosci 30:2960–2966.

Badler JB, Heinen SJ (2006) Anticipatory Movement Timing Using Prediction and External Cues. J Neurosci 26:4519–4525.

Bestmann S, Duque J (2015) Transcranial Magnetic Stimulation: Decomposing the Processes Underlying Action Preparation. Neurosci:1–14.

Castellote JM, Van Den Berg MEL, Valls-Sole J (2013) The startreact effect on self-initiated movements. Biomed Res Int 2013.

Chen R, Corwell B, Hallett M (1999) Modulation of motor cortex excitability by median nerve and digit stimulation. Exp Brain Res 129:77–86.

Chen R, Hallett M (1999) The time course of changes in motor cortex excitability associated with voluntary movement. Can J Neurol Sci 26:163–169.

Churchland MM, Cunningham JP, Kaufman MT, Ryu SI, Shenoy K V. (2010) Cortical Preparatory Activity: Representation of Movement or First Cog in a Dynamical Machine? Neuron 68:387–400.

Duque J, Greenhouse I, Labruna L, Ivry RB (2017) Physiological Markers of Motor Inhibition during Human Behavior. Trends Neurosci 40:219–236.

Duque J, Labruna L, Cazares C, Ivry RB (2014) Dissociating the influence of response selection and task anticipation on corticospinal suppression during response preparation. Neuropsychologia 65:287–296.

Duque J, Lew D, Mazzocchio R, Olivier E, Ivry RB, Louvain D, Brussels B-, Clinica N (2010) Evidence for Two Concurrent Inhibitory Mechanisms during Response Preparation. J Neurosci 30:3793–3802.

Greenhouse I, Sias A, Labruna L, Ivry RB (2015) Nonspecific Inhibition of the Motor System during Response Preparation. J Neurosci 35:10675–10684.

Haith AM, Pakpoor J, Krakauer JW (2016) Independence of Movement Preparation and Movement Initiation. J Neurosci 36:3007–3015.

Hannah R, Cavanagh XSE, Tremblay S, Simeoni S, Rothwell XJC (2018) Selective Suppression of Local Interneuron Circuits in Human Motor Cortex Contributes to Movement Preparation. 38:1264–1276.

Hasbroucq T, Kaneko H, Akamatsu M, Possamaı¨ C-A (1997) Preparatory inhibition of cortico-spinal excitability: a transcranial magnetic stimulation study in man. Cogn Brain Res 5:185–192.

Janssen P, Shadlen MN (2005) A representation of the hazard rate of elapsed time in macaque area LIP. Nat Neurosci 8:234–241.

Kaufman MT, Churchland MM, Ryu SI, Shenoy K V. (2014) Cortical activity in the null space: Permitting preparation without movement. Nat Neurosci 17:440–448.

Kaufman MT, Seely JS, Sussillo D, Ryu SI, Shenoy K V., Churchland MM (2016) The Largest Response Component in the Motor Cortex Reflects Movement Timing but Not Movement Type. eNeuro 3.

Lara AH, Elsayed GF, Zimnik AJ, Cunningham J, Churchland MM (2018) Conservation of preparatory neural events regardless of how movement is initiated. Elife:7:e31826.

Lebon F, Greenhouse I, Labruna L, Vanderschelden B, Papaxanthis C, Ivry RB (2016) Influence of Delay Period Duration on Inhibitory Processes for Response Preparation. Cereb Cortex 26:2461–2470.

Nickerson RS (1973) Intersensory facilitation of reaction time: Energy summation or preparation enhancement? Psychol Rev 80:489–509.

Omrani M, Kaufman MT, Hatsopoulos NG, Cheney PD (2017) Perspectives on classical controversies

about the motor cortex. J Neurophysiol:jn.00795.2016.

Pascual-Leone A, Brasil-neto J, Valls-Sole J, Cohen LG, Hallett M (1992a) Simple reaction time to focal transcranial magnetic stimulation. Brain 115:109–122.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Brasil-neto J, Cohen LG, Hallett M (1992b) Effects of Focal Transcranial Magnetic Stimulation on Simple Reaction Time To Acoustic , Visual and Somatosensory stimuli. Brain 115:1045–1059.

Prut Y, Fetz EE (1999) Primate spinal interneurons show pre-movement. Nature 401:590–594.

