Exploration of Corticospinal Activity During Movement Preparation

by

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DISSERTATION ABSTRACT

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Title: Exploration of Corticospinal Excitability During Movement Preparation

Action preparation is a vital component of healthy goal-directed movement. While several studies have found evidence of transient inhibition of the motor system prior to simple finger movements, the functional role and putative source of this inhibition is not well understood. We explored corticospinal activity during the preparatory state under a number of different manual task types and conditions to investigate the nature of movement preparation. We used single-pulse Transcranial Magnetic Stimulation (TMS) in combination with electromyography (EMG) to measure summary analogues of the motor output pathway during three different studies. The first was a delayed-response task involving button-presses with the index finger while the contralateral hand held a tonic contraction. Here we showed a release of inhibition in the non-responding hand, as evidenced in shorter cortical silent periods. Experiment two involved a two-dimensional reach across a tablet surface to acquire targets. Here, motor evoked potentials (MEPs) measured during reach preparation did not change from baseline. Experiment three was a delayed-response task involving a choice between an out-and-back reach across the tablet surface and a button press using the thumb and forefinger of the same hand. Here also MEPs during the preparatory period were unchanged from baseline. While these findings stand in contrast to previous findings, they may suggest that certain task-related parameters, such as feedback and task complexity may influence whether preparatory inhibition is observable. They also add to a small but growing body of work that challenges the proposed models of preparatory inhibition.

This dissertation includes previously published co-authored material.

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CHAPTER I
INTRODUCTION

Successful goal-directed behavior depends on a delicate harmony of several neural processes that work together to plan, prepare, execute and refine desired actions. The neural mechanisms involved in this procession of movement have garnered considerable interest within neurophysiology and motor psychology. In humans, electrophysiology has led to many important insights by allowing for non-invasive recording of neural activity, both at rest as well as during the completion of behavioral tasks. One important application of such methods has been the investigation into preparatory activity and its role in healthy goal-directed movement.

Transcranial Magnetic Stimulation & Inhibition

Transcranial Magnetic Stimulation (TMS), which offers a non-invasive means of probing the motor system at precise time points, has been used to investigate the relationships between corticospinal (CS) activity and the manifestation of behavior. When applied over primary motor cortex (M1), TMS can elicit a measurable deflection in the electromyogram (EMG) of a targeted muscle. The amplitude of this voltage wave, known as the motor evoked potential (MEP), provides an index of the momentary excitability state of the CS pathway - a resultant analog of all excitatory and inhibitory influences[1]. In addition to providing a reliable within-subjects measurement of intrinsic CS excitability, TMS can be combined with other behavioral or kinematic metrics to draw conclusions about movement planning, preparation and execution along with associated physiological processes.

Several TMS studies have shown that action preparation during a delay period is characterized by transient inhibition of the motor-output pathway. Such studies require participants to press buttons in response to visual stimuli. Often a preparatory cue informs the participant of the forthcoming response and a subsequent imperative cue elicits the response. TMS can be delivered at specific time-points relative to these cues - typically either at the onset of the preparatory cue (baseline) or 100ms before the imperative cue (delay). Relative to baseline, delay period MEPs are reduced after an informative cue when measured in task-relevant muscles - that is, in primary agonists or potential agonists[2-5]. This phenomenon, known as preparatory inhibition, was first thought to play an important role in *impulse control* by

preventing premature movement. However, a similar inhibitory effect was also found in task-irrelevant muscles[3,5], which prompted a *competition-resolution model* - one in which inappropriate or undesired responses are inhibited prior to action.

A newer model, dubbed the *Spotlight Model of Inhibition*[5], does well to unify both processes into a single model by imagining preparatory inhibition as a beam of light. The focus of the spotlight is on the primary agonist, where inhibition is strongest, and the light that spreads out radially indicates a gradually weakening radius of inhibition throughout the motor system. This model captures both impulse control (inhibition of selected muscle) and competition-resolution (inhibition of non-selected muscle), while helping to explain why muscles completely unrelated to the task also exhibit preparatory inhibition.

While the findings described above point to a reliable suppression in the motor system when humans are preparing actions, other studies have produced evidence that challenges our understanding of preparatory inhibition, as well as its role in goal-directed movement. In a study that investigated the effect of task context on corticospinal excitability, MEP suppression was found in the task-irrelevant muscle, but not in the primary agonist or the potential agonist[6]. In fact, the primary agonist exhibited an increase in excitability (facilitation) during the preparation of movement. The authors interpreted this contrasting result to be attributable to elements of the task design that were distinct from previous work - an important consideration for novel task paradigms.

The source of preparatory inhibition also remains a question of considerable interest. Whether observed preparatory inhibition results from intracortical or subcortical influences is unclear. Using a paired pulse TMS protocol to measure short-interval intracortical inhibition (SICI), Duque et al.[4] showed evidence of a release of intracortical inhibition during action preparation. This finding motivates the search for either a separate cortical source or subcortical source that explains the observation of preparatory inhibition. A candidate electrophysiological measure is the cortical silent period (CSP), which occurs in a tonically active muscle following TMS. When the M1 representation is stimulated, the electromyogram of the active muscle will show an MEP followed by a period of inactivity. While the early portion of the CSP is thought to reflect spinal influences, the latter portion likely reflects intracortical inhibition. With both the amplitude of the MEP and the duration of the CSP, one may be able to unravel the relative

contributions of cortical and subcortical influences on corticospinal excitability during preparation. For example, a reduction in CSP duration would indicate a release of intracortical inhibition - similar to the findings related to SICI – and would thus converge on a subcortical source of preparatory inhibition. This would revise our current hypotheses about the candidate mechanisms of preparatory inhibition and motivate further investigations into the role of inhibition in movement more broadly.

A separate methodological limitation exists within the TMS literature. Most, if not all TMS studies investigating movement preparation have employed simple, ballistic finger or wrist movements. While employing highly constrained movements has experimental advantages, it fails to capture the richness and complexity of natural human behavior. Moreover, a large body of research on action preparation in animal models has examined more complex actions, which has complicated our ability to draw inferences between human TMS work in this area and findings in animals. We propose that studies of goal-directed reaching in humans is an optimal - and novel - direction for TMS-related investigations into the mechanisms of action preparation as well as the relationships between CS excitability and behavior, more broadly.

Goal-directed Reaching

Goal-directed reaching is a universal skill that allows humans to interact with and manipulate the external environment. As one of the most studied behaviors in Motor Physiology, Psychology, and Neuroscience research, reaching has produced a rich and extensive literature that includes a variety of methodologies.

Many studies have investigated reaching with a behavioral approach, employing sensorimotor adaptation tasks to explore the nature of motor learning and control[7-9]. In general, healthy humans (and monkeys) are extremely adept at performing goal-directed movements with our limbs. Regardless of the complexity of the task, a novice performer will optimize her/his movement pattern, reduce movement variability and become an expert[10]. The mechanisms that govern this process of adapting to reduce variability are of great interest. Optimal feedback control is the established model to explain the reflexive, automatic responses made to correct for variability in one's movement. This framework encapsulates both short-latency (~25ms) corrections, controlled by spinal reflex arcs, as well as long-latency (50-200ms) corrections,

controlled by subcortical and cortical mechanisms[11]. A number of perturbation types have been employed, mainly force-field perturbations - which involve fast-acting proprioceptive systems - and visual perturbations[12]. The use of prism goggles and virtual workspaces, for instance, introduces a lingering visual perturbation that the motor system must factor into the motor plan in order to successfully complete a task. A common method in this literature is to introduce a visuomotor rotation in the virtually-presented visual feedback. Several authors have found that, in response to the rotation, error-reduction occurs incrementally and exponentially, with pronounced aftereffects once the rotation is removed[13-15]. This gradual recalibration, known as sensorimotor adaptation, reflects a process of implicit motor learning[9]. However, the exact neural mechanisms involved are not entirely clear, and whether these mechanisms exert influence over the CS output pathway is unknown.

In a related body of work, direct neural recordings from non-human primates have produced insights into the neural activity at the level of a single neuron[16,17]. Employing a delayed response reaching task, such studies have found that preparatory neural activity is predictive of direction, extent, and speed of movement[18]. That is to say that single cortical neurons are individually and selectively tuned to the basic parameters of a given movement before movement onset occurs. Importantly, this preparatory activity is not simply a subthreshold version of movement-related activity, as one might expect. In fact, the tuning of individual neurons during preparation is dissimilar to movement-related tuning[19,20]. Clearly, preparatory activity is functionally relevant to goal-directed movement, but its exact role is still in question. Some have argued that preparatory activity exists in a *null space* which permits neural activity without triggering movement[21]. A Dynamical systems model posits that preparatory activity sets the initial state in an ever-changing system[20], and thus serves an important mechanistic role in goal-directed actions.

Importantly, the potential relationship between the preparatory activity of single-neurons and the corticospinal dynamics observed in TMS studies has not yet been explored. Furthermore, to our knowledge, single-pulse TMS has not yet been applied to reaching paradigms to explore action preparation in humans.

Cerebellar Influences

The role of the cerebellum in producing smooth, accurate movements has long been established. Connections to prefrontal and motor cortices, thalamus, brainstem and spinal cord point to a broad network of functions related to goal-directed actions. While the cerebellum receives several inputs from mossy and climbing fibers, its output via Purkinje cells is relatively straightforward, with simple spike activity governing cerebellar outflow. Single-unit studies have found that Purkinje cell activity is tuned for limb position and direction of forthcoming arm movements[22]. Importantly, cerebellar activity precedes movement-onset, especially when the movement is elicited by visual or auditory stimuli[23].

Much of what is known about the functional role of the cerebellum is informed by clinical work in patients with cerebellar lesions and experiments that modulate cerebellar output. Cerebellar patients exhibit a suite of symptoms most frequently illustrated by ataxia - discoordination of actions - and dysmetria - inaccurate distancing of actions. When asked to reach for a target, for example, cerebellar patients with dysmetria most often overshoot (hypermetria) and sometimes undershoot (hypometria) an intended target[24]. Furthermore, individuals with cerebellar lesions exhibit a decrease in excitability of the contralateral motor cortex, indicating a permissive influence of cerebellar output on motor regions.

Electrophysiological studies have probed the cerebello-thalamo-cortical pathway in healthy participants using magnetic stimulation. Using a paired-pulse TMS protocol, with one coil over cerebellum and a second over M1, the connectivity between the two regions can be interrogated. When a conditioning stimulus is applied over the cerebellum 5-7ms prior to a test stimulus over M1, the induced muscle response is attenuated - often referred to as Cerebellar-Brain-Inhibition (CBI)[25]. This protocol is thought to measure the inhibitory projection from the cerebellar cortex to the dentate, which reduces M1 activity[26]. During adaptation tasks, CBI has been shown to decrease – ie. less inhibition. In fact, reductions in CBI have been shown to scale with the magnitude of learning in a locomotor adaptation task[27] and a visuomotor adaptation task[28]. Surprisingly, this effect is seen in both a relevant agonist as well as a task-irrelevant muscle[29]. How this body of work relates to investigations of action preparation using single-pulse TMS is not yet clear.

In Summary

The neural mechanisms involved in goal-directed behavior are of considerable relevance to our understanding of both the motor system and neurophysiology. While several studies have observed transient inhibition of the motor-output pathway during action preparation, many questions remain regarding the functional role of inhibition and its putative sources. While the Spotlight Model of Inhibition suggests that undesired movement channels are suppressed to increase the gain of the desired signal, recent evidence suggests a more nuanced process. TMS-derived measures of intracortical inhibition suggest that while overall MEP amplitudes are suppressed, a coinciding release of intracortical inhibition may occur, evident in both a release of short – interval intracortical inhibition[3] and shorter durations of cortical silent periods[30]. These combined pieces of evidence may implicate subcortical sources of preparatory inhibition.

A likely source of subcortical inhibition is input from cerebellar- thalamocortical pathways. Evidence from dual-coil TMS that involve a conditioning stimulus over cerebellum and a test stimulus over M1 reliably show a decrease in MEP amplitudes[31]. This suggests cerebellar inputs to M1 have a modulatory influence over corticospinal output, and thus could be one of the sources of preparatory inhibition.

The neural mechanisms involved in goal-directed behavior are of considerable relevance to our understanding of both the motor system and neurophysiology. TMS offers a non-invasive means of exploring the relationships between CS activity and the unfolding of actions. While several studies have observed transient inhibition of the motor-output pathway during action preparation, its functional role is still unclear. Since previous TMS studies have constrained tasks to simple finger and wrist movements, goal-directed reaching is a promising behavior to further explore this relationship. To our knowledge, single-pulse TMS has not yet been applied to reaching paradigms to explore action preparation in humans. This paradigm may provide insight into the selectivity and extent of preparatory inhibition. Another dimension to be explored is how motor learning affects CS excitability during preparation. While it has been shown that during visuo-motor adaptation, cerebellar-mediated inhibition on M1 is reduced, how this dynamic relates to action preparation is unclear.

The following experiments contribute to our understanding of intracortical inhibition in action preparation and represent a novel direction for TMS investigations of preparatory inhibition. By implementing reaching behavior with single-pulse TMS methodology, we were able to probe CS excitability during the preparation of a complex behavior. We also provide a flexible framework for future investigations that wish to explore potential relationships between reaching and corticospinal excitability.

Overview of the Following Chapters

Chapter II describes an investigation into preparatory mechanisms and the putative sources of preparatory inhibition. This study is a previously published paper with co-authors Kara Ormiston and Dr. Ian Greenhouse. Chapter III describes a novel approach for probing the motor output pathway during 2-dimensional reaching with transcranial magnetic stimulation. This is a previously published methods paper with co-authors Serena Orsinger, Dr. Hyosub Kim and Dr. Ian Greenhouse. Chapter IV describes unpublished work investigating the presence of preparatory inhibition during reaching behaviors, as well as the effect of adaptation on corticospinal excitability. Chapter V describes an unpublished follow-up study further exploring the preparatory state during reaching and button-press movements, as well as the potential influence of task-relevancy. Chapter VI is the final conclusionary chapter of the dissertation. This chapter summarizes previous chapters and offers interpretations of results and final takeaways.

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CHAPTER II
RESPONSE PREPARATION INVOLVES A RELEASE OF INTRACORTICAL INHIBITION IN TASK-IRRELEVANT MUSCLES

Contributions

This work was published in volume 125, issue two of the Journal of Neurophysiology in February of 2021. Isaac N. Gomez and Ian Greenhouse conceived of and designed the project. Isaac N. Gomez and Kara Ormiston performed experiments and analyzed data. All authors contributed to the writing, editing and reviewing of the manuscript.

Response Preparation Involves a Release of Intracortical Inhibition in Task-Irrelevant Muscles

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Summary

Action preparation involves widespread modulation of motor system excitability, but the precise mechanisms are unknown. In this study, we investigated whether intracortical inhibition changes in task-irrelevant muscle representations during action preparation. We used transcranial magnetic stimulation (TMS) combined with electromyography in healthy human adults to measure motor-evoked potentials (MEPs) and cortical silent periods (CSPs) in task-irrelevant muscles during the preparatory period of simple delayed response tasks. In *experiment 1*, participants responded with the left index finger in one task condition and the right index finger in another task condition, whereas MEPs and CSPs were measured from the contralateral nonresponding and tonically contracted index finger. During *experiment 2*, participants responded with the right pinky finger whereas MEPs and CSPs were measured from the tonically contracted left index finger. In both experiments, MEPs and CSPs were compared between the task preparatory period and a resting intertrial baseline. The CSP duration during response preparation decreased from baseline in every case. A laterality difference was also observed in *experiment 1*, with a greater CSP reduction during the preparation of left finger responses

compared to right finger responses. Despite reductions in CSP duration, consistent with a release of intracortical inhibition, MEP amplitudes were smaller during action preparation when accounting for background levels of muscle activity, consistent with earlier studies that reported decreased corticospinal excitability. These findings indicate that intracortical inhibition associated with task-irrelevant muscles is transiently released during action preparation and implicate a novel mechanism for the controlled and coordinated release of motor cortex inhibition. In this study, we observed the first evidence of a release of intracortical inhibition in task-irrelevant muscle representations during response preparation. We applied transcranial magnetic stimulation to elicit cortical silent periods in task-irrelevant muscles during response preparation, and observed a consistent decrease in the silent period duration relative to a resting baseline. These findings address the question of whether cortical mechanisms underlie widespread modulation in motor excitability during response preparation.

Introduction

Successful goal-directed behavior depends on the ability to use information in the environment to prepare the motor system for action. However, action preparation is not a pure state of readiness to act. Successful preplanning of actions relies on the monitoring of ever-changing levels of uncertainty in the environment to successfully predict future outcomes of actions and to evaluate the ability to cancel actions when they become inappropriate (1). Determining how this balance is achieved in the nervous system during action preparation is an essential piece for understanding the neural mechanisms involved in goal-directed behavior. Inhibitory mechanisms are directly implicated in maintaining this balance. Several transcranial magnetic stimulation (TMS) studies have found evidence for inhibition of the motor output pathway during the preparation of actions, referred to as preparatory inhibition, measured as a reduction in motor-evoked potential (MEP) amplitudes, following an informative cue in a delayed response task (2–13). Further evidence suggests preparatory inhibition is widespread, influencing not only task-relevant muscles but also task-irrelevant muscles (3, 5). Whether this widespread decrease in motor system excitability during action preparation reflects the influence of local intracortical, long-distance transcortical, or subcortical mechanisms is unclear.

The cortical silent period (CSP) is the suppressed activity in the electromyogram (EMG) of a tonically active muscle following a single TMS pulse (14). Early and late portions of the CSP reflect spinal and cortical inhibitory mechanisms, respectively (15, 16). Pharmacological studies suggest that a mixture of $GABA_A$- and $GABA_B$-mediated intracortical inhibitory mechanisms contribute to the latter portion of the CSP (17–20). The most common approach used to measure the CSP includes the complete epoch from the administration of the TMS pulse until the resumption of EMG activity, and this includes the MEP. Isolating the contribution of the MEP duration to the CSP may provide additional insight into the recruitment of specific intracortical mechanisms. MEP duration increases during tonic contraction, is dissociable from MEP amplitude, and strongly correlates with short-interval intracortical inhibition, suggesting MEP duration reflects the influence of cortical mechanisms (21, 22). It is unclear whether MEP duration may explain CSP effects commonly reported in the literature and whether the latter portion of the CSP, after the MEP has resolved, reflects distinct intracortical mechanisms.

During action preparation, the duration of the CSP measured from a muscle involved in the planned response progressively shortens over the course of the fore-period—likely the result of increasing neural drive approaching movement onset (2). This release of inhibition, seen in the CSP, occurs in parallel with a decrease in MEP amplitude (2). The collective existing evidence suggests that a local release of intracortical inhibition occurs in the context of a more widespread decrease in corticospinal excitability, possibly via a subcortical or intercortical mechanism. However, although previous CSP studies have focused on task-involved muscles, to our knowledge, no previous work has investigated the CSP in task-irrelevant muscles, and the question of whether changes in intracortical inhibition are specific to task-involved muscles remains unanswered. Furthermore, investigating the CSP in task-irrelevant muscles avoids issues related to changes in neural drive associated with response execution.

In addition, a growing body of literature addresses the putative functions of ipsilateral primary motor cortex (M1) during unilateral responses (23–26). The precise contributions of M1 ipsilateral to the responding muscles remain unclear, with some work suggesting the recruitment of a transcallosal inhibitory mechanism that suppresses M1 ipsilateral to the response hand (27) and other work suggesting cross-activation [28, also see Cabibel et al. (29) for a review]. CSP measurements taken during the preparation of unimanual responses and acquired with TMS

administered ipsilateral to the responding hand, may help to determine the role of inhibition within ipsilateral M1.

In the current study, we sought to assess whether intracortical inhibitory mechanisms contribute to the widespread modulation of motor system excitability during action preparation. To this end, we examined MEP amplitudes and CSP durations in a task-irrelevant muscle during action preparation in a delayed response task. Participants maintained a tonic contraction in the nonresponding hand in order to investigate the CSP in that hand.

We tested three competing hypotheses: *1*) CSP duration would be shorter during preparation when compared to a resting baseline, reflecting a widespread release of intracortical inhibition during action preparation. Such a pattern would parallel the pattern observed in a muscle selected for a forthcoming response, implicating a possible common mechanism. Although, more than one mechanism could account for this pattern as well. *2*) CSP duration would be longer during preparation when compared to baseline. Such a pattern would be consistent with the recruitment of widespread intracortical inhibition during action preparation, implicating a specific cortical mechanism for preparatory inhibition. *3*) There would be no change in CSP duration measured in a task-irrelevant muscle during action preparation, consistent with earlier findings using paired-pulse TMS (3), and providing support against the widespread involvement of intracortical mechanisms in the modulation of corticospinal excitability during action preparation.

Materials and Methods

Participants

A total of 22 healthy, self-reported right-handed participants (10 females, 12 males, 24 ± 5 yr old) were included in the study. Data were collected in two experiments (n = 14 in each). Six participants completed both experiments, and 8 participants were unique to each experiment. All participants were screened for contraindications to TMS and provided written informed consent per a protocol approved by the Institutional Review Board of the University of Oregon.

Experimental Setup

Participants were seated comfortably in front of a computer monitor with both hands placed palm-down on the surface of a table. USB-interfaced response buttons were fixed to a button box such that button presses could be executed starting from a resting hand position. The configuration of the response buttons differed between *experiments 1* and *2* (Fig 2.1). Visual stimulus presentation was controlled by Psychtoolbox 3.0, and both EMG recording and the timed administration of TMS pulses were controlled by the VETA toolbox (30) in MATLAB. All experimental task code, analysis code, and data are available for download through the Open Science Framework at https://osf.io/jvnmq/.

Transcranial Magnetic Stimulation and Electromyography

Surface EMG was recorded using bipolar electrodes placed over both first dorsal interosseous (FDI) muscles for *experiment 1*, and the left FDI and right abductor digiti minimi (ADM) in *experiment 2*. A ground electrode was placed over the ulnar styloid process of the left arm. EMG was sampled at 5,000 Hz, amplified ×1,000, and bandpass filtered (50–450 Hz; Delsys). At the start of the experiment, maximum voluntary contraction (MVC) of the target FDI muscle was determined using a foam squeeze ball placed between the left index finger and thumb. Participants executed four consecutive 4 s contractions separated by 1 s of rest, and the maximum peak-to-peak amplitude of the EMG activity was calculated. Subsequently, participants were trained in maintaining a tonic contraction of near 25% MVC while holding the foam squeeze ball and visualizing the live EMG trace with markers indicating the target amplitude. In *experiment 1*, the determination of MVC was done separately for the left and right FDI corresponding to the two response conditions. In *experiment 2*, MVC was determined for the left FDI only.

TMS was administered using a Magstim 200-2 stimulator with a 7-cm diameter figure-of-eight coil. The center of the TMS coil was positioned over the left M1 to elicit MEPs and CSPs in the right FDI muscle (*experiment 1* only) and over the right M1 to elicit MEPs and CSPs in the left FDI muscle (*experiments 1* and *2*). A standard hot-spotting and thresholding procedure was used while the participant remained at rest.

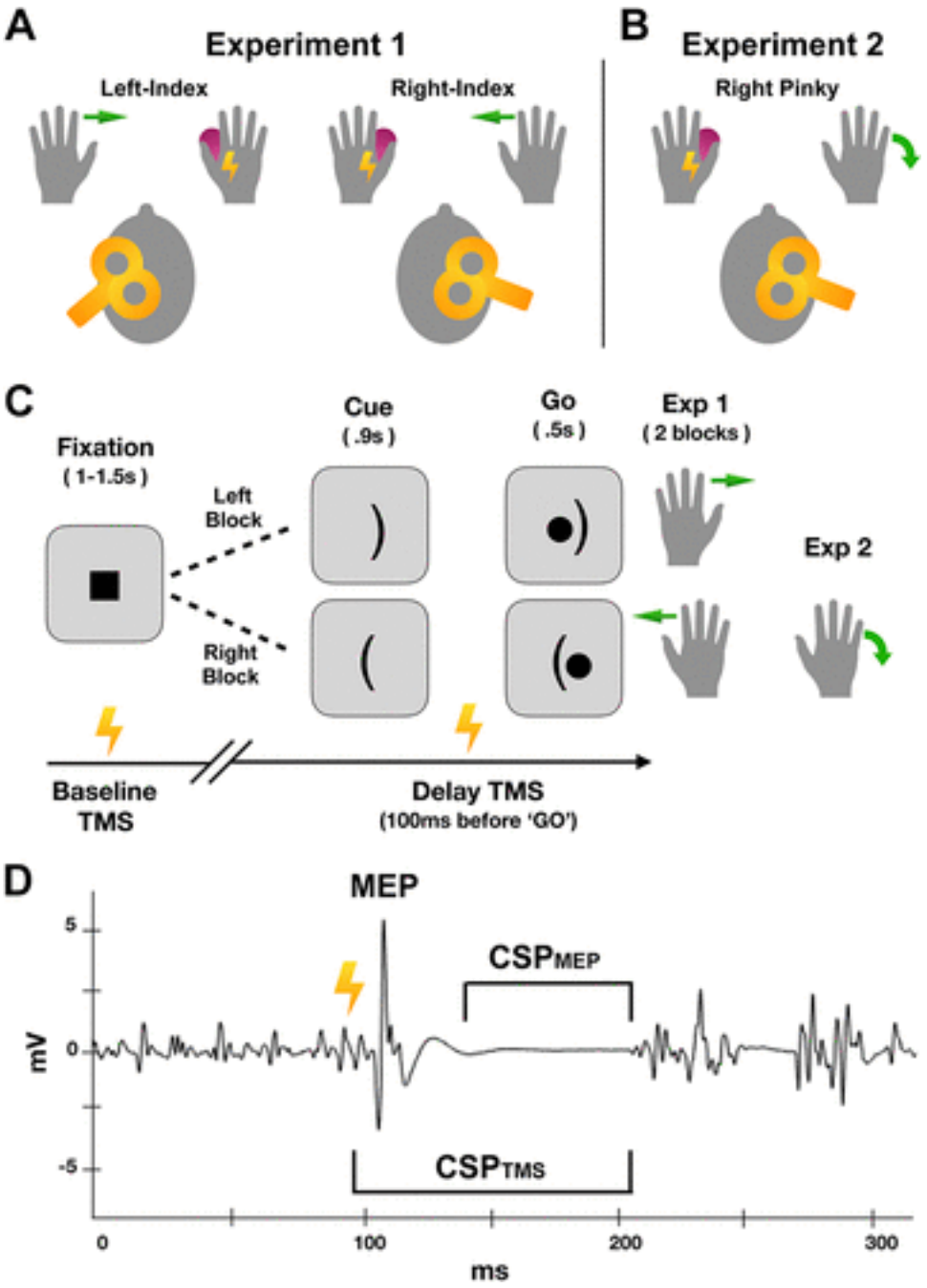

Figure 2.1 Left index and right index response conditions relative to transcranial magnetic stimulation (TMS) coil placement in *experiment 1*. The coil was positioned contralateral to the nonresponding hand, which held a foam squeeze ball (*A*). Right pinky response condition relative to TMS coil placement in *experiment 2* (*B*). Timing of visual stimuli and TMS administration for the delayed response task (*C*). Example EMG trace showing the motor-evoked potential (MEP), and the cortical silent period (CSP) measured from the TMS artifact (CSP_{TMS}) and from MEP offset (CSP_{MEP}) (*D*).

First, the coil was positioned ~2 cm anterior and 5 cm lateral to the vertex, over the hemisphere contralateral to the target muscle, and with the coil oriented ~45° off the midline to induce a current in the posterior to anterior direction. Second, the TMS intensity was adjusted and the coil was repositioned in incremental adjustments of ~1 cm until consistent MEPs were elicited from the targeted FDI. During this hot-spotting procedure, TMS pulses were administered once every 4 s. Third, once the optimal coil position and orientation were determined, a felt-tip marker was used to trace the coil position directly on the participant's scalp. Finally, the resting motor threshold (RMT) was determined as the intensity of TMS, which elicited MEPs with amplitudes of at least 50 μV on five out of ten attempts. During subsequent testing, TMS was administered at 115% RMT. The average RMTs for *experiments 1* and *2* were 47% ± 7% and 46% ± 9% of maximum stimulator output, respectively.

Unimanual Delayed Response Task

Participants completed a unimanual delayed response task while maintaining a tonic contraction with the nonresponding hand (Fig 2.1, *A* and *B*). Each trial of the task consisted of a 200 ms baseline fixation cue, followed by a 900 ms preparatory cue and a 500 ms imperative Go stimulus (Fig 2.1*C*). Each block consisted of 44 Go trials and six randomly interspersed catch trials, in which the preparatory cue remained on the screen through the end of the trial and the Go stimulus never appeared. Catch trials were included to discourage premature responses. Participants were instructed to keep the responding hand at rest between trials and to respond as quickly as possible to the Go stimuli.

Tonic contraction at 25% MVC was maintained in the nonresponding hand throughout each experimental block of the task, and the live EMG traces were monitored by the experimenter on an adjacent display. Verbal feedback was provided to participants, by the experimenter, if the EMG associated with the tonic contraction was outside the 25% MVC range. Participants were successful at maintaining this level of contraction for the duration of the experimental block.

TMS was delivered on 32 of 50 trials, either at the onset of the fixation cue (baseline) or 100 ms before the imperative Go stimulus (delay), and at only one time point on a given trial. The trial order was randomized so that participants could not predict the administration or timing of TMS or whether the trial was a Go or catch trial.

Experiment 1

In *experiment 1*, participants completed two task blocks, one with each hand, within a single testing session (Fig 2.1*A*). During one block, participants responded to the imperative stimulus by making a lateral abduction with the left index finger to depress the response button, and the 25% MVC contraction was maintained in the right hand. In the other block, the setup was reversed, such that participants responded to the imperative stimulus by making a lateral abduction with the right index finger to depress the response button, and the 25% MVC contraction was maintained in the left hand. The block order was counterbalanced across participants. TMS was always delivered to M1 contralateral to the nonresponding, tonically contracted hand, yielding MEP and CSP measurements from the task-irrelevant FDI muscle. With this setup, the nonresponding FDI targeted by TMS was "homologous" to the responding muscle.

Experiment 2

Experiment 2 consisted of a single task block in which the right abductor digiti minimi (ADM) was the responding muscle and the left FDI was the nonresponding muscle (Fig 2.1*B*). Participants made downward pinky movements (toward the table) to depress a button on a custom-built response device designed for the right hand. As in *experiment 1*, the nonresponding left hand maintained a tonic contraction near 25% MVC and TMS was administered over the right M1 to elicit MEP and CSP measurements from the task-irrelevant left FDI. In contrast to *experiment 1*, this setup represents the "nonhomologous" case, in which the responding muscle (right ADM) is contralateral but "nonhomologous" to the tonically contracted left FDI muscle targeted by TMS.

Data Analysis

Offline analysis of EMG data was performed using the VETA toolbox and custom-automated procedures within MATLAB. Dependent variables of interest included CSP duration, MEP duration, MEP peak-to-peak amplitude, MEP area, button press RT, EMG burst onset RT, and the percentage of failed catch trials. MEP duration was estimated using the MATLAB "findchangepts.m" function in the window from 18 ms to 100 ms following the TMS artifact. The first point was identified as the MEP onset and the last point as the MEP offset (30). CSP duration was estimated using two different approaches differing only in the identified onset of the CSP (Fig 2.1*D*). For the first approach, the CSP was estimated as the period from the TMS artifact through the resurgence of EMG activity, as in many previous studies. We refer to this estimate of the CSP duration as the CSP_{TMS} as it begins with the TMS artifact. For the second approach, the CSP was estimated as the period of MEP offset through the resurgence of EMG activity. We refer to this estimate of the CSP duration as the CSP_{MEP}. The latter approach accounts for the possibility that the CSP may not be measurable until after the MEP has resolved and depends on the calculation of the MEP duration (21, 22). Delay period CSP_{TMS}, CSP_{MEP}, MEP duration, and MEP amplitudes were calculated as a percentage of the respective baseline measurements. Button press and EMG burst onset RTs were calculated separately for no TMS, baseline TMS, and delay period TMS trials. Background EMG was quantified using the root mean square (RMS) of the raw EMG signal in the 100 ms preceding TMS pulses.

We implemented four statistical approaches in our analysis. *1*) We used a linear mixed model to analyze the effects of the response hand (left and right) and TMS epoch (baseline and delay) in *experiment 1*. This model included the background RMS EMG as a covariate because this measure was acquired at each level of the two main effects factors and can influence MEP and CSP measurements. *2*) We used paired two-tailed *t* tests to compare between conditions of *experiment 2*, with Cohen's *d* effect sizes reported where appropriate. Both the linear mixed model and *t* test analyses were performed in SPSS, v 26. *3*) To compare between the two experiments, we used a *t* test for partially overlapping samples (31) conducted in *R*. *4*) Finally, we used Bayesian repeated-measures ANOVAs and Bayesian paired *t* test to derive Bayes Factors (BF) to evaluate evidence for data derived from overlapping distributions. These

analyses were performed for MEP amplitudes, RTs, and EMG burst onset RT and were conducted in JASP (32).

Results

We present the results of both experiments side-by-side to facilitate comparisons. All participants contributed at least 13 trials for MEP and CSP measures across all conditions measured in each experiment. In *experiment 1*, the average number of MEPs and CSPs for the left response baseline and delay conditions were 15.3 ± 0.7 and 15.1 ± 0.9, respectively, and for the right response baseline and delay conditions were 15.8 ± 0.6 and 15.2 ± 0.6, respectively. In *experiment 2*, the average number of MEPs and CSPs for the baseline and delay conditions were 15.7 ± 0.5 and 14.2 ± 1.0, respectively.

CSP_{TMS} Duration

Baseline CSP_{TMS} durations were 135 ± 38 ms for the left index, 173 ± 32 ms for the right index, and 139 ± 34 ms for right pinky response conditions (Fig 2.2*A*). Delay period CSP_{TMS} durations were 121 ± 15 ms for left index, 160 ± 15 ms for right index, and 133 ± 18 ms for right pinky response conditions. Thus, CSP_{TMS} duration at baseline was longer for the right index response condition than the other two conditions [vs. left index, $t(12) = 8.49$, $P < 0.01$, $d = 2.35$; and vs. right pinky, $Z(16.1) = 3.40$, $P < 0.01$]. This was despite the fact that CSPs were measured from the left FDI in both the right index (*experiment 1*) and right pinky (*experiment 2*) response conditions, suggesting a potential effect of homology at baseline. The means ± SD of CSP_{TMS} duration within-subjects were 17.4 ms for baseline and 16.0 ms for delay measurements.

CSP_{TMS} duration during the preparatory delay period decreased from baseline for left index (89.9% ± 8%), right index (92% ± 7%), and right pinky (96% ± 5%) response conditions. In *experiment 1*, main effects of TMS epoch [$F(1,22) = 8.7$, $P < 0.01$] and response hand [$F(1,13) = 23.9$, $P < 0.001$] were significant. No significant interaction [$F(1,13) = 13.2$, $P = 0.9$] was observed. In *experiment 2*, CSP_{TMS} duration was also significantly reduced from baseline [$t(13) = 2.73$, $P < 0.05$, $d = 0.73$] during the preparation of right pinky responses. Post hoc

comparisons between *experiment 1* and *experiment 2* revealed that the left index response condition showed greater modulation than the right pinky response condition [$Z(16.1) = 2.2$, $P < 0.05$, uncorrected], but no significant difference in modulation between the right index and right pinky condition ($P = 0.16$) was found (Fig 2.2*B*).

To check whether CSP changes could have arisen from changes in the background tonic EMG during the response period, we analyzed the RMS of the tonic EMG signal in the 100 ms epoch following the EMG burst onset in the opposite hand (response epoch) and compared it with the 100 ms epoch preceding the TMS during the delay period. The RMS EMG in the right FDI during the left index response epoch (0.23 ± 0.16 mV) did not increase relative to the delay epoch (0.22 ± 0.13 mV; $P = 0.87$). However, the RMS EMG in the left FDI was significantly increased during the right index response (0.21 ± 0.12 mV) relative to the delay epoch [0.17 ± 0.09 mV; $t(13) = 4.1$, $P < 0.01$, $d = 1.08$, uncorrected]. A similar pattern was observed for the right-pinky response condition which showed a significant increase in RMS EMG during the response epoch (0.11 ± 0.05 mV) relative to the delay epoch (0.09 ± 0.04 mV) [$t(13) = 3.6$, $P < 0.01$, $d = 0.96$, uncorrected]. Thus, although the pattern of CSP durations did not differ across response conditions, there was a laterality difference in background EMG activity from the delay period to the response.

CSP_{MEP} Duration

The pattern for CSP_{MEP} duration was similar to that observed for CSP_{TMS} duration, suggesting that differences in the estimated MEP duration did not greatly influence CSP estimates. Baseline CSP_{MEP} duration was 75 ± 29 ms for the left index, 109 ± 30 ms for the right index, and 78 ± 30 ms for the right pinky response conditions (Fig 2.2*C*). Consistent with CSP_{TMS} results, CSP_{MEP} baseline duration was greater in right index responses than in left index [$t(13) = 6.0$, $P < 0.01$, $d = 1.60$, uncorrected] and right pinky [$Z(17) = 3.64$, $P < 0.01$, uncorrected] responses. Delay period CSP_{MEP} durations were 59 ± 17 ms for left index, 97 ± 14 ms for right index, and 72 ± 20 ms for right pinky response conditions. The SDs of CSP_{MEP} duration were 19.4 ms for baseline and 17.1 ms for delay measurements, closely matching that of CSP_{TMS}.