Quoilin C, Lambert J, Jacob B, Klein PA, Duque J (2016) Comparison of motor inhibition in variants of the instructed-delay choice reaction time task. PLoS One 11:1–16.

Rohenkohl G, Cravo AM, Wyart V, Nobre AC (2012) Temporal Expectation Improves the Quality of Sensory Information. J Neurosci 32:8424–8428.

Rossi S, Hallett M, Rossini P, Pascual-Leone A (2011) Screening questionnaire before TMS: An update. Clin Neurophysiol 122:1686.

Schneider C (2004) Timing of cortical excitability changes during the reaction time of movements superimposed on tonic motor activity. J Appl Physiol 97:2220–2227.

Shenoy K V., Sahani M, Churchland MM (2013) Cortical Control of Arm Movements: A Dynamical Systems Perspective. Annu Rev Neurosci 36:337–359.

Shimazu H, Kaji R, Murase N, Kohara N, Ikeda A, Shibasaki H, Kimura J, Rothwell JC (1999) Pre-movement gating of short-latency somatosensory evoked potentials. Neuroreport 10:2457–2460.

Tanji J, Evarts E V (1976) Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. J Neurophysiol 39:1062–1068.

Terao Y, Ugawa Y, Suzuki M, Sakai K, Hanajima R, Gemba-Shimizu K, Kanazawa I (1997) Shortening of simple reaction time by peripheral electrical and submotor-threshold magnetic cortical stimulation. Exp Brain Res 115:541–545.

Valls-Sole J, Kumru H, Kofler M (2008) Interaction between startle and voluntary reactions in humans. Exp Brain Res 187:497–507.

Valls-Sole J, Rothwell JC, Goulart F, Cossu G, Muñoz E (1999) Patterned ballistic movements triggered by a startle in healthy humans. J Physiol 516:931–938.

Wasaka T, Hoshiyama M, Nakata H, Nishihira Y, Kakigi R (2003) Gating of somatosensory evoked magnetic fields during the preparatory period of self-initiated finger movement. Neuroimage 20:1830–1838.

Ziemann U, Tergau F, Netz J, Hömberg V (1997) Delay in simple reaction time after focal transcranial magnetic stimulation of the human brain occurs at the final motor output stage. Brain Res 744:32–40.

Figure 1. Movement initiation tasks and TMS recordings. (A) In PT movements -experiment 1- participants had to press a button with their index finger after the end of a 1-s countdown (the time it took the 4 white circles to reach the centre of the cross). (B) In RT movements -experiment 1- participants had to perform the same movement as in the PT task, but here they were specifically instructed to perform movements in response to seeing the 4 white circles suddenly appearing on the centre of the cross. During the 1-s delay period in this case, circles moved randomly along the arms of the cross. Both in PT and RT movements, single-pulse TMS was delivered when the four circles appeared on the screen at the beginning of the delay period (BASELINE), half way through the delay period and 200/60/30 ms before the average movement times in each subject and for each task. (C) In experiment 2, participants performed SP movements consisting in simultaneously pressing two keyboard 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.

Figure 2. Means and SEs of the movement times (relative to non-TMS trials) for the RT and PT tested in Experiment 1. (** indicates $P < 0.01$)

Figure 3. Normalized means and SEs of the FDI (left panel) and ADM (right panel) MEP amplitudes in RT and PT movements tested in Experiment 1. The found significant difference in MEPs between baseline and -200 ms TMS time conditions is indicated as well. (** indicates $P < 0.01$)

Figure 4. Upper and lower confidence limits (black lines) of the observed intervals between the TMS trigger times and the subsequent movement onset times in the SP paradigm. The simulated frequencies based on the non-TMS trials are represented at the back. The horizontal bars at the bottom of the figure represent time points in which a significant ($P < 0.01$) difference between the two distributions is observed.

Figure 5. Changes in the FDI (A) and ADM (C) MEPs across time before movements in the SP paradigm. Dots show normalized MEP amplitudes (all subjects and trials). Solid traces represent the upper and lower confident limits obtained using a bootstrap analysis with all data points ($p < 0.01$). Bars at the bottom identify three intervals of interest used to extract MEPs for a subsequent group level rmANOVA. These are aimed to contain MEPs observed at baseline, and during the periods of reduced excitability and movement initiation phase. Normalized group means and SEs of FDI (B) and ADM (D) MEP amplitudes in the three selected intervals of interest.