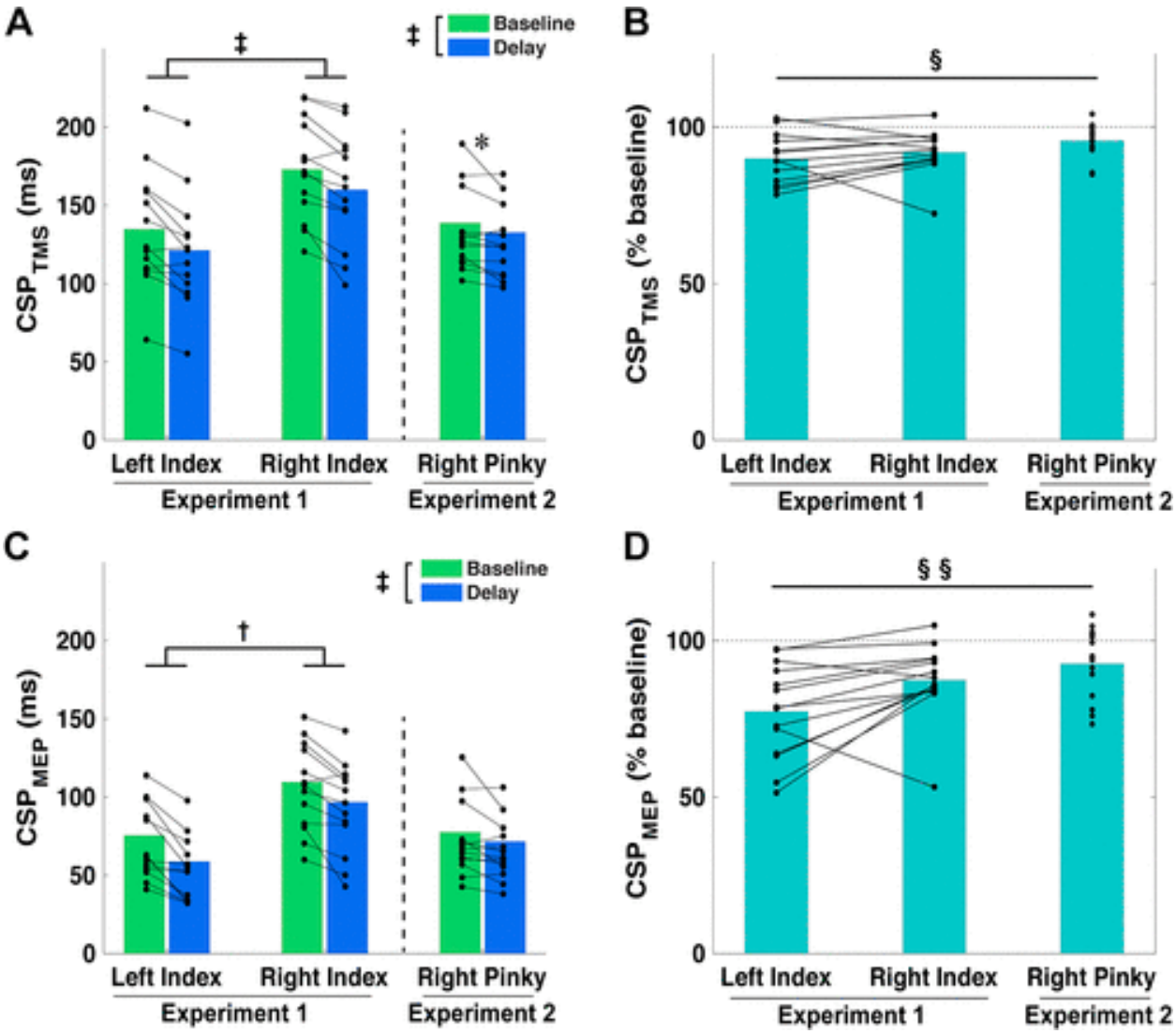

Figure 2.2 Cortical silent period (CSP) duration was measured at baseline and delay transcranial magnetic stimulation (TMS) epochs for all three response conditions. When measured from the TMS artifact (CSP_{TMS}), significant main effects of hand and TMS epoch (‡$P < 0.01$) were found for *experiment 1*, and a within-subjects test was significant (*$P < 0.05$) for *experiment 2* (*A*). Comparisons between *experiment 1* and *experiment 2* involving partially overlapping samples reveal a significant difference in modulation between left index and right index (§$P < 0.05$) responses (*B*). When measured from the end of the motor-evoked potential [MEP (CSP_{MEP})], main effects of hand (†$P < 0.05$) and TMS epoch (‡$P < 0.01$) remained significant in *experiment 1*, however, the within-subjects test was not significant for *experiment 2* (*C*). Comparisons between *experiment 1* and *experiment 2* revealed significant differences in modulation between left index and right pinky (§§$P < 0.01$) response conditions (*D*).

In *experiment 1*, CSP_{MEP} duration decreased from baseline to delay. Significant main effects of TMS epoch [$F(1,22) = 18.1$, $P < 0.001$] and response hand [$F(1,14) = 6.0$, $P < 0.05$] were found, without a significant interaction [$F(1,14) = 0.26$, $P = 0.6$] effect. In *experiment 2*, the change in CSP_{MEP} duration trended toward significance in the right pinky response condition [$t(13) = 2.1$, $P = 0.06$]. Post hoc comparisons between *experiment 1* and *experiment 2* revealed that the left index response condition showed greater modulation than the right pinky response condition [$Z(17) = 3.0$, $P < 0.01$, uncorrected], but no significant difference in modulation between the left index and right pinky condition ($P = 0.26$, Fig 2.2*D*).

MEP Duration

Baseline MEP duration was 36 ± 7 ms for the left index, 41 ± 4 ms for the right index, and 37 ± 7 ms for the right pinky response conditions. Baseline MEP duration was significantly shorter for left index than for right index [$t(13) = 2.64$, $P < 0.05$, $d = 0.7$, uncorrected; Fig 2.3*A*] conditions. Neither condition in *experiment 1* differed from right pinky [vs. left index, $Z(17) = 0.27$, $P = 0.8$, vs. right index, $Z(17) = 1.75$, $P = 0.1$] responses in *experiment 2*. Delay period MEP durations were 40 ± 8 ms for left index, 41 ± 3 ms for right index, and 37 ± 8 ms for right pinky response conditions.

In *experiment 1*, MEP duration increased from baseline to delay, in contrast to the observed shortening of CSP duration. The main effect of TMS epoch was significant [$F(1,14) = 12.5$, $P < 0.01$], whereas the effect of responding hand was not [$F(1,21) = 0.24$, $P = 0.6$]. A significant interaction [$F(1,18) = 8.2$, $P = 0.01$] indicated that the effect of TMS epoch was stronger for the left response condition. In *experiment 2*, MEP duration did not differ between baseline and delay ($P = 0.6$). Post hoc comparisons revealed no differences between right pinky and left index ($P = 0.23$) or right index ($P = 0.87$) response conditions (Fig 2.3*B*).

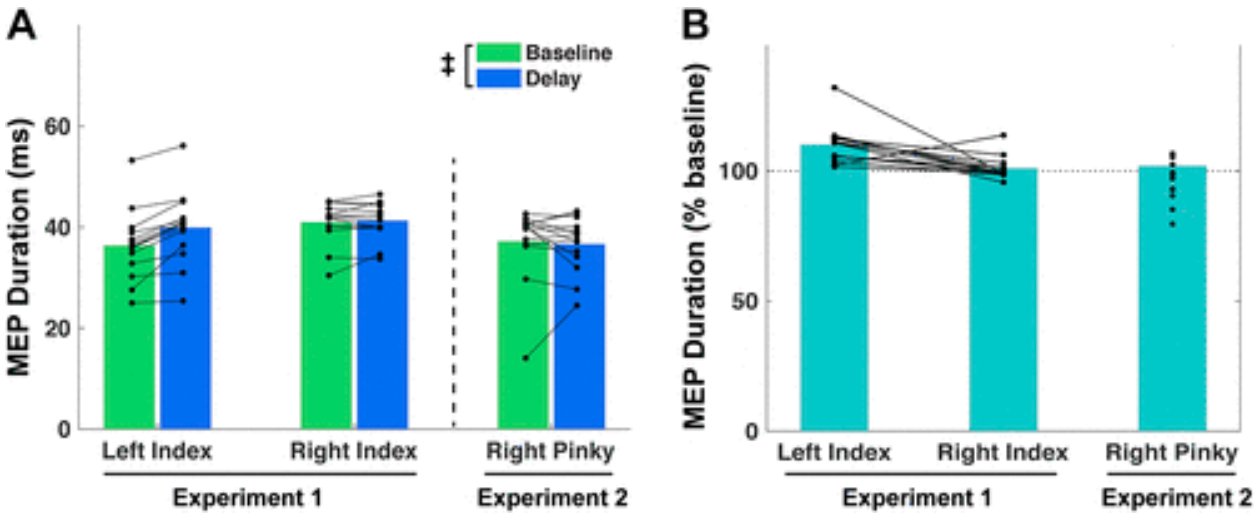

Figure 2.3 Motor-evoked potential (MEP) duration was measured at baseline and delay transcranial magnetic stimulation (TMS) epochs in three different response conditions. In *experiment 1*, the main effect of TMS epoch (‡$P < 0.01$) was significant but the effect of hand was not ($P = 0.6$), and in *experiment 2*, a within-subjects test showed no difference ($P = 0.6$) between baseline and delay (*A*). Comparisons between *experiment 1* and *experiment 2* found no differences (P's > 0.2) between conditions (*B*).

MEP Amplitudes

Baseline MEP amplitudes were 6.2 ± 2.5 mV in the left index, 6.8 ± 2.3 mV in the right index, and 4.2 ± 2.1 mV in the right pinky response conditions. Delay period MEP amplitudes were 6.1 ± 1.5 mV in the left index, 6.8 ± 0.8 mV in the right index, and 4.0 ± 1.2 mV in the right pinky response conditions (Fig 2.4*A*). Delay period MEP amplitudes as a percentage of baseline were 99% ± 8% for the left index, 98 ± 11% for the right index, and 93 ± 11% for the right pinky response conditions. Consistent with previous work, MEP amplitudes in *experiment 1* were significantly reduced, albeit a small percentage, during the delay period relative to baseline [$F(1,17) = 11.6$, $P < 0.01$] with no significant main effect of hand [$F(1,22) = 0.21$, $P = 0.7$] and no significant interaction [$F(1,17) = 0.01$, $P = 0.9$]. However, the effect of TMS epoch only reached significance when background EMG was included as a covariate in the statistical model. MEPs in *experiment 2* exhibited a trend for reduced amplitude during the delay relative to baseline, [$t(13) = 2.09$, $P = 0.06$]. The comparison in *experiment 1* did not reach significance when background EMG was excluded as a covariate and did not differ between response conditions (P's > 0.15, Fig 2.4*B*). Moreover, the Bayesian repeated-measures ANOVA showed

moderate evidence in support of there being no difference in MEP amplitudes between baseline and delay in *experiment 1* [$BF_{10} = 0.27$; BF_{10} indicates the Bayes factor in favor of the hypothesis (H1) over the null hypothesis (H0)], whereas a Bayesian paired samples *t* test provided anecdotal evidence in favor of a difference in MEP amplitudes between baseline and delay in *experiment 2* ($BF_{10} = 1.425$).

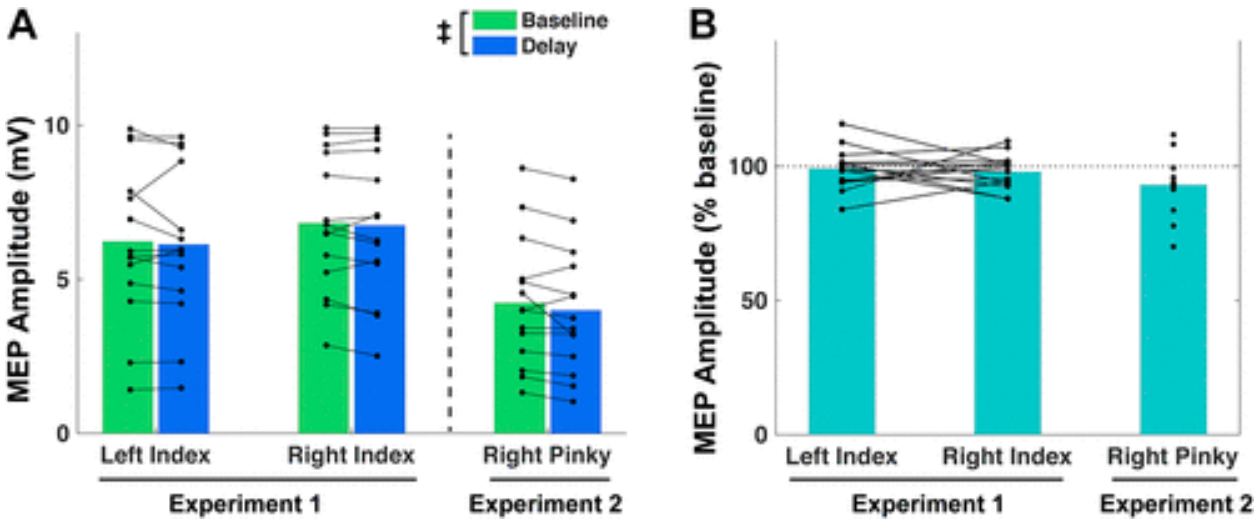

Figure 2.4 Motor-evoked potential (MEP) amplitudes were measured at baseline and delay transcranial magnetic stimulation (TMS) epochs for all three response conditions. In *experiment 1*, the main effect of TMS epoch (‡$P < 0.01$) was significant but the effect of hand was not ($P = 0.7$), and in *experiment 2*, a within-subjects test showed no significant difference ($P = 0.06$) between baseline and delay (*A*). Comparisons between the two experiments did not reach significance (P's > 0.15). No differences were observed in MEP amplitudes as a percentage of baseline (*B*).

MEP Area

Baseline MEP area was 6.3 ± 4.4 mV for left index, 10.8 ± 3.7 mV for the right index, and 4.7 ± 2.2 mV for right pinky response conditions. Delay MEP areas were closely matched to baseline, with 6.3 ± 4.1 mV for left index, 9.9 ± 3.8 mV for right index, and 4.2 ± 1.5 mV for right pinky response conditions. MEP area did not differ between baseline and delay for *experiment 1*, as neither main effects of TMS epoch [$F(1,13) = 1.9$, $P = 0.19$] nor hand [$F(1,18) = 0.05$, $P = 0.8$] were significant, and no interaction [$F(1,18) = 0.94$, $P = 0.3$] was found. The same was true

for *experiment 2*, with no difference between baseline and delay ($P = 0.12$) for right pinky responses.

Overall and Pre-TMS Tonic EMG

The maximum tonic EMG in the nonresponding hand averaged across all trials was 1.35 ± 0.71 mV in the left index response condition in *experiment 1*, 1.34 ± 0.81 mV in the right index response condition in *experiment 1*, and 0.73 ± 0.30 mV for *experiment 2*. RMS of the EMG signal in the 100 ms preceding TMS for the left index response condition was not significantly different between baseline (0.22 ± 0.14 mV) and delay periods (0.22 ± 0.13 mV; $P = 0.66$). However, there were significant differences between the baseline and delay periods for the right index response condition [baseline: 0.19 ± 0.12 mV and delay: 0.17 ± 0.1 mV; $t(13) = 2.44$, $P < 0.05$, uncorrected, $d = 0.65$] and right pinky response condition [baseline: 0.10 ± 0.04 mV and delay: 0.09 ± 0.04 mV; $t(13) = 2.69$, $P < 0.05$, uncorrected, $d = 0.72$]. Therefore, in the left index response condition, background EMG differences did not account for the observed differences in the CSPs or MEPs. In the other two cases, the pattern indicates a decrease in background EMG activity during the preparatory period, which one might expect to correspond to an increase rather than a decrease in CSP duration. Moreover, our linear mixed model analysis, which included background EMG as a covariate, indicated that background EMG did not account for differences in the CSP or MEP, with the exception of MEP amplitude in *experiment 1*.

Button Press and EMG Burst Onset RTs

Button press RTs for *experiment 1* did not differ between left index (noTMS: 342 ± 26 ms; baselineTMS: 338 ± 26 ms; delayTMS: 338 ± 26 ms) and right index (noTMS: 335 ± 25 ms; baselineTMS: 332 ± 26 ms; delayTMS: 327 ± 45 ms) responses, and there was no effect of TMS or TMS timing (all P's > 0.05; effect of hand: $BF_{10} = 0.46$; effect of TMS: $BF_{10} = 0.14$; Fig 2.5*A*). Right pinky response RTs in *experiment 2* (noTMS: 341 ± 38 ms; baselineTMS: 340 ± 49 ms; delayTMS: 341 ± 50 ms) did not differ from left index or right index responses in *experiment 1* (all P's > 0.05). There was also no effect of TMS in *experiment 2* (all P's > 0.05; $BF_{10} = 0.18$; Fig 2.5*B*).

A similar pattern was found for EMG onset RTs. There was no effect of response finger in *experiment 1* ($P > 0.05$; $BF_{10} = 0.45$), no effect of TMS for either experiment (all P's > 0.05; *experiment 1*: $BF_{10} = 0.14$; *experiment 2*: $BF_{10} = 0.88$), and no difference in EMG burst onset RTs between experiments (all P's > 0.05). Thus, the CSP and MEP results were not explained by differences in response performance.

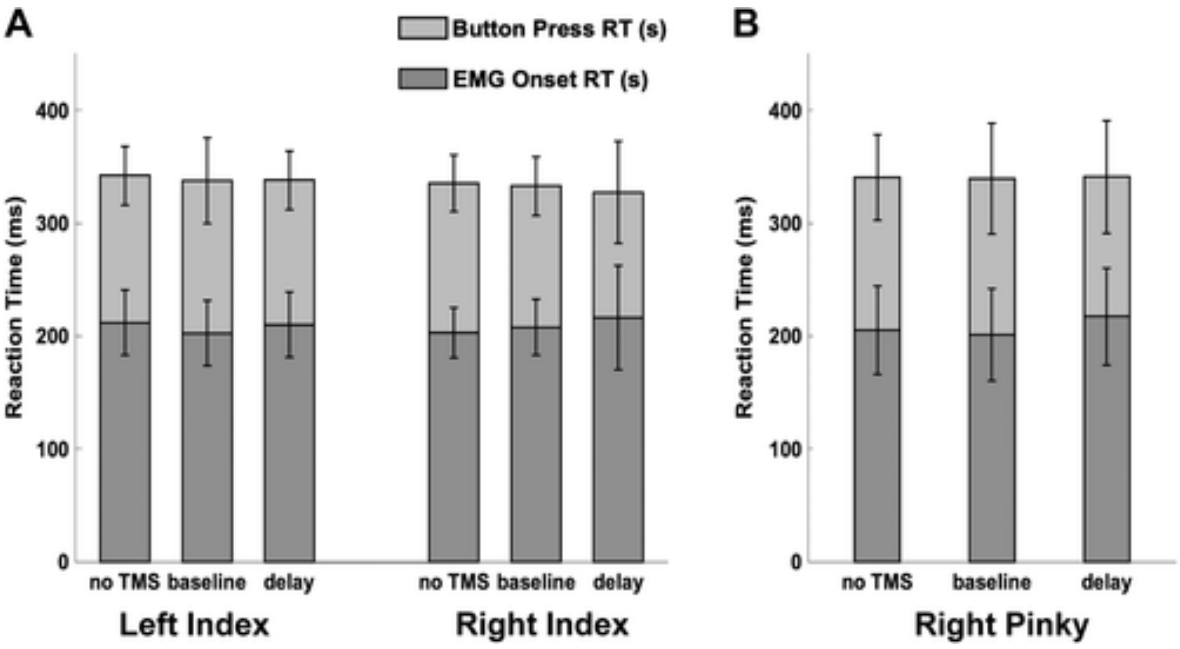

Figure 2.5 Button press reaction times (RTs) and electromyogram (EMG) burst onset RTs did not show differences across hands or transcranial magnetic stimulation (TMS) conditions in *experiment 1* (*A*) or across TMS conditions in *experiment 2* (*B*). There were no differences between *experiment 1* and *experiment 2* as well.

Discussion

In this study, we tested the competing hypotheses that intracortical inhibition increases, decreases, or remains stable in task-irrelevant muscles during the preparation of actions. We found compelling evidence for a decrease in intracortical inhibition during action preparation. Specifically, we observed reductions in the CSP duration measured in a task-irrelevant muscle whether it was homologous (*experiment 1*) or nonhomologous (*experiment 2*) to the contralateral responding muscle. This pattern was not explained by MEP amplitude, MEP duration, MEP area,

or background EMG activity levels. The observed changes in CSP duration implicate a mechanism involved in the cortical release of GABA-ergic inhibition during action preparation. Although the observed pattern could reflect a mechanism distinct from those that influence properties of the MEP, it is possible the release of intracortical inhibition is offset by an opposing inhibitory mechanism that scales in a manner that either stabilizes or reduces the MEP amplitude.

Interpretation of Reduced Intracortical Inhibition

Several studies have characterized the spinal and cortical contributions to the CSP. Seminal work attributed the first 50–80 ms of the CSP to a spinal origin (16, 33) and the later portion to the interruption of voluntary cortical drive (16). These early findings would later be supported by epidural recordings (15) and pharmacological studies (20, 34–36). Although the spinal contribution remains relatively stable, the cortical contribution is thought to reflect GABA-ergic intracortical mechanisms and to be primarily responsible for changes in the CSP duration (20, 35). Whether such effects are specific to $GABA_A$- or $GABA_B$-mediated mechanisms remains the subject of debate (18, 19). Nevertheless, given the existing evidence for sources of the CSP, we believe our results are best explained by a release of inhibition at the cortical level.

Short-interval intracortical inhibition (SICI) is another TMS-derived index of cortical inhibition, which manifests as a reduction in the MEP amplitude when a conditioning TMS pulse is administered 1–5 ms before a MEP-eliciting test pulse (37) and is widely accepted to reflect fast-acting $GABA_A$-dependent inhibition (38, 39). SICI is decreased during action preparation, consistent with a transient release of fast intracortical inhibition (3). Similarly, long-interval intracortical inhibition (LICI) manifests as a reduction in MEP amplitude when a suprathreshold conditioning stimulus precedes a test stimulus by ~100–200 ms (40, 41). In a warned reaction time task, SICI and LICI in the responding muscle were reduced during a short preparatory fore-period, indicating a release of inhibition (12). These results suggest that intracortical inhibition is reduced during action preparation in task-relevant muscles. However, task-irrelevant muscles were not investigated in these studies.

In the context of this previous work, our findings suggest that there is a release of intracortical inhibition during action preparation involving multiple GABA-dependent mechanisms acting across different time scales and with potentially different spatial distributions. Our data show that the release of intracortical inhibition in the form of reduced CSP duration, previously observed in task-relevant (2) muscles, extends to task-irrelevant muscles as well. Further studies should investigate whether SICI and LICI change in task-irrelevant muscles during response preparation to address the question of whether the spatial extent of the release of intracortical inhibition is shared across multiple mechanisms or could dissociate between different cortical inhibitory mechanisms.

Motor-Evoked Potentials, Laterality Differences, and Ipsilateral Modulation

MEP duration also showed an interesting pattern, exhibiting a significant increase from baseline to delay, but only for left index response trials. We also note that there were marked laterality differences in CSP duration. These likely reflect the influence of hand-dominance, as CSPs were shorter in the dominant hand. This finding replicates previous work that reported hand-dominance effects on CSP without changes in MEP amplitude, latency, and threshold (42). MEP amplitudes did not exhibit a similar pattern, pointing to an interesting dissociation between MEP duration and MEP amplitude.

Interestingly, we observed a decrease in the RMS of the EMG activity immediately preceding the TMS pulse between baseline and delay period measurements, but this was only the case for right-hand responses, i.e., when tonic EMG was recorded from the left FDI. We also observed increased tonic EMG activity during the transition from the delay period into the response phase but only for right-hand responses. These patterns may reflect laterality differences, although opposite to the pattern we observed for MEP duration. Moreover, this did not appear to influence MEP amplitudes.

Although hand-dominance may explain the differences between left index and right index responses, we were unable to compare the differences between the index and pinky response conditions within participants. Therefore, differences between the right index and right pinky response conditions may be explained by intersubject differences.

Our results have additional relevance for the mechanisms engaged in the ipsilateral motor cortex during unilateral actions. There remains debate about whether ipsilateral motor cortex is activated or inhibited during action (23–29). Our observation of a release of intracortical inhibition ipsilateral to the responding hand is consistent with a role for activation.

Alternative Interpretations and Limitations

The CSPs and MEPs were measured from tonically active muscles, which complicates comparisons of our results to previous investigations of preparatory inhibition. We observed a decrease in MEP amplitudes during response preparation, a finding from multiple previous studies (3–5, 7–10, 12). However, this was only significant when background EMG was included as a covariate and the proportional decrease in MEP amplitudes was smaller than in the majority of previous studies. This could be explained by the level of tonic EMG maintained in the muscle from which CSPs were measured. By asking participants to remain in a tonically active state, we may have diminished the commonly observed preparatory MEP suppression and, in exchange, uncovered a decrease in CSP duration. Previously, authors measured MEPs in task-irrelevant muscles while at rest, which deliberately avoids the possible interference introduced by tonic EMG activity. We note that one previous study observed reduced MEP amplitudes during response preparation in a tonically active muscle (6) although the muscle was task-relevant and tonic contraction was 5%–10% MVC, a lower intensity than we used here. In contrast, the design of the current study, which required participants to maintain 25% MVC, likely introduced interhemispheric effects and stronger descending corticospinal drive. Interestingly, previous work suggests that a low-intensity, but not a high-intensity, contraction results in decreased MEP amplitudes in a task-irrelevant homologous muscle (43).

We also note that RMT served as the reference for determining the TMS intensity used for CSP measurements, rather than an active motor threshold (AMT) as has been frequently used in other studies. This yielded MEP amplitudes larger than those found in most previous studies. The higher level of activation may have further impacted our ability to detect changes in MEPs during the task. On a separate note, even with these large MEP amplitudes, we did not observe a difference in reaction times between trials with and without TMS, raising questions about the

possible source of RT differences reported in previous work. In the response hand, this pattern of faster RTs in the presence of TMS may reflect the influence of a shortened CSP.

As we only measured EMG from hand muscles, we cannot make strong claims about the widespread nature of CSP modulation in other task-irrelevant muscles. Similarly, it is unclear whether the CSP results from $GABA_A$ and $GABA_B$ mechanisms. Comparing SICI, LICI, and CSP measurements will help to elucidate which of these mechanisms may be responsible for preparatory modulation in task-irrelevant muscles.

Conclusion

We observed evidence of a non-focal release of intracortical inhibition during the preparation of actions evident in the form of decreased duration of CSPs measured from task-irrelevant muscles. Our findings are consistent with a model in which response preparation involves a widespread release of cortical inhibition and extend those of previous studies that reported changes in other TMS-derived markers of intracortical inhibition in muscles involved in the task. Furthermore, we wish to highlight that MEP amplitudes did not show a pattern consistent with a release of inhibition. Instead, our results suggest that the release of intracortical inhibition might arise from a mechanism that operates independent of other mechanisms that influence corticospinal excitability or from one that is offset by an additional inhibitory mechanism that influences the MEP. These findings have clinical relevance for diseases that impair response initiation, such as Parkinson's disease, stroke, and trauma, by providing insight into potentially affected mechanisms. Intracortical inhibition is abnormal in movement disorders, including Parkinson's disease, dystonia, and stroke, that are also characterized by impaired response initiation. The functional significance of abnormal intracortical inhibition in these cases remains uncertain. The application of our approach in these populations may be useful for determining whether the release of intracortical inhibition during response preparation relates to specific symptoms in these populations.

Disclosures

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

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Bridge

This chapter represents one of the few published studies that show evidence of disinhibition during movement preparation. A reduction in MEP amplitude coupled with a shortened CSP duration indicates two opposing processes, and implicates a non-cortical source of preparatory inhibition. Previous work has shown that both MEP and CSP measures change over the course of motor adaptation during reaching tasks. However, whether these same physiological markers change during the preparation of reaches in a manner similar to finger responses has not been investigated. In the following chapter, we outline novel methods for using single-pulse TMS in conjunction with 2-dimensional reaching on a tablet surface. These methods offer a general and flexible approach to using non-invasive electrophysiology to investigate the motor system under the widely studied behavioral context of reaching. These methods attempt to bridge two disparate areas of research, and form the basis for the experiments outlined in subsequent chapters.

CHAPTER III
ASSESSING CORTICOSPINAL EXCITABILITY DURING GOAL-DIRECTED REACHING BEHAVIOR

Contributions

This work was published in the Journal of Visualized Experiments in December of 2022. Isaac N. Gomez and Ian Greenhouse conceived of and designed the project. Isaac N. Gomez and Kara Ormiston performed experiments and analyzed data. All authors contributed to the writing, editing and reviewing of the manuscript.

Assessing Corticospinal Excitability During Goal-Directed Reaching Behavior

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Summary

Reaching is a widely studied behavior in motor physiology and neuroscience research. While reaching has been examined using a variety of behavioral manipulations, there remain significant gaps in the understanding of the neural processes involved in reach planning, execution, and control. The novel approach described here combines a two-dimensional reaching task with transcranial magnetic stimulation (TMS) and concurrent electromyography (EMG) recording from multiple muscles. This method allows for the noninvasive detection of corticospinal activity at precise time points during the unfolding of reaching movements. The example task code includes a delayed response reaching task with two possible targets displayed ± 45° off the midline. Single pulse TMS is delivered on the majority of task trials, either at the onset of the preparatory cue (baseline) or 100 ms prior to the imperative cue (delay). This sample design is suitable for investigating changes in corticospinal excitability during reach preparation. The sample code also includes a visuomotor perturbation (i.e., cursor rotation of ± 20°) to investigate the effects of adaptation on corticospinal excitability during reach preparation. The

task parameters and TMS delivery can be adjusted to address specific hypotheses about the state of the motor system during reaching behavior. In the initial implementation, motor evoked potentials (MEPs) were successfully elicited on 83% of TMS trials, and reach trajectories were recorded on all trials.

Introduction

Goal-directed reaching is a fundamental motor behavior that allows humans to interact with and manipulate the external environment. The study of reaching in the fields of motor physiology, psychology, and neuroscience has produced rich and extensive literature that includes a variety of methodologies. Early studies of reaching used direct neural recordings in non-human primates to investigate neural activity at the level of single neurons[1,2] . More recent studies have investigated reaching using behavioral paradigms that employ sensorimotor adaptation to explore the nature of motor learning and control[3-5] . Such behavioral tasks combined with functional magnetic resonance imaging and electroencephalography can measure whole brain activity during reaching in humans[6,7] . Other studies have applied online TMS to investigate various features of reach preparation and execution[8-14]. However, there remains a need for an open-source and flexible approach that combines the behavioral assessment of reaching with TMS. While the utility of combining TMS with behavioral protocols is very well established[15], here, we specifically examine the application of TMS within the context of reaching using an open-source approach. This is novel in that other groups who have published using this combination of methods have not made their tools readily available, prohibiting direct replication. This open-source approach facilitates replication, data sharing, and the possibility of multi-site studies. Additionally, should others wish to pursue novel research questions with similar tools, the open-source code can act as a launch pad for innovation, as it is readily adaptable.

TMS offers a noninvasive means of probing the motor system at precisely controlled time points[16]. When applied over the primary motor cortex (M1), TMS can elicit a measurable deflection in the electromyogram of a targeted muscle. The amplitude of this voltage wave, known as the motor evoked potential (MEP), provides an index of the momentary excitability

state of the corticospinal (CS) pathway-a resultant analog of all excitatory and inhibitory influences on the CS pathway[17]. In addition to providing a reliable within- subject measurement of intrinsic CS excitability, TMS can be combined with other behavioral or kinematic metrics to investigate the relationships between CS activity and behavior in a temporally precise manner. Many studies have utilized a combination of TMS and electromyography (EMG) to address a variety of questions about the motor system, particularly since this combination of methods makes it possible to investigate MEPs under a vast array of behavioral conditions[15]. One area where this has proven particularly useful is in the study of action preparation, most often through the study of single-joint movements[18]. However, there are comparatively fewer TMS studies of naturalistic multi-joint movements such as reaching.

The current goal was to design a delayed-response reaching task that includes behavioral kinematics, online single-pulse TMS administration, and simultaneous EMG recording from multiple muscles. The task includes a two- dimensional point-to-point reaching paradigm with online visual feedback using a horizontally oriented monitor such that visual feedback matches reach trajectories (i.e., a 1:1 relationship during veridical feedback and no transformation between visual feedback and motion). The current design also includes a set of trials with a visuomotor perturbation. In the provided example, this is a 20° rotational shift in the cursor feedback. Previous studies have used a similar reaching paradigm to address questions about the mechanisms and computations associated with sensorimotor adaptation[19-25] . Furthermore, this approach makes it possible to assess motor system excitability dynamics at precise time points during online motor learning.

Because reaching has proven to be a fruitful behavior for investigating learning/adaptation, assessing CS excitability in the context of this behavior has enormous potential to shed light on the neural substrates involved in these behaviors. These may include local inhibitory influences, changes in tuning properties, the timing of neural events, etc., as have been established in non-human primate research. However, these features have been more difficult to quantify in humans and clinical populations. Neural dynamics can also be investigated in the absence of overt movement in humans using the combined TMS and EMG approach (i.e., during the preparation of movement or at rest).

The tools presented are open-source, and the code is easily adaptable. This novel paradigm will produce important insights into the mechanisms involved in the preparation, execution, termination, and adaptation of reaching movements. Moreover, this combination of methods has the potential to uncover relationships between electrophysiology and reaching behavior in humans.

Protocol

All methods detailed here were performed in compliance with IRB protocol and approval (University of Oregon IRB protocol number 10182017.017). Informed consent was obtained from all subjects.

Reaching apparatus

1. Place a large graphics tablet flat on a desktop.
2. Use an adjustable 80-20 aluminum frame to position the task monitor 6-8 in above the tablet in parallel, with the screen facing upward (for a blueprint, check here: https://github.com/greenhouselab/ Reach_TMS and **Supplementary Figure 3.3**). Note: This setup allows for participants to reach across the tablet and acquire targets presented on the task monitor while occluding vision of their reaching arm.
3. Use the setup described in Kim et al.[3] as a reference.

Machine Interfaces

1. Connect the tablet to the computer via a USB port. Connect the task monitor to the computer *via* the HDMI port. Connect the rear TMS port to the computer *via* a DB-9 cable.
2. Connect the EMG system to the computer *via* a PCI-6220 card DAQ. Connect the photodiode to the EMG system *via* a BNC cable.

Photodiode Sensor

4. Attach a photodiode sensor to the BNC cable. Secure the photodiode sensor with tape to the top-right corner of the task monitor, with the sensor facing the screen, ≤ 1 cm away.

Note: This will record the timing of stimuli presented on the task monitor as analog data in an independent input channel.

Software

1. Download VETA Toolbox (https://github.com/greenhouselab/Veta) for Matlab 2018 to interface with the hardware for data collection.
2. Download the reaching task code (https://github.com/greenhouselab/Reach_TMS) developed for the control of experimental parameters and interfacing with the tablet.

Participant Screening and Informed Consent

1. Screen the subject for contraindications to TMS. Exclusion criteria include a personal or family history of seizure, headache, brain trauma, fainting, chronic stress or anxiety, problems with sleep, and any neuroactive medication. Additional exclusion criteria include any metal implants in the brain or skull and any recreational drug or alcohol use in the 24 h prior to testing. Inclusion criteria included right-handedness and age between 18 and 35 years.

2. Provide a written explanation of the procedure and associated risks, clarifying any further questions the participant may have.
3. Obtain informed consent from participants.

Subject Setup

1. Position the subject in a comfortable chair facing the tablet. Ensure that the knees are flexed to 90° with the legs under the desk.
2. Prepare the skin and place EMG electrodes.
    1. Use fine-grain sandpaper to gently abrade the skin at the site of the right first dorsal interossei (FDI), extensor carpi radialis, and anterior deltoid muscles, as well as the C4 prominence at the base of the neck, to detect electrical artifacts produced by the TMS pulse. Note: Muscle recording sites can be adapted based on user needs.

2. Swab each abraded area with an alcohol prep pad once per electrode site to clean.
3. Place one EMG electrode on each site. Ensure that the electrodes run perpendicular to the muscle fibers. Place the ground electrode on the bony prominence of the right elbow.
4. Secure each electrode with medical tape.

3. Check the quality of the EMG recording. Use the VETA toolbox to visualize all EMG traces and ensure they are free of artifacts. If EMG traces are noisy, ensure the ground is properly placed and that all electrodes make proper contact with the skin.

Transcranial Magnetic Stimulation

1. Turn on the TMS machine.

2. Find the TMS hot spot of the right FDI muscle *via* stimulation of the left M1.

    1. Place the coil ~5 cm lateral and 2 cm anterior to the vertex of the head, oriented ~45° off the midline.
    2. Administer TMS pulses once every 4 s while repositioning the coil in increments of approximately 5 mm in the anterior-posterior and medial-lateral plane.
    3. Beginning at 30% maximum stimulator output, gradually increase the TMS intensity by 2% increments until MEPs are observed.
    4. Once the optimum location is identified, at which MEPs can be reliably elicited on the majority (~75%) of pulses at the lowest possible stimulator intensity, determine the resting motor threshold (RMT) by finding the intensity level that produces MEPs with a peak-to-peak amplitude of >50 μV on five out of 10 pulses.
    5. Mark the position by gently placing thin strips of reflective tape on the participant's head along the perimeter of the coil. Maintain coil positioning either by manually holding the coil or using a stand to support it.

Reaching Task Setup

1. Put a Velcro glove on the right hand of the participant to facilitate a relaxed power grip posture.
2. Attach the stylus to the glove and advise the subject to keep the hand relaxed between reaching movements.
3. Communicate the task instructions, which are as follows: Guide the cursor to the home position on the bottom of the screen. You will see a cue at one of two target locations. When the target fills in with color, reach through the target as fast and as accurately as possible. Then return to the home position. Indicate locations of home positions, cues, and targets (**Fig 3.1A**).
4. Coach the participant to slice through targets with the stylus as quickly and as accurately as possible. Turn off the lights in the task room to obscure the participant's vision of arm movements and improve the visibility of the task monitor.

Task Design

1. Control visual stimulus presentation with Psychtoolbox 3.0 in Matlab 2018.
2. Use the following parameters to match the current data: 20 practice trials; 270 test trials; TMS on 4/5 of test trials; TMS either coincides with the preparatory cue onset (baseline TMS) or 100 ms before the imperative cue (delay TMS) with equal frequency; 1/10 of total trials are catch trials, in which the imperative cue does not appear; the home position is a circle with a 2 cm radius positioned in the bottom center of the workspace; two circular targets with 1 cm radius are positioned 15 cm from the home position at +45° and -45° away from the midline.
3. Set the event order and durations as follows: preparatory cue at 900 ms and imperative cue at 900 ms.