Figure 1

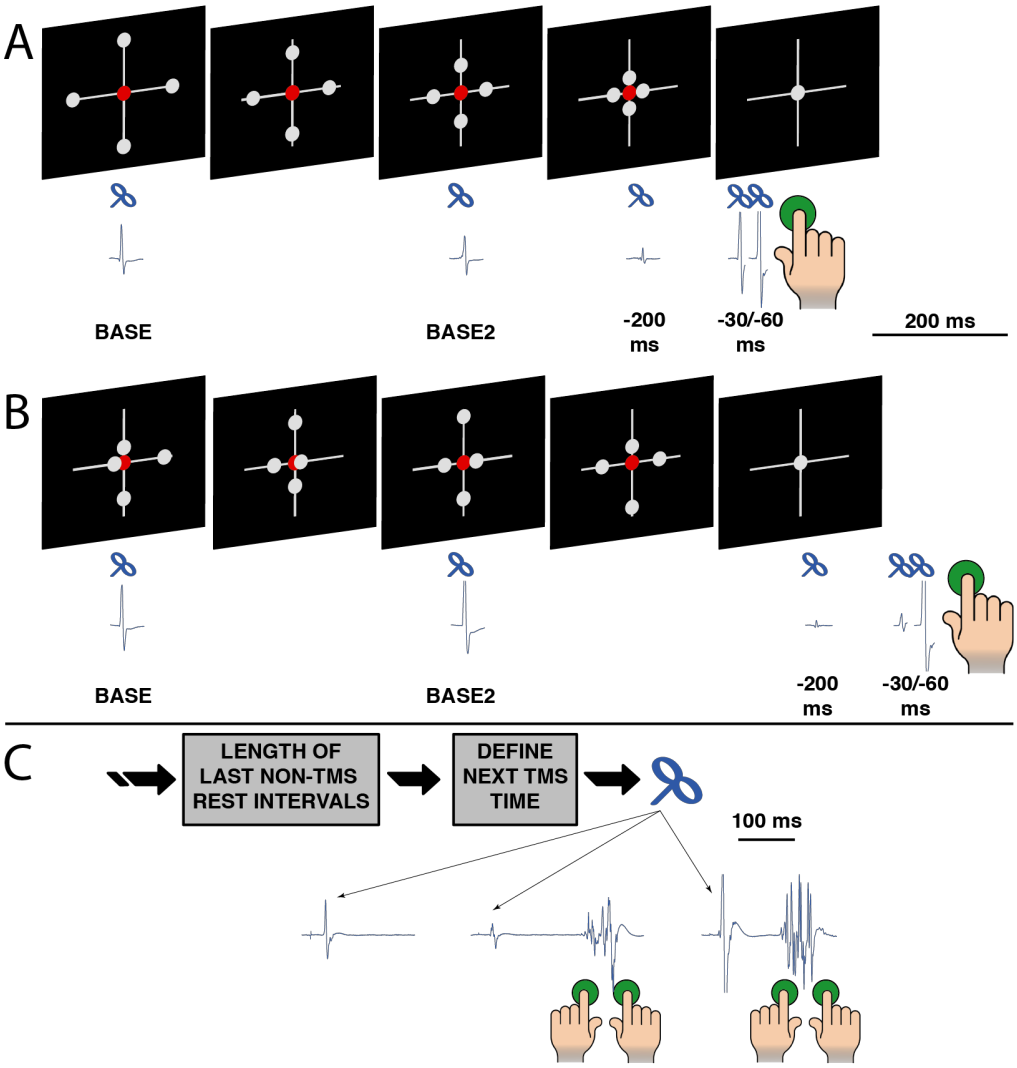


Figure 2

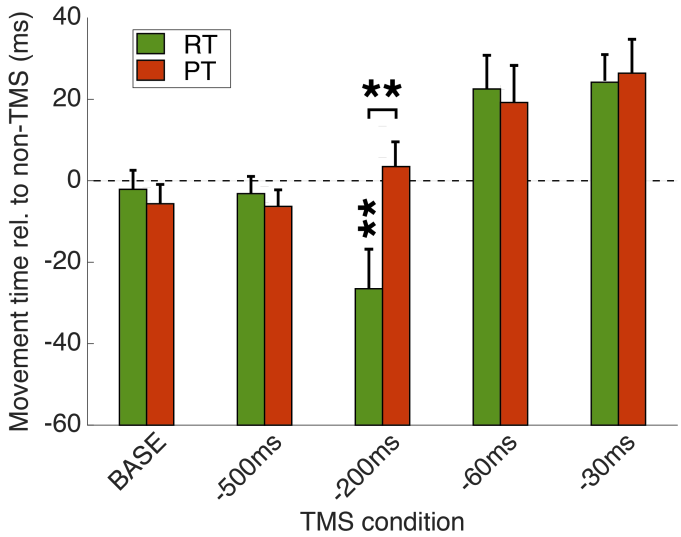


Figure 3

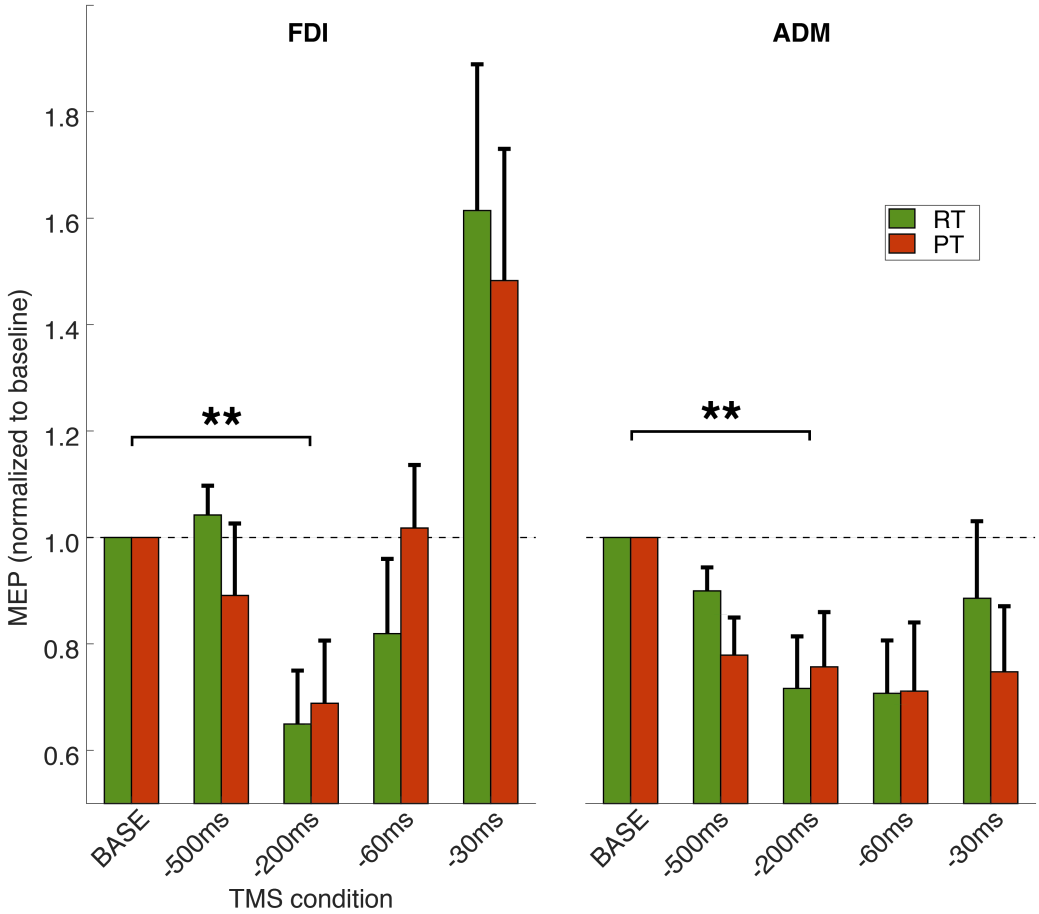


Figure 4