TMS Administration

1. The VETA toolbox concurrently administers TMS and records EMG https://github.com/greenhouselab/Veta.
2. Control the timing of the TMS pulses with the VETA toolbox to coincide with the chosen behavioral events (i.e., the onset of the preparatory cue or 100 ms preceding target onset).

3. Deliver TMS with sufficient frequency to ensure a sufficient number of MEPs for analysis. Note: As written, the task code will deliver a TMS pulse on 4/5 of total trials either at the onset of the preparatory cue-to elicit baseline MEPs-or 100 ms before the imperative cue-to elicit delayed MEPs. Parameters can be adjusted in the code according to user needs. Trials without TMS can be used to evaluate behavioral performance in the absence of TMS. This is useful for determining any possible influence of TMS on performance.

Representative Results

Successful execution of the described methods includes the recording of tablet data, EMG traces, and reliable elicitation of MEPs. An experiment was completed that included 270 test trials with TMS delivered on 4/5 of the trials (216 trials).

Data were collected from 16 participants (eight females; eight males) aged 25 ± 10 years, all of whom self-reported as right-handed. We assessed the effectiveness of the visual perturbation on behavioral performance by deriving a learning function for one representative participant. These data are presented in **Figure 3.1B** and show that the participant's hand target error adjusted to the perturbation and washout conditions as expected. We also evaluated the standard deviation of the target error during baseline reaches, which approximated 4.5° (**Fig 3.1B**). This is consistent with previous studies[24].

One TMS pulse was delivered on each trial. Half of the pulses were delivered at baseline, and half were delivered during a preparatory delay period (**Fig 3.2A**). An average of 91 ± 23 baseline and 88 ± 20 delay MEPs were successfully recorded per participant, corresponding to 84% and 81% success rates, respectively. MEPs were counted only when amplitudes exceeded .05 mV. Reach trajectories were successfully captured from the graphics tablet on all trials, excluding catch trials (i.e., trials in which the "go" cue was not presented and trials in which participants either failed to initiate a reach or initiated before the imperative cue).

The average delay period (duration between the preparatory and imperative cue) was 915 ± 0.5 ms (mean ± standard deviation). Baseline TMS was administered 26 ± 8 ms after preparatory cue onset, and delay TMS was 126 ± 3 ms prior to imperative cue onset (**Fig 3.2B**).

The consistent deviation from the intended TMS administration time in each case indicates that further optimization is needed to account for undesired latencies introduced by hardware or software components. However, the relatively low proportional variance in these latencies suggests these are mostly fixed delays that can be controlled with additional pilot testing and indicate that the timing of events is generally reliable across trials.

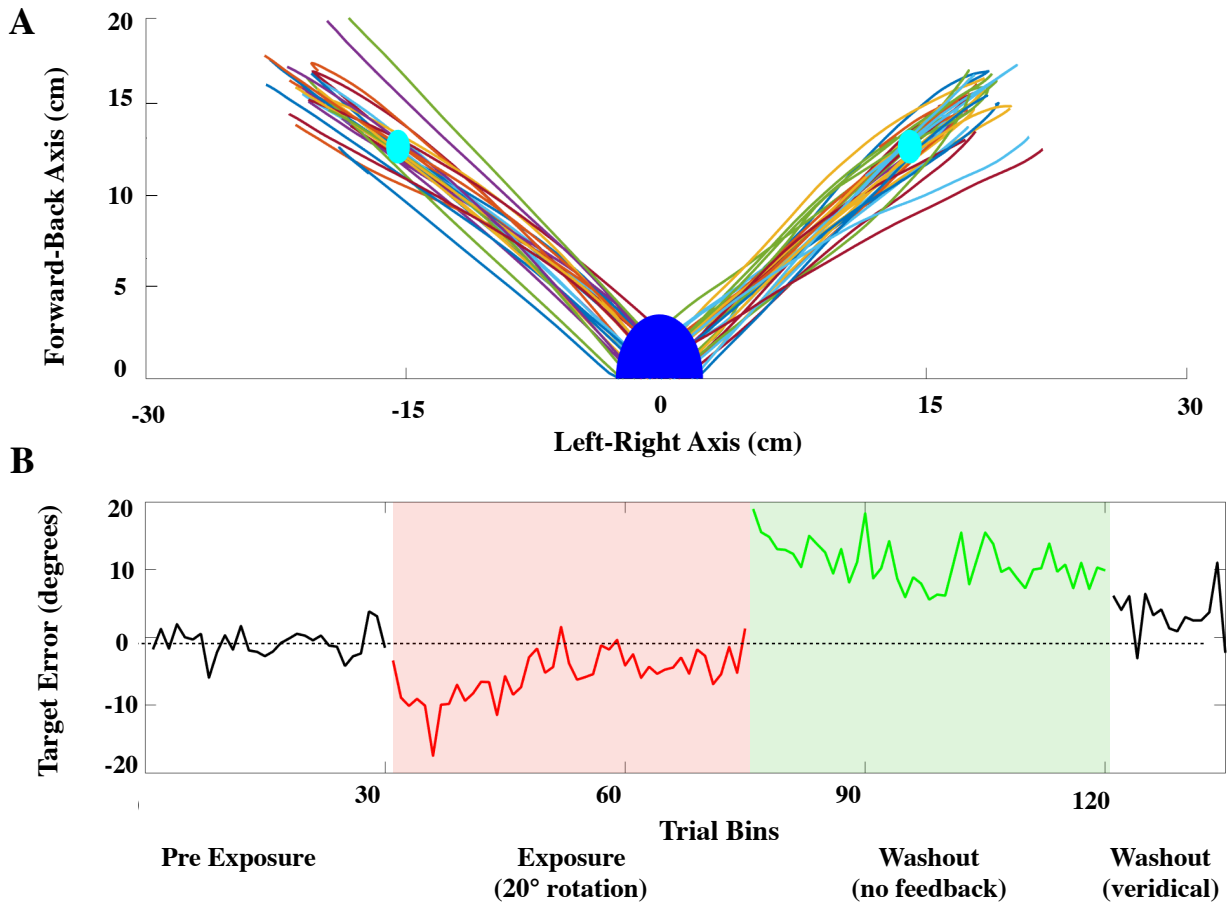

Figure 3.1 Behavioral data collected from the tablet. (**A**) The workspace includes the home position (dark blue), two targets (cyan), and a representative set of reach trajectories from the pre-exposure block of a single participant. (**B**) Target error was calculated as the distance in degrees from the endpoint of the reach to the center of the target. Trial bins are the mean of two consecutive trials per bin, and the data are separated by experimental blocks: Pre-exposure (unshaded), exposure (red), washout in the absence of feedback (green), and washout with veridical feedback (unshaded).

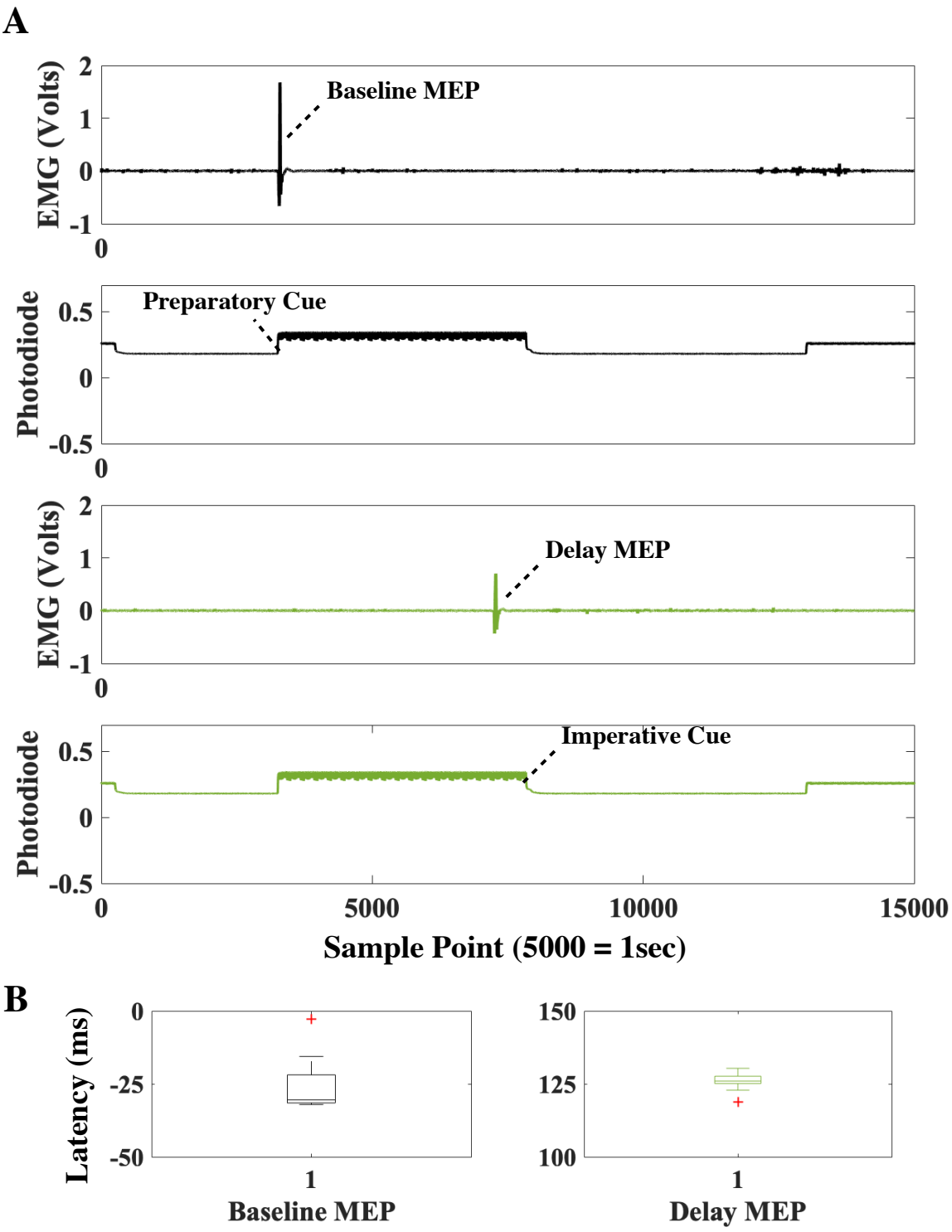

Figure 3.2 Example MEP traces. (**A**) Representative MEPs and corresponding photodiode trace for both experimental epochs (baseline and delay). (**B**) Negative baseline MEP latency (-26 ± 8 ms) indicates that the TMS stimulus arrived after the preparatory cue, while positive delay MEP latency (126 ± 3 ms) indicates that the TMS stimulus arrived before the desired time point (100 ms prior to the imperative cue). Latencies are averaged across all participants (n = 16).

Discussion

The methods outlined above offer a novel approach to studying motor preparation in the context of reaching behaviors. Although reaching represents a popular model task in the study of motor control and learning, there is a need for precisely evaluating the CS dynamics associated with reaching behavior. TMS offers a noninvasive, temporally precise method of capturing CS activity at discrete time points during reaching. The approach described here combines two independent subfields-TMS and reaching-into a single paradigm that involves the simultaneous recording of kinematic and electrophysiological metrics.

While the methods described have the potential to reveal important insights into action control in the context of reaching, there are certain limitations and considerations. Most importantly, the reliability of MEP measurements depends on the stability of the EMG activity prior to TMS administration, as well as the number of MEPs captured[27]. It is critical that EMG data quality be assessed prior to data collection. For sufficient statistical power, a minimum of 20 MEP measurements per task condition are recommended. Additionally, while changes in the MEP represent a quantitative change in CS excitability, the nature of TMS and the resultant MEP produce a rather crude, summary metric of CS activity, and their causal relationship to behavior should be interpreted with caution[15]. Furthermore, the graphics tablet requires that the stylus maintain contact with the tablet surface, which limits the range of reaching tasks and grip apertures that can be employed.

Despite the limitations of this specific protocol, the combination of TMS and EMG for indexing motor system excitability during behavioral tasks other than reaching is well established[15]. Advantages of this combined approach include the ability to measure CS excitability dynamics even in the absence of overt movement, as well as in task- irrelevant muscles. This approach also offers high temporal precision, on the order of milliseconds. Additionally, the protocol described here can be adapted to work with any number of EMG devices that interface directly with a stimulus presentation computer *via* the listed input/output devices.

Given these advantages, the protocol can help bridge the gap between human and animal studies. A large body of research in non-human primates has examined the electrophysiological mechanisms associated with reaching and motor learning in the context of reaching. Further investigations in humans using the combined TMS and EMG approach can help to bridge non-human electrophysiology and human behavioral findings. Previous studies of MEPs in the context of reaching have shown a facilitatory effect of TMS during reach and grasp preparation when the parietal cortex, premotor cortex, and parietal-M1 circuits were stimulated prior to movement[8,14]. However, the amplitudes of resting evoked potentials measured with electroencephalography 75 to 150 ms after TMS over the M1 were reduced following force field adapatation[13]. The nuanced relationship between reaching preparation, adaptation, and changes in CS warrants further investigation. Moreover, by using the same set of tools and methods across laboratories, replication will be more achievable, and this will aid the interpretability of study results.

While the focus here is on TMS of the M1, several studies have utilized dual-site TMS to investigate interactions between cortical areas (e.g., parietal cortex and M1). While many of these studies were conducted during rest, a handful of studies examined cortico-cortical interactions in the context of reach planning and execution. Dual-site TMS showed stimulation of the posterior parietal cortex facilitated M1 excitability at 50 ms and ~100 ms following an auditory "go" cue to initiate a prepared contralateral reach[28]. Additional methods have been established for dual coil TMS approaches that include applications during goal-directed reach-to-grasp behaviors[29]. The protocol described here complements these previous studies and methods and can be readily adapted for dual-site TMS studies as well.

The example task code consists of a delayed response task with two potential targets. Parameters such as trial numbers, target and cursor characteristics, visual feedback, and TMS delivery can be adjusted to address a variety of research questions. Data recorded with this approach include behavioral kinematics from the tablet and electrophysiological measurements from the EMG. Preliminary results revealed that TMS and behavioral measurements exhibit reliable timing and sufficient sensitivity to variability in reach directions across trials. These

methods and results stand as proof of concept for future investigations into the neural mechanisms of reaching *via* TMS using this approach.

Disclosures

All authors declare that there are no conflicts of interest

Acknowledgments

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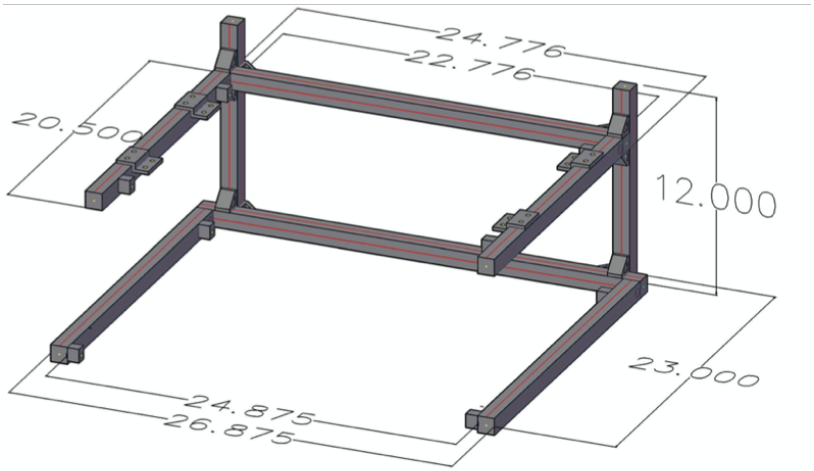

Supplementary Figure 3.3

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Bridge

The methods described in the previous chapter represent a flexible framework that can be used for further explorations into the relationships between corticospinal activity and reaching. The following chapter describes the first experimental application of said methods, and our first investigation into preparatory activity during 2-dimensional reaching. We probe the corticospinal (CS) pathway using transcranial magnetic stimulation during the preparation of reaches. We also test the potential influence of adaptation on the CS pathway. By doing so, we hoped to extend previous observations of preparatory inhibition to reaching behaviors, and to further explore the potential sources of this phenomenon.

CHAPTER IV
INVESTIGATION OF PREPARATORY INHIBITION DURING REACHING

Introduction

Movement preparation and the underlying neural mechanisms involved have been widely studied using a variety of approaches. Transcranial Magnetic Stimulation (TMS) has been a popular choice for non-invasively probing motor circuitry under particular task conditions. Several TMS studies have observed preparatory inhibition[1-4] during a delayed-response task. This phenomenon is thought to be involved in gain-modulation, suppressing the noise in the motor system so that the appropriate response can be selected and executed.

Most of this work has employed simple, ballistic finger or wrist movements due to the low degrees of freedom such movement allows, as well as the convenient location of the hand area in primary motor cortex (M1). While constraining responses to highly controlled movements has its advantages, it fails to capture the richness and complexity of natural human behavior. We argue that goal-directed reaching is an optimal - and novel - direction for TMS-related investigations into the mechanisms of action preparation.

Previous TMS studies involving simple manual behavior have examined CS excitability in the context of motor adaptation and learning. This work suggests that CS excitability increases following visuomotor adaptation for finger[5,6] and wrist movements[7,8]. Other studies have found an association between the degree of learning and increased CS excitability[9-12]. In addition, more complex movements, such as reaching, have also been employed to investigate the relationship between learning and CS excitability. For instance, Xivry et al.[13] found that MEPs increased in amplitude following a bout of an abrupt force-field perturbation but not a gradual one. Further studies suggest the learning process may affect CS correlates, such as the MEP and the CSP, in different ways. For example, Sarwary et al.[12] showed that changes in the MEP were associated with a fast adaptation process while changes in the CSP were associated with a slow learning process. While the evidence here is clear that motor adaptation is associated with greater CS excitability, these investigations involve muscles at rest, between or after bouts of learning. The association between motor adaptation and CS excitability in the preparatory state has not yet been established. Although, in general, these studies show an increase in CS excitability during

and after adaptation, the question of whether CS excitability dynamically adjusts during the preparation of more complex movements in the context of gradual motor adaptive processes remains unanswered.

We assessed CS excitability during a two-dimensional reaching task in which participants acquired one of two targets in a virtual workspace. We also tested the influence of sensorimotor adaptation on corticospinal excitability by gradually introducing a perturbation of 20 degrees. While adaptation is known to play a role in motor learning and control, whether this implicit process influences the CS pathway during preparation is unknown. Our novel approach allows us to explore the relationships between learning processes and CS activity, potentially revealing electrophysiological markers of implicit motor learning.

Single-pulse TMS was administered to the left M1 on a trial-by-trial basis either during a resting inter-trial baseline or a preparatory delay period. MEP amplitudes were measured using EMG placed on the right FDI. We expected to observe preparatory inhibition, measured as a reduction in MEP amplitude from baseline to delay epochs. This would be consistent with previous work that studied finger and wrist movements, as well as extend the phenomenon of preparatory inhibition to include reaching behaviors.

Furthermore, we hypothesized motor adaptation during exposure to a visuomotor perturbation would be associated with less inhibitory influence from the cerebellum on M1 during, corresponding to greater MEP amplitude. This hypothesis is based on previous work that found reductions in cerebellar-brain inhibition (CBI) associated with implicit learning[14-16]. Because this cerebellar-mediated effect seems to influence the CS pathway generally over longer timescales, we expected both inter-trial baseline MEPs and delay period MEPs to be similarly modulated. Thus, we expected no difference between trial epochs, i.e. baseline and delay, but rather an overall increase in MEP amplitude during the blocks of the task in which behavioral adaptation was evident.

Methods

Participants

A total of 16 (8 females, 8 males, mean age 26 ± 10 yrs) completed testing. Participants were recruited from the University of Oregon through the Human Subjects Pool and online flyers through the Human Physiology department. A pre-screening process excluded individuals with contraindications to TMS and those who are not right-handed. Exclusion criteria included any history of neurological disorder, use of psychoactive medication, and recent drug use. All participants provided written informed consent before the start of testing. All procedures were approved by the University of Oregon, IRB.

Electromyography & Transcranial Magnetic Stimulation

Surface EMG was measured from three muscles of the right arm: first dorsal interossei (FDI), extensor carpi radialis (ECR), anterior deltoid (AD). A ground electrode was placed on the epicondyle of the elbow, and an additional electrode was placed on the dorsal aspect of the neck to detect electrical artifacts for marking TMS pulse times. After the completion of EMG setup, a standard TMS hotspotting and thresholding procedure was performed. First, the coil was positioned approximately 2 cm anterior and 5 cm lateral to the vertex, over the hemisphere contralateral to the target muscle, and with the coil oriented approximately 45 degrees off the midline to induce a current in the posterior to anterior direction. Second, the TMS intensity was adjusted, and the coil repositioned in incremental adjustments of ~1 cm until consistent MEPs were elicited from the target muscle. During this hotspotting procedure, TMS pulses were administered once every 4 s. Third, once the optimal coil position and orientation was determined, athletic tape was placed on the head to mark the location of the coil. Finally, the resting motor threshold (RMT) was determined as the intensity of TMS which elicits MEPs with amplitudes of at least 50 μV on 5 out of 10 attempts. During subsequent testing, TMS was administered at 115% RMT. 20 baseline MEP measurements were taken prior to the start of the task. EMG recording and the timed administration of TMS pulses were controlled by the VETA toolbox (Jackson & Greenhouse, 2019) in Matlab.

Virtual Workspace

The virtual workspace (Fig 4.1) includes a home position (4cm-diam) in the bottom-center, two circular targets (1cm-diam), and a cursor (0.5cm-diam) to represent the current position of the stylus. The two targets were presented 15cm in euclidean distance away from the center of the Home Position at -45 and +45 degrees off the midline. Only one target position was displayed per trial. Visual stimulus presentation of the targets and cursor were controlled by Psychtoolbox 3.0 for Matlab 2018.

Reaching Task

Each trial consisted of a resting phase, preparatory phase, and an out-and-back reaching phase (Fig 4.1). On each trial, participants had to maintain the cursor within the Home Position for a period of .5-1.5s (random, uniform distribution). Then, the outline of the forthcoming target would appear, representing the preparatory cue. After a delay of 900ms the target would fill-in, representing the imperative signal to initiate a reach. Participants were instructed to 'slice' through the target as quickly and accurately as possible. Once the euclidean distance of 15cm was surpassed, the target would disappear, prompting a return to Home Position. If the cursor did not travel the 15cm distance within the allotted time of 1sec, participants would receive a 'too slow' message to encourage high speed. Cursor feedback was altered, contingent on the task condition, across blocks.

The task consisted of four blocks for a total of 290 trials. The first was a Practice block of 20 trials to familiarize participants with the virtual work space and the desired reaching distance and speed. During this block, the cursor feedback was veridical and always visible to the participant. The Practice block was followed by Pre-Exposure, Exposure, Washout, and Post-Exposure blocks, respectively.

The Pre-Exposure block included 60 trials of out-and-back reaches with veridical feedback. Once the reach distance threshold was passed, the cursor disappeared and would not be visible to the participant until the cursor returned within 3 cm from the home position. In the Exposure block (90 trials), the cursor feedback displayed a gradual rotational shift (direction counterbalanced across participants) of 2 degrees per trial to a maximum of 20 degrees for the remainder of the block. This stepwise introduction was to prevent participant awareness of the

rotation. Aside from the rotation, the cursor visibility matched the Pre-Exposure block. In the Washout-No-Feedback block (60 trials), the cursor would disappear at the onset of the imperative signal, thus concealing visual feedback of the cursor during the reach phase for the entirety of the block. In the Washout-Feedback block (60 trials), the cursor returned and cursor position was veridical, matching the Pre-Exposure block.

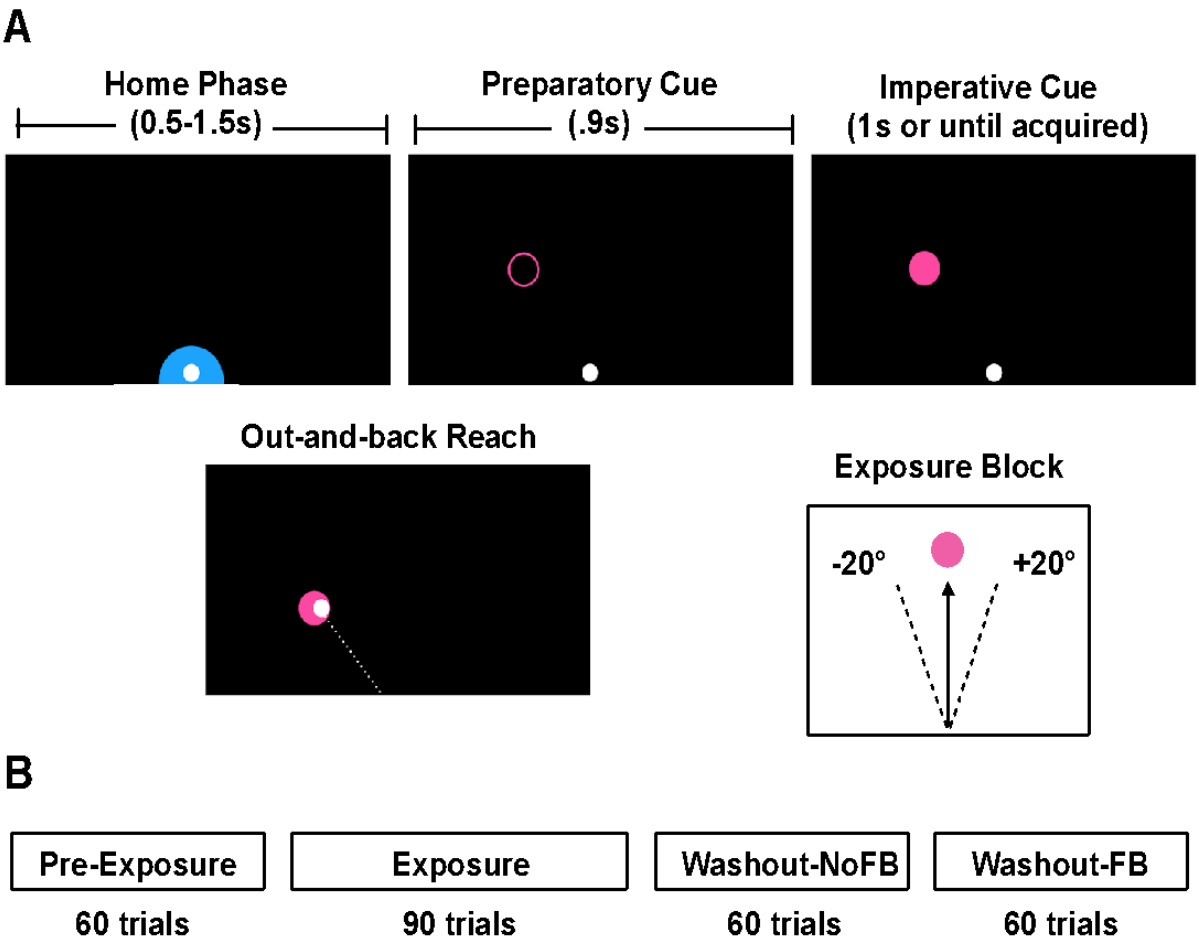

Figure 4.1 (A) Representation of the virtual workspace during a left-target trial. Home position (blue) was centered on the bottom of the screen. Visual targets (magenta) were fixed 45° off the midline to the left (shown here) and right, on a trial-by-trial basis. The cursor (white) provided live-feedback of the position of the stylus. (B) Progression of the task through four continuous blocks which differed only in the visual feedback of the cursor. Pre-Exposure and Washout-FB represented veridical positioning of the cursor, while Exposure introduced a 20° rotation of the cursor position and Washout-NoFB hid the cursor position during the out-and-back reach phase.

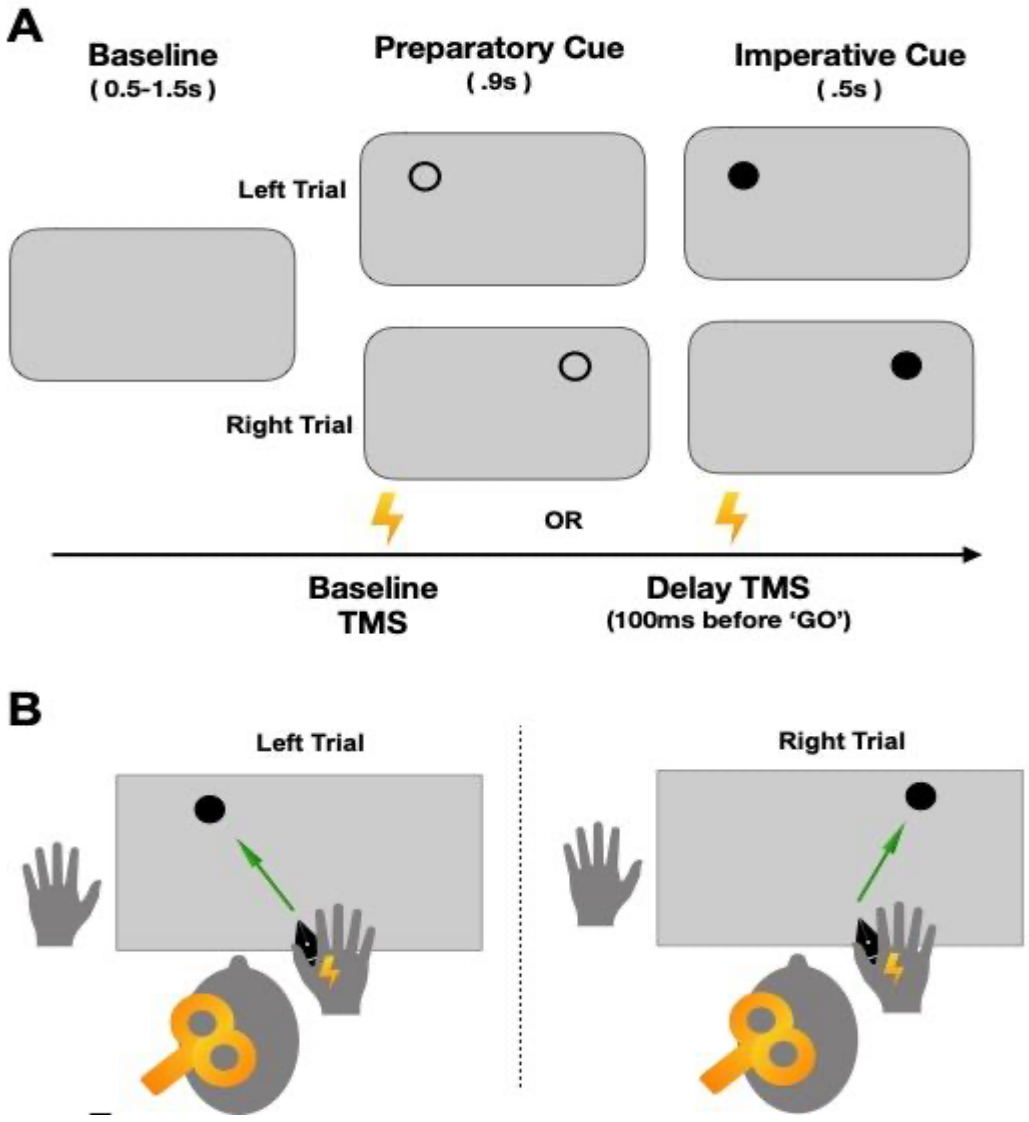

Figure 4.2 Diagram of the timeline of TMS administration relative to the task. (A) Single-pulse TMS was delivered either at the onset of the preparatory cue (baseline TMS) or 100ms prior to the imperative cue (delay TMS). (B) TMS over left primary motor cortex elicited MEPs in the right FDI.

Dependent Measures & Analysis

During the task, TMS was administered (Fig 4.2) either at the onset of the preparatory cue (baseline) or 100ms before the onset of the imperative cue (delay). The proportion of TMS measurements across trials within blocks was as follows: ⅖ baseline; ⅖ delay; ⅕ none. Trials without TMS were used to assess behavioral performance in the absence of TMS. Within each block, MEP amplitudes for each timepoint (baseline vs. delay) were directly compared to assess

whether preparatory inhibition was present, then compared across blocks to determine whether the preparatory state of the output motor pathway changed with task conditions, e.g. Exposure to a visuomotor rotation influences the degree of MEP modulation. MEPs that did not meet a threshold of .05mV were not included for analysis. The average number of MEPs measured from each participant were 92 ± 23 at baseline and 91 ± 19 at delay.

Results

Reaching Accuracy

Reaching trajectories were analyzed on a trial-by-trial basis to calculate the euclidean distance between the center of the target and the end-point of the reach trajectory (target error), then converted into degrees for comparability. The average target error for the Pre-Exposure and Washout-Feedback blocks were 0.45 ± .8 degrees and 2.0 ± 6 degrees, respectively, indicating accurate acquisition of targets when visual feedback was veridical. Given the nature of the visual feedback in the Exposure and Washout-no-Feedback blocks, we grouped sequential trials into bins of two trials and report the average target error for each bin (*Fig 4.3*).

To confirm successful adaptation, we grouped trials within the Exposure block into bins of 5 trials each. A repeated measures ANOVA confirmed a significant effect across bins ($F(1,18) = 5.2$, $p < .001$), and no effect of the direction of rotation (positive or negative) ($p = .5$). As expected, target error increased sharply at the beginning of the Exposure block due to the onset of the 20 degree rotation. Adaptation can be observed as target error was reduced towards baseline as the Exposure block proceeded. A subsequent spike in error was brought on by the Washout-No-FB block, in which the rotation was removed and participants reached in the dark, without visual feedback of the stylus position. A return to baseline was then brought on by the Washout-FB block, in which participants regained visual feedback of the stylus position. This observed evolution of target error is consistent with previous work on visuomotor perturbations.

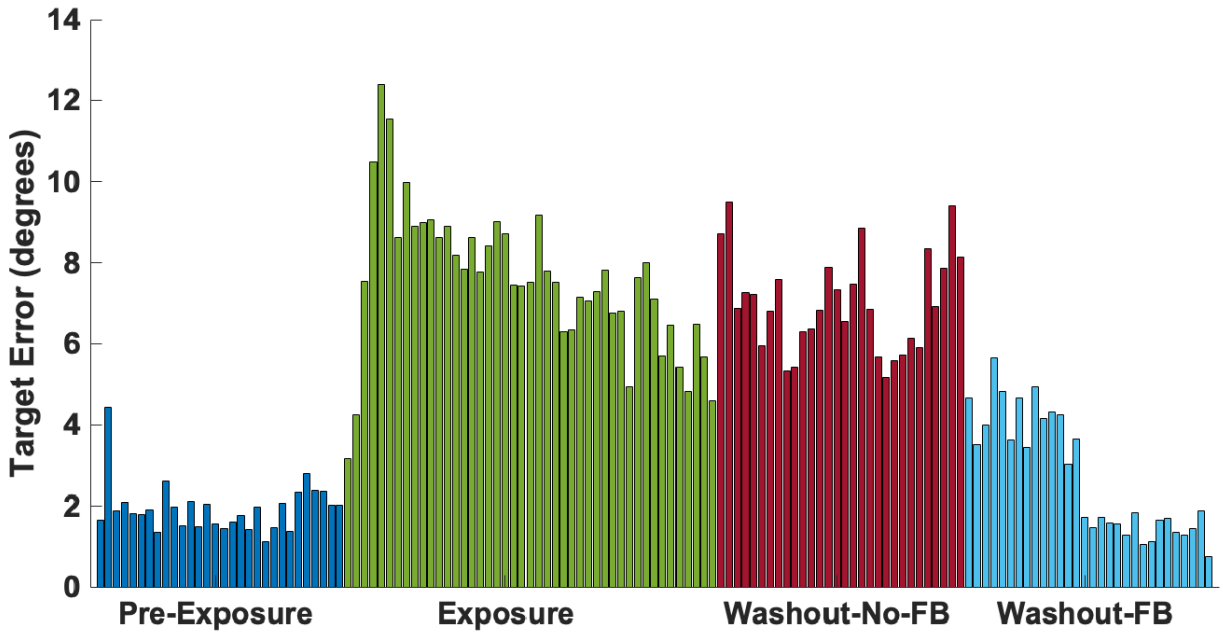

Figure 4.3 Target error was measured as the absolute distance between the center of the target and the cursor position once the target distance threshold (15cm) was surpassed. Here we report target error in averaged bins of two consecutive trials for each block.

EMG Reaction Time

Reaction time was calculated as the duration between the imperative cue and the EMG onset of the deltoid muscle. A one-way ANOVA revealed that TMS had a significant effect on Reaction Time ($F(2,45) = 7.38$, $p = .002$). Responses were slowest on trials without TMS (278 ± 51ms), and faster on trials with baseline ($t(15) = 5.07$, $p < .001$) and delay ($t(15) = 3.04$, $p = .008$) TMS. Responses were faster ($t(15) = 2.39$, $p = .03$) when TMS was delivered at baseline (226 ± 40ms) compared to delay (244 ± 30ms), indicating an effect of TMS as well as the timing of administration (Fig. 4.4). Reaction times did not differ significantly across the experimental blocks ($p = .3$), indicating consistent behavior throughout the task.