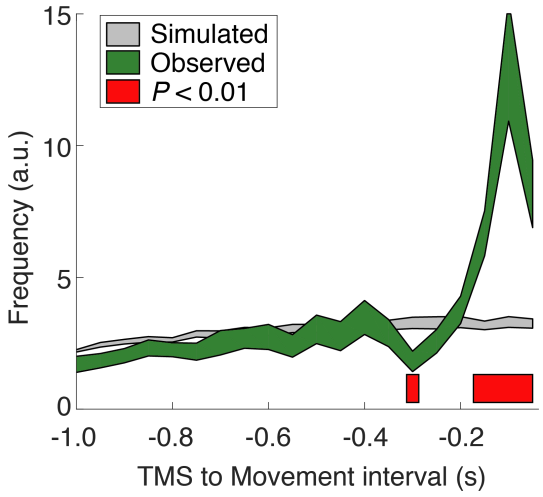
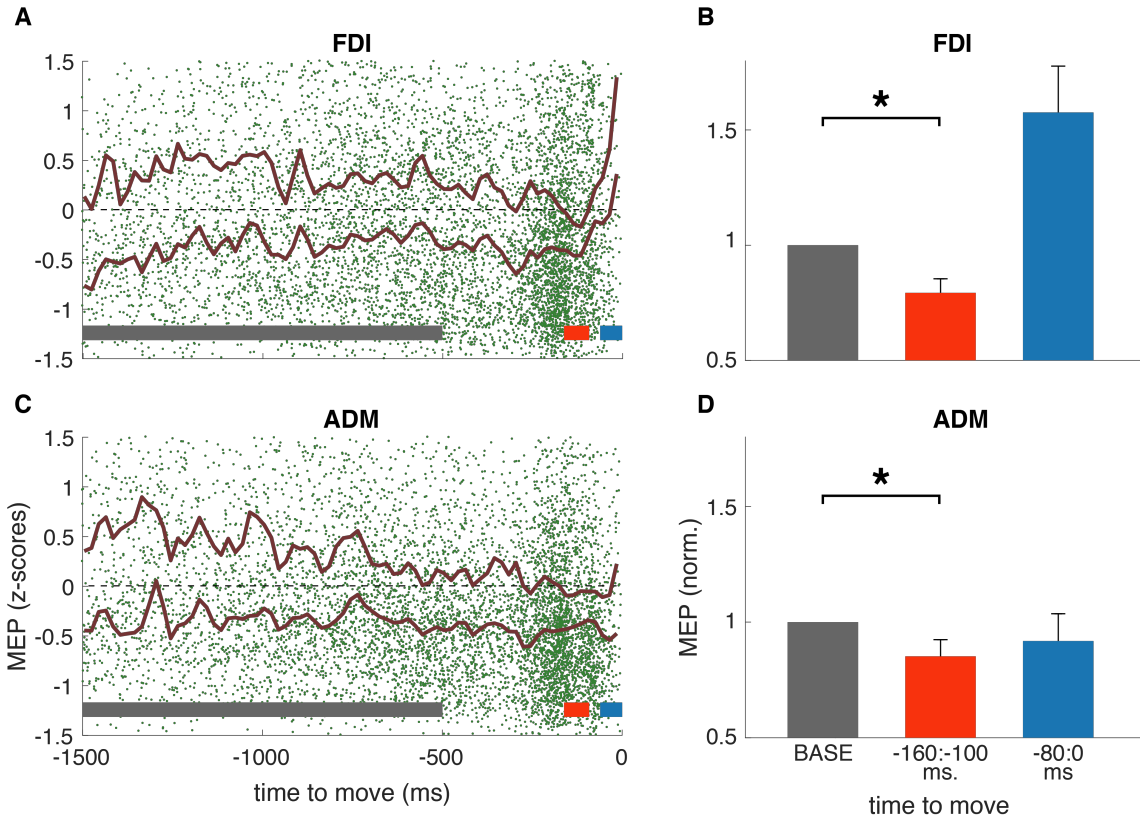

Simulated
Observed
P < 0.01
Frequency (a.u.)
15
10
5
0
-1.0
-0.8
-0.6
-0.4
-0.2
TMS to Movement interval (s)

Figure 5


A
FDI
B
FDI
C
ADM
D
ADM
MEP (z-scores)
MEP (norm.)
1.5
1
0.5
0
-0.5
-1
-1.5
-1500
-1000
-500
0
time to move (ms)
BASE
-160:-100 ms.
-80:0 ms
time to move