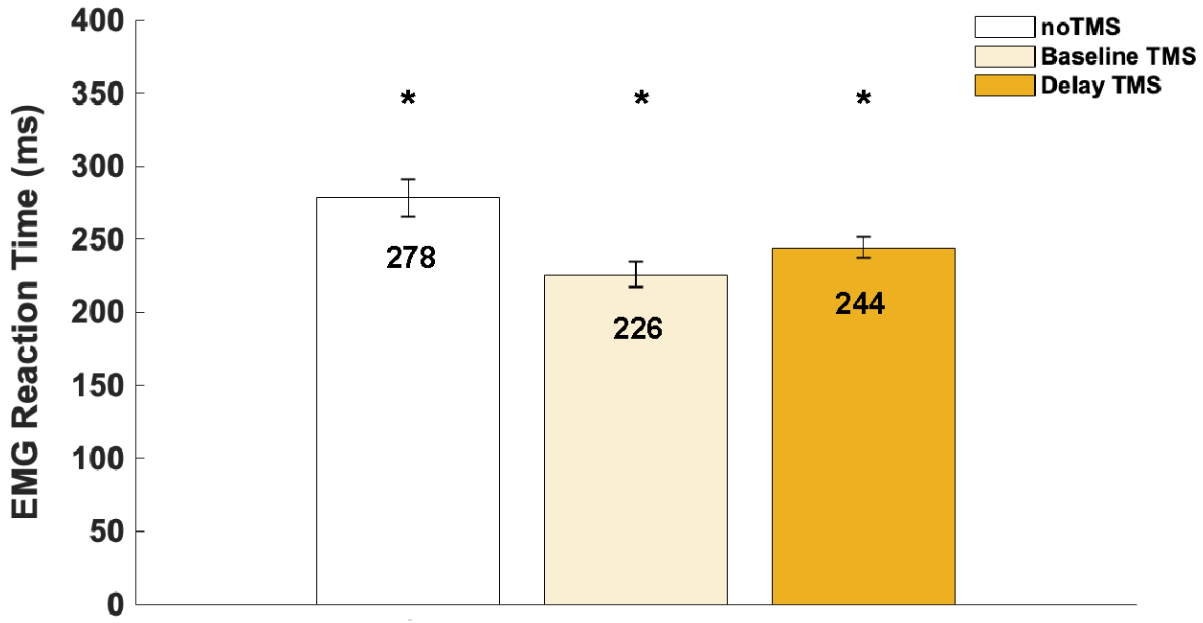

Figure 4.4 EMG Reaction Times as measured by the onset of the anterior Deltoid muscle relative to the imperative cue. Trials that included baseline TMS (226 ± 40ms) were associated with the fastest responses, followed by trials with delay TMS (244 ± 30ms), and then trials without TMS (278 ± 51ms). *All comparisons were significant ($p < .05$).

FDI MEP Amplitude

MEP amplitudes were recorded from the task-relevant FDI muscle at two different epochs (baseline and delay) and averaged across experimental blocks (*Fig 4.5*). A repeated measures ANOVA revealed no effect of TMS epoch ($F(1,15) = 1.9$, $p = .2$) or Task Block ($F(1,15 = .63$, $p = .6$). Contrary to our predictions, our results do not support the presence of preparatory inhibition during reaching.

Baseline MEP amplitudes were stable across Pre-Exposure (1.0 ± 0.76mV), Exposure (.91 ± .68mV), Washout-No-Feedback (.95 ± .84mV), and Washout-Feedback (.95 ± .72mV) blocks. This consistent baseline suggests there was no general shift in motor system excitability related to fatigue or time in task. Similarly, delay period MEP amplitudes did not change across Pre-Exposure (1.0 ± .64mV), Exposure (0.94 ± .74mV), Washout-No-Feedback (0.88 ± .8mV), and Washout-Feedback (0.87 ± .72mV) blocks.

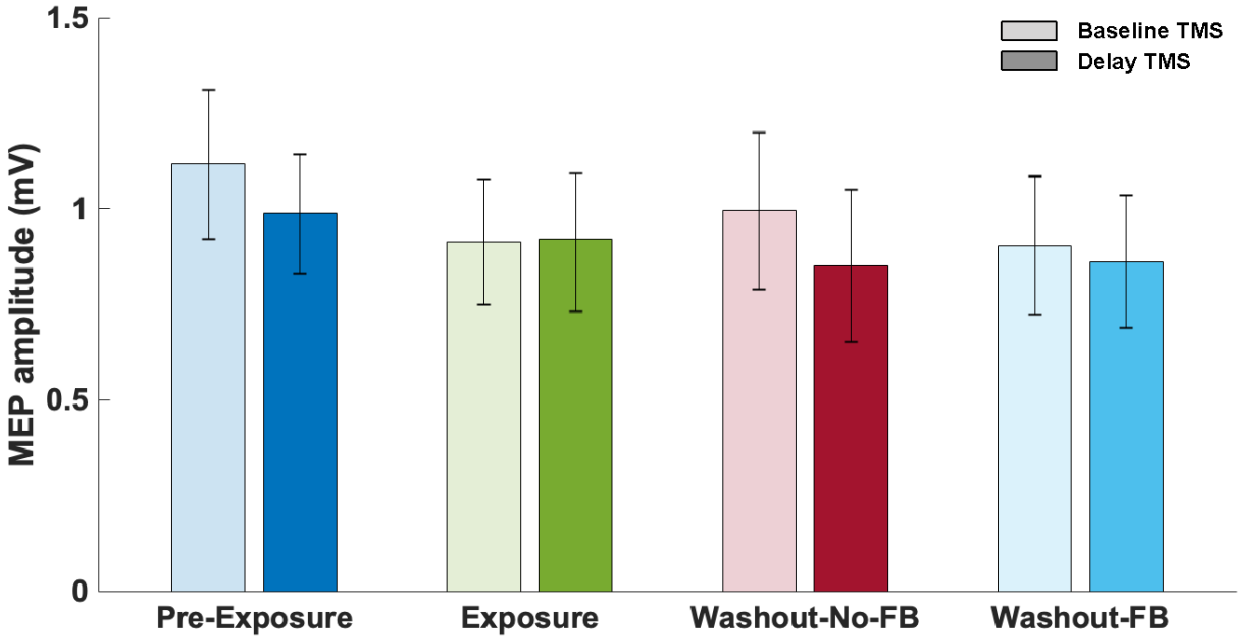

Figure 4.5 Mean MEP amplitudes elicited at baseline and delay epochs are shown for Pre-Exposure (1.0 ± 0.76mV; 1.0 ± .64mV), Exposure (.91 ± .68mV; 0.94 ± .74mV), Washout-NoFB (.95 ± .84mV; 0.88 ± .8mV) and Washout-FB (.95 ± .72mV; 0.87 ± .72mV) blocks. No significant effect of TMS epoch (p = .2) or experimental block (p = .6) was observed.

Correlations

MEP amplitudes scale with background EMG activity. To ensure the lack of differences between baseline and preparatory delay period MEP amplitudes was not simply explained by background EMG activity, we assessed pre-MEP EMG activity from the FDI in the 100ms window preceding TMS administration. We calculated the root mean square of the EMG activity preceding TMS administration and correlated this metric with MEP amplitude (Fig 4.6). Pre-TMS RMS was significantly correlated with baseline MEP amplitude (r = .65, p = .007) and delay MEP amplitude (r = .58, p = .02). When experimental blocks were analyzed separately, Exposure (baseline: r = .61, p = .01; delay: r = .64, p = .008), Washout-No-Feedback (baseline: r =.78, p < .01; delay: r = .71, p < .01), and Washout-Feedback (baseline: r = .58, p = .02; delay: r = .3, p = .2) blocks exhibited a relationship between Pre-TMS EMG activity and MEP amplitude, with larger MEP amplitudes associated with greater EMG activity. This relationship was not observed in the Pre-Exposure block (baseline: r = .31, p = .24; delay: r = .31, p = .25).

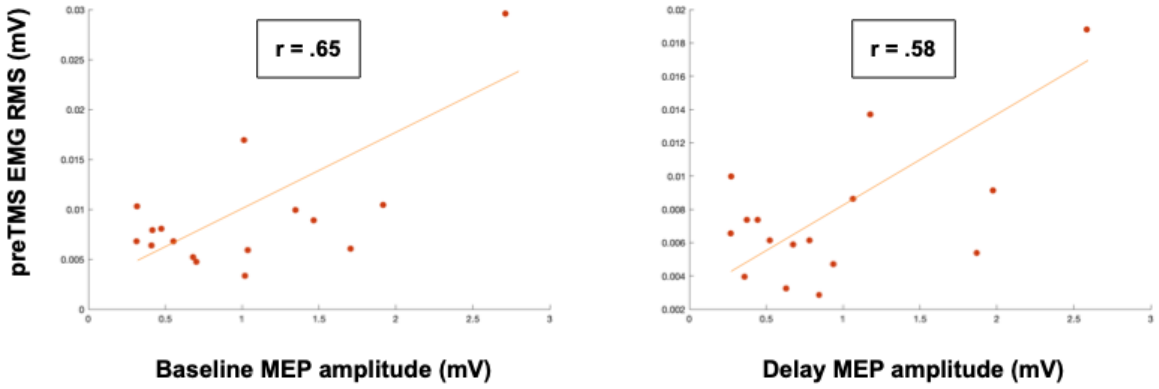

Figure 4.6 Root mean square of the FDI muscle activity in the 100ms window prior to TMS delivery was positively correlated with the elicited MEP for both baseline (r = .65, p = .007) and delay (r = .58, p = .02) epochs.

While the association between Pre-TMS EMG and MEP amplitude is not surprising in and of itself, the fact that it coincides only with phases of the experiment that involved some level of adaptation is interesting. Perhaps the cerebellar activity during implicit learning modulates cortical activity in a way that strengthens the association between corticospinal excitability and surface-level EMG activity. Nonetheless, since both baseline and delay MEPs were correlated with background EMG activity to a similar extent, we do not suspect that this relationship impacted our MEP amplitude comparisons.

Discussion

Our results did not support our predictions, as MEP amplitudes were similar across TMS epochs and experimental blocks. Specifically, we did not observe differences in MEP amplitudes between inter-trial rest and preparatory delay periods and did not observe an influence of exposure to visuomotor perturbation. These results fail to extend previous evidence of preparatory inhibition in finger and wrist movements to reaching behaviors. These studies have shown relatively consistent patterns of decreased MEP amplitudes[1-4,17-21] during response preparation relative to inter-trial baseline measurements. There are several possible reasons why preparatory inhibition may not have been observed in this experiment.

Lack of Preparatory Inhibition during Delayed Reaching

Some argue that the observation of preparatory inhibition depends on task-related variables. Quoilin et al.[22] showed that MEP suppression in task-relevant effectors depended on the nature of task feedback. This is critical to the interpretation of our data since the nature of the feedback was the primary independent variable of interest. Notably, we did not observe preparatory inhibition during the exposure or no-exposure blocks of the task, suggesting the presence or absence of visual feedback is not a critical factor. Additionally, Quoilin et al.[21] found more reliable MEP suppression when the task involved a choice between two hands compared to when only one response was required. While our task involved a choice between two targets, the same agonists were responsible for both response options - albeit with variation. However, other work has shown that preparatory inhibition is present in the primary effector even when there is no choice involved in a task[4]. Additional experiments are needed to determine whether feedback-related or response selection-related elements of the experimental design might unveil preparatory inhibition.

Another possible explanation for the negative result is the complexity of the reaching movement. Perhaps the increased complexity, compared to finger abduction, introduces more noise into the motor system, and thus any modulation in the CS pathway is washed out by a general shift in excitability throughout the task. However, previous work examining the influence of complexity on preparatory inhibition showed greater preparatory inhibition was associated with the preparation of more complex manual responses[4] requiring coordination of multiple effectors. Nevertheless, increased noise associated with reaching might explain our high degree of variability in MEP amplitudes and a lack of any prevailing trend between the two TMS timepoints. Future analyses can account for this variability as a factor and evaluate whether individuals with decreased MEP amplitude variability also exhibit a pattern more consistent with preparatory inhibition.

Corticospinal Excitability during Visuomotor Adaptation and Cerebellar Influence

Previous studies have examined the effect of visuomotor adaptation on corticospinal (CS) excitability. This literature has shown that CS excitability is enhanced following visuomotor adaptation involving finger movements[5] and wrist movements[8]. Similar investigations that involve motor learning without adaptation have also found an association between the degree of

learning and increased CS excitability[9-12]. Surprisingly, the facilitation in MEP size is even evident after mere observation of motor learning[23].

The cerebellum is strongly implicated in mediating visuomotor adaptation[24] for reaching in particular[25,26]. Evidence suggests cerebellar-thalamocortical projections reduce M1 activity[27,28]. Conditioning pulses of TMS over the cerebellum reduce MEP amplitudes elicited from M1, a phenomenon referred to as cerebellar inhibition[29]. However, after adaptation in a reaching task, this cerebellar-mediated inhibition is reduced[14,15]. This effect has been shown in the primary agonist of a trained behavior as well as a task-irrelevant muscle[16].

Here, consistent with previous findings described above, we predicted a release of preparatory inhibition and greater MEP amplitudes overall during the Exposure task block. Our results do not support this prediction, as MEPs of the Exposure block were similar to those of other blocks. While previous work has exhibited changes in CS excitability due to adaptation, in these studies TMS was delivered outside of the task context while participants were at rest, whereas the current study probed CS excitability inside the context of the task on a trial-by-trial basis.

Although we did observe behavioral evidence for adaptation, we did not observe an influence of perturbation exposure on CS excitability at baseline or during the preparatory delay periods of our task. This suggests online changes are too subtle to detect with MEPs or that MEP measurements taken during epochs of the task may have been too noisy to provide sufficient sensitivity to effects reported in previous studies.

TMS Effects on Reach-related EMG Onsets

One surprising result was the effect of TMS on EMG reaction time. While the effects of delay TMS have been shown to hasten forthcoming responses[4,30], here we showed that TMS administered at baseline, in addition to delay, significantly increased response speed. Given the temporal distance between the delivery of baseline TMS and the onset of the deltoid muscle, this effect is surprising. Since trials without TMS and trials with TMS are otherwise identical, it is unlikely to be caused by anticipation of the relatively fixed task events.

One possible explanation for this effect is that baseline TMS somehow oriented participants to the behavioral task. For example, TMS may have heightened participant sensitivity to their arm position or primed them for a visual stimulus. Another possible

explanation is that TMS at baseline removed ambiguity about whether or not a TMS pulse would arrive later in the trial. Once TMS was delivered on a trial, there was no possibility of additional stimulation until the subsequent trial. Increased certainty that there were no immediately forthcoming TMS pulses may have freed up additional attentional resources to devote to the reaching task.

Limitations

Some limitations complicate the interpretation of the current data. These include the relatively small sample size which may have limited power for specific analyses. Our estimated sample size was based on previous investigations of preparatory inhibition. However, we did not account for the potential influence of certain task differences that may have increased noise in our MEP measures relative to previous studies. Additionally, while we observed robust statistical effects for our EMG onset measures, we may not have had sufficient sensitivity to detect more subtle MEP effects, and this may have been particularly true across behavioral task blocks.

Future studies may include larger samples. It is also possible that an extended Exposure block would have induced stronger or more complete adaptation in our sample. A stronger behavioral effect of adaptation may be more likely to reveal changes in CS excitability. Finally, we only measured MEPs in task-relevant muscles in the reaching arm. Preparatory inhibition has been shown to extend to task-irrelevant muscles, and whether muscles outside the responding limb exhibit modulation in excitability during response preparation is an important lingering question.

Conclusion

Overall, our results did not support the presence of preparatory inhibition during reaching behaviors in the presence or absence of a visual perturbation. The lack of preparatory inhibition may be the consequence of task elements such as the nature of feedback used or the muscles from which MEPs were measured. Whether reaching is prepared differently than simple finger or wrist movements, as commonly investigated in previous studies, is questionable, however, and further studies comparing the two task paradigms are needed. Furthermore, while our results regarding the effects of visuomotor adaptation on action preparation are not supportive of our hypothesis, this novel paradigm may still prove useful in exploring the relationship between CS

excitability and preparatory activity during reaching. This paradigm may have special utility in clinical populations, including individuals with cerebellar related dysfunction.

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Bridge

Results from the experiment in the current chapter indicate a lack of inhibition preceding reaching movements. In addition, we found no effect of motor adaptation on corticospinal activity during preparation. Given the presumed involvement of cerebellar-M1 projections, these findings are surprising. Whether reaching involves a separate preparatory mechanism than that of button-pressing using fingers, however, remains doubtful. Nonetheless, a more direct comparison between the two movement types is motivated. In the following chapter, we investigate corticospinal activity during a delayed response task involving a choice between a forward reach and a button press. This follow-up study simplifies the behavioral task to a single reaching target, but adds a choice between a reach and a button press using the index finger to more directly compare the two behavioral contexts.

CHAPTER V
EFFECT OF MOVEMENT TYPE ON CORTICOSPINAL EXCITABILITY

Introduction

Corticospinal excitability (CS) during action preparation has been the focus of many electrophysiological studies. Preparatory inhibition has emerged as a phenomenon of the motor system, although its exact role is still in question[1-4]. The vast majority of these studies employed single-joint finger, wrist, or ankle movements to probe the CS tract under controlled conditions. Whether preparatory inhibition extends to more complex behaviors is yet unknown.

We investigated the effects of response type on CS excitability by combining the button-press task, traditionally used in the TMS literature, with a forward reaching task. Previous work comparing preparatory inhibition across movements types showed that preparation of more complex actions involving multiple effectors[4] was associated with stronger preparatory inhibition. However, this study only involved finger movements and did not address actions engaging arm and hand muscles together. Our paradigm allows for a direct comparison between those two movement types in one sitting. The chosen button press response engages the joints of the index and thumb, whereas the reach response engages shoulder, elbow, wrist, and finger joints. Whether the total degrees of freedom about these joints for the two types of responses differently modulates preparatory CS excitability is an interesting and important question and may help to determine the functional significance of preparatory activity adjustments. Specifically, equivalent levels of modulation across the two tasks would suggest a uniform process for preparing forthcoming actions. Alternatively, greater modulation in one context than the other could point to greater modulation of CS excitability for specific movements involving a different number of joints.

We also tested whether task-relevance of the targeted muscle played a role in CS modulation. Many previous TMS studies have revealed reliable evidence of transient inhibition during the preparation of simple, ballistic movements. Importantly, this phenomenon has been observed in both task-relevant and task-irrelevant muscles[2,4]. While these findings seem to support a widespread preparatory mechanism, we have yet to replicate these results in reaching behaviors. Thus, the inclusion of task-relevancy as a factor in this study may help to reveal the selectivity of any observed preparatory modulation.

We conducted a two-part experiment in one sitting in order to investigate the presence of preparatory inhibition during reaching and the degree to which task-relevance is a factor. We employed a delayed-response task that involved a choice between reaching and button-press with the right hand. In Part 1, TMS was administered to the left primary motor cortex (M1) to elicit MEPs in the right FDI (Task-Relevant). In Part 2, TMS was administered to the right M1 to elicit MEPs in the left FDI (Task-Irrelevant). The task was performed with the right hand on both occasions, and the order was counterbalanced across participants.

We expected to observe preparatory inhibition in the targeted FDI for both button presses and reaching movements, seen as a reduction in MEP amplitude from baseline to delay. This would be in line with previous findings using button-press tasks, while expanding our understanding of preparatory mechanisms to include reaching behavior. This would suggest that complex and simple movements share a similar preparatory process, thereby supporting the idea that inhibition is global and widespread.

With regards to task-relevance, we expected to observe stronger inhibition in the task-relevant condition and weaker inhibition in the task-irrelevant condition. This would support the hypothesis of the spotlight model of preparatory inhibition, in which modulation is focused upon the responding agonist and wanes somatotopically.

Methods

Participants

A total of 17 participants (9 female, 8 male, 23 ± 3 y.o.) completed testing. Participants were recruited from the University of Oregon Human Subjects Pool and via online flyers. A pre-screening process excluded individuals with contraindications to TMS and those who are not right-handed. Exclusion criteria included any history of neurological disorder, use of psychoactive medication, and recent drug use. All participants provided written informed consent before the start of testing. All procedures were approved by the University of Oregon, IRB.

Electromyography & Transcranial Magnetic Stimulation

Surface EMG was measured from three muscles of the responding right arm – first dorsal interossei (FDI), extensor carpi radialis (ECR), anterior deltoid (AD) – and two muscles of the resting left arm – FDI and ECR. A ground electrode was placed on the epicondyle of the right elbow, and an additional electrode was placed on the dorsal aspect of the neck to detect electrical artifacts for marking TMS pulse times. After the completion of EMG setup, a standard TMS hotspotting and thresholding procedure was performed. First, the coil was positioned approximately 2 cm anterior and 5 cm lateral to the vertex, over the hemisphere contralateral to the target muscle, and with the coil oriented approximately 45 degrees off the midline to induce a current in the posterior to anterior direction. Second, the TMS intensity was adjusted, and the coil repositioned in incremental adjustments of ~1 cm until consistent MEPs were elicited from the target muscle. During this hotspotting procedure, TMS pulses were administered once every 4 s. Third, once the optimal coil position and orientation was determined, athletic tape was placed on the head to mark the location of the coil. Finally, the resting motor threshold (RMT) was determined as the intensity of TMS which elicits MEPs with amplitudes of at least 50 μV on 5 out of 10 attempts. During subsequent testing, TMS was administered at 115% RMT. The hotspotting procedure was performed on each hemisphere to determine the hotspot and RMT of both left and right FDI target muscles. EMG recording and the timed administration of TMS pulses were controlled by the VETA toolbox[5] in Matlab.

Virtual Workspace

The virtual workspace (Fig 5.1) included a home position (4cm) in the bottom-center, and a cursor (0.5cm) to represent the current position of the stylus. Visual cues and targets were presented in the center of the screen, 15cm straight ahead from home position. Visual stimulus presentation of the targets and cursor were controlled by Psychtoolbox 3.0 for Matlab 2018.

Button & Reach Choice Task

Participants used the right hand with a fisted-grip to manipulate a custom stylus that interfaced with the tablet workspace. Upon navigating to the Home position, a resting period of .5 to 1.5 seconds began. Next, one of two preparatory cues informed the participant of the forthcoming response, either a button press or a forward reach. Following a delay period of 0.9

seconds, the preparatory cue flashed with color, indicating the imperative cue, or 'Go' signal to execute either a button-press or a forward reach (Fig 5.1).

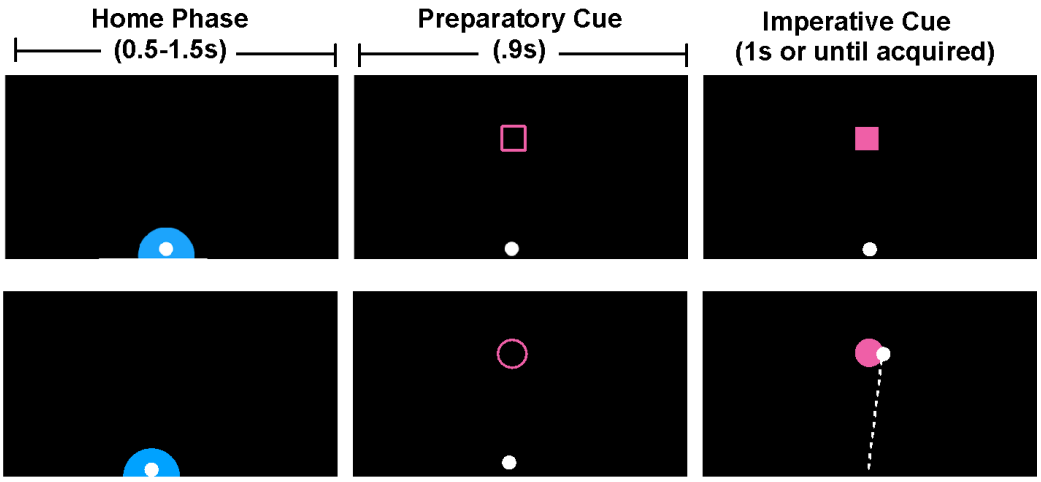

Figure 5.1 Representation of the virtual workspace for button trials (top) and reach trials (bottom). Home position (blue) was centered on the bottom of the screen. Visual cues (magenta) were presented as a square to elicit button-press responses and a circle to elicit out-and-back reaches. The cursor (white) provided live feedback of the position of the stylus.

On Button-press trials, participants depressed a mechanical button on the stylus by abducting the index finger while maintaining the cursor within the home position. On Reach trials, the imperative cue acted as a positional target for out-and-back reaches. Participants were instructed to slice-through the target with speed and accuracy before returning to the home position. A total of 120 trials were completed, including an equal number of Button-press and Reach trials.

Dependent Measures & Analysis

During the task, TMS was administered (Fig 5.2) either at the onset of the preparatory cue (baseline) or 100ms before the onset of the imperative cue (delay). The proportion of TMS measurements across the experiment were ⅖ baseline; ⅖ delay; ⅕ none. Trials without TMS were used to assess behavioral performance in the absence of TMS. Within each block, MEP amplitudes for each timepoint (baseline vs. delay) were directly compared to assess whether preparatory inhibition was present, then compared across blocks to determine whether the

preparatory state of the output motor pathway changed with movement type and task-relevancy. MEPs that did not meet a threshold of .05mV were not included for analysis. Average number of MEPs measured from each participant were 85 ± 11 for the Task-Relevant block and 88 ± 8 for the Task-Irrelevant block.

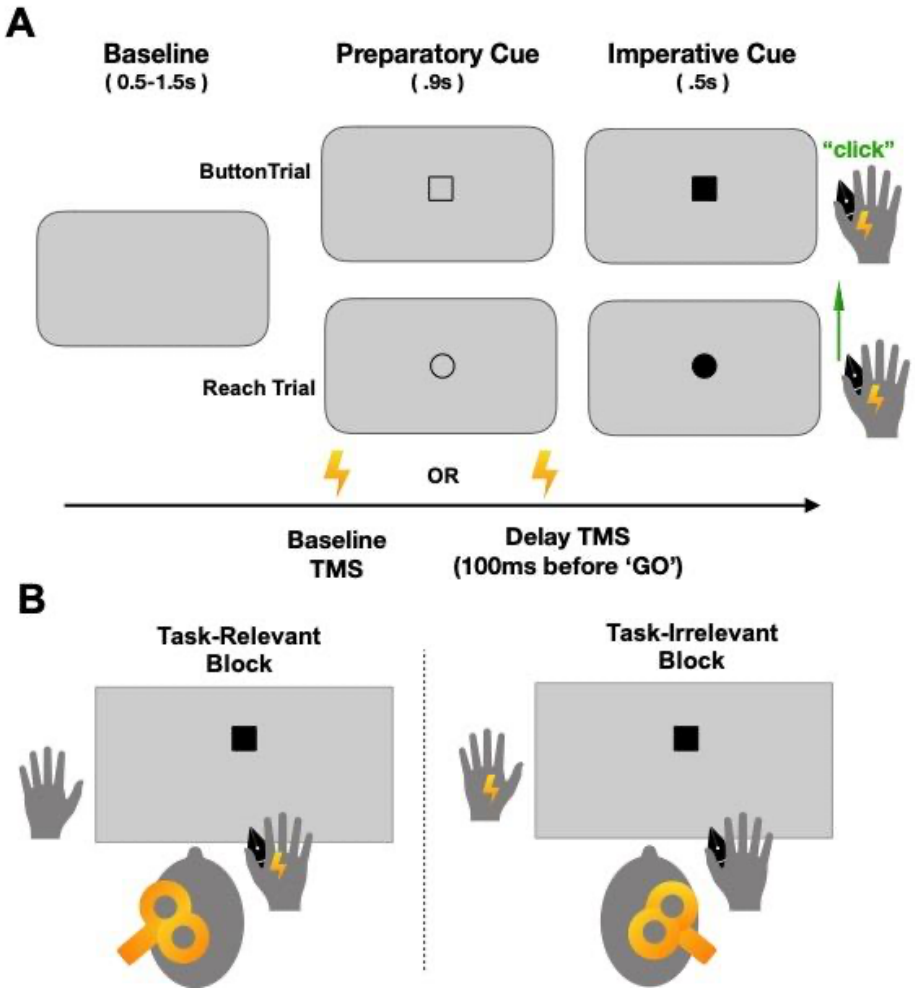

Figure 5.2 Diagram of the timeline of TMS administration relative to task. (A) Single-pulse TMS was administered either at the onset of the preparatory cue (baseline TMS) or 100ms prior to the imperative cue (delay TMS). (B) In part one, TMS was administered to the left M1 to elicit MEPs in the right FDI (task-relevant). In part two, TMS was administered to the right M1 to elicit MEPs in the left FDI (task-irrelevant).

Results

Reach Accuracy

Mean reach error was similar between Task-Relevant TMS (1.1 ± 1.6 degrees) and Task-Irrelevant TMS (0.8 ± 3.69 degrees) blocks, indicating a relatively accurate performance throughout the experiment. Overall, participants completed the appropriate response choice (button vs. reach) on 95% of trials, indicating high levels of focus throughout the task.

EMG Reaction Time

Overall EMG Reaction Times (Fig 5.3A) were measured from the right FDI (262 ± 56ms), right ECR (232 ± 77ms), and right AD (250 ± 49ms). Since the task involved differential involvement of the three muscles monitored, we analyzed button trials and reach trials separately for each muscle (Fig 5.3B). EMG RT measured from the right FDI during Button trials was 261 ± 80ms, slightly slower than that of the right AD(250 ± 48ms) during Reach Trials, although this difference was not significant (p = .49). In the Task-Irrelevant block, reaction times were similar for the right FDI (238 ± 61ms; p = .6), right ECR (264 ± 35ms; p = .3), and right AD (226 ± 34ms; p = .08).

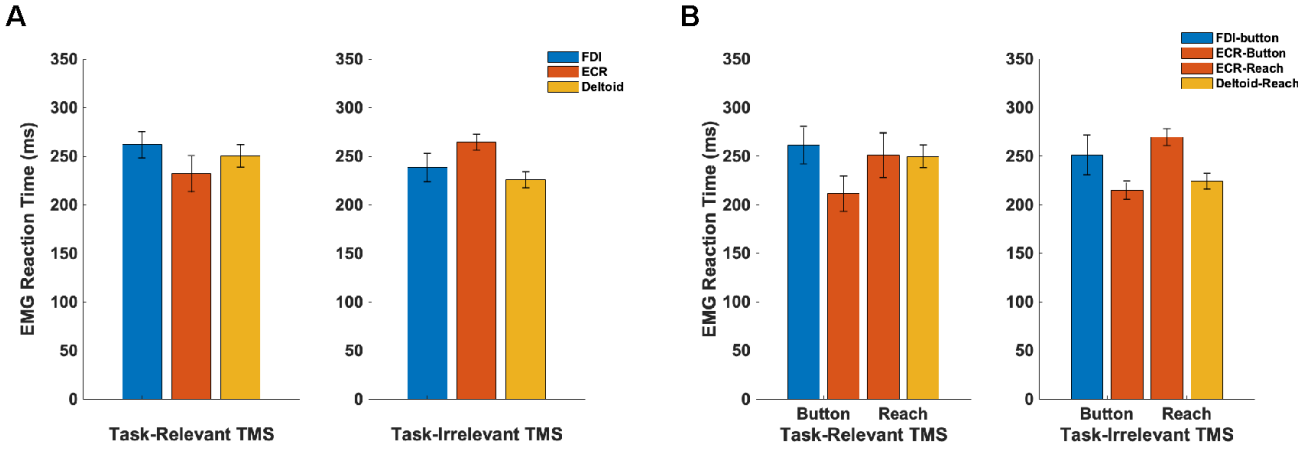

Figure 5.3 EMG Reaction Time was measured as the duration between the imperative cue and the onset of muscle activity. (A) Overall EMG RTs were similar in the task-relevant block for the FDI (262ms), ECR (232ms), and AD (250ms), as well as in the task-irrelevant block (238ms; 264ms; 226ms, respectively). (B) When muscles responses times were parsed out by Button and Reach trials, the overall trend was maintained.

We then analyzed response times in relation to TMS administration (Fig 5.4). An ANOVA revealed an effect of TMS ($F(1,16)$ = 3.5, p = .04) for the right FDI, as trials with no TMS (300 ± 53ms) were slower than trials with baseline TMS (247 ± 54ms; p < .01) and delay TMS (259 ± 70ms; p = .02). This effect was also seen in the right AD ($F(1,16)$ = 3.0, p = .05, as trials with no TMS (279 ± 55ms) were slower than trials with baseline TMS (234 ± 59ms; p < .01) and delay TMS (247 ± 52ms; p < .01). This observed increase in speed with TMS trials was not seen in the ECR muscle. Perhaps the relative use of each muscle in relation to the task made the FDI (the primary mover during button-press) and deltoid (the primary mover during reach) particularly sensitive to the effects of TMS

.

In the Task-Irrelevant block (Fig 5.4), the effect of TMS timing on EMG RT was absent for the right FDI (p = .4) and the right ECR (p = .7), but present for the right AD ($F(1,16)$ = 5.4, p < .01). The trend observed here was similar to that in the Task-Relevant block, as trials with no TMS (261 ± 52ms) were slower than trials with baseline TMS (217 ± 29ms; p < .01) and delay TMS (220 ± 47ms; p < .01). In this case, TMS delivered to the left hand was associated with faster responses only in the contralateral deltoid.

FDI MEP Amplitudes

MEPs were elicited from the right FDI in the Task-Relevant block and the left FDI in the Task-Irrelevant block, while the task was performed with the dominant right hand on both occasions. A repeated measures ANOVA was performed to test whether MEP amplitudes differed by TMS epoch (baseline vs. delay), Response type (button-press vs. reach) and Target hand (Task-Relevant vs. Task-Irrelevant). The analysis revealed no significant effects of TMS epoch ($F(1,16)$ = .77, p = .4), Response type ($F(1,16)$ = .29, p = .6), or Task-Relevance ($F(1,16)$ = .52, p = .6). These results suggest an absence of preparatory modulation in both traditional button-press and the novel reaching paradigm whether the Task-Relevant (right) FDI or Task-Irrelevant (left) FDI was the target of TMS.

Overall MEP amplitudes (Fig 5.5) elicited in the right hand at baseline (1.2 ± .9mV) were similar to those elicited during the delay epoch (1.1 ± .7mV). The Task-Irrelevant block also yielded similar MEP amplitudes measured at baseline (0.9 ± 1mv) and delay (1.0 ± 1mv). Given

the dual nature of the task, we analyzed MEPs from each response type separately (Fig 5.5), however, similar amplitudes were found at each TMS epoch for both Button (baseline: 1.2 ± 1.1mV ; delay: 1.1 ± .7mV) and Reach (baseline: 1.2 ± .9mV; delay: 1.2 ± .8mV). Task-Irrelevant MEPs were also similar for Button (baseline: 1.0 ± 1mV; delay: 0.9 ± 1mV) and Reach (baseline: 1.0 ± 1mV; delay: 1.0 ± 1mV) trials.

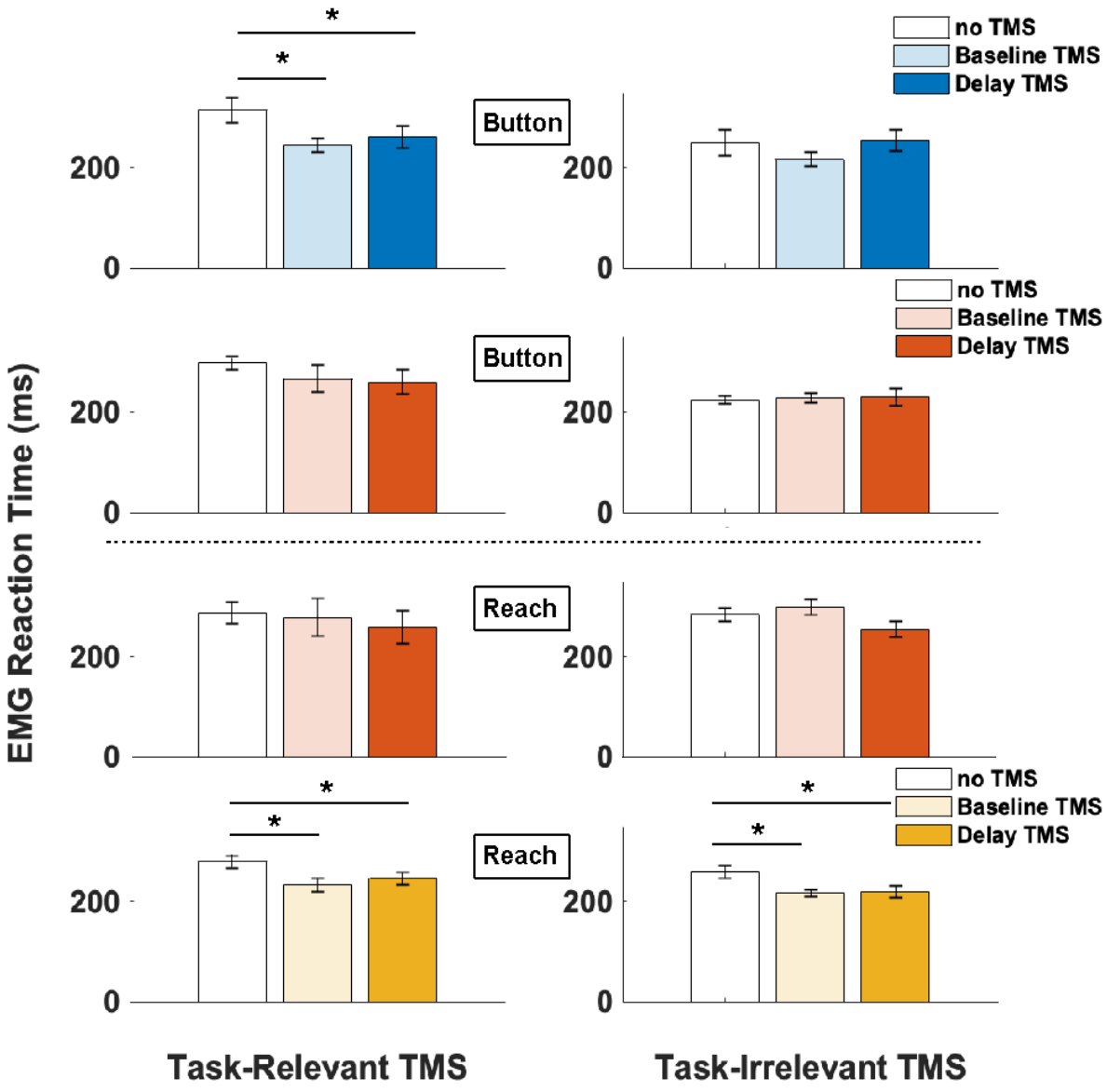

Figure 5.4 EMG Reaction Times with regards to response type and TMS administration. In the Task-Relevant block, a significant effect of TMS was observed in the FDI-button trials ($F(1,16) = 3.5$, $p = .04$) and AD-Reach trials ($F(1,16) = 3.0$, $p = .06$) but not ECR trials. In the Task-Irrelevant block, a similar effect was observed but only in the AD-reach trials ($F(1,16) = 5.4$, $p < .01$).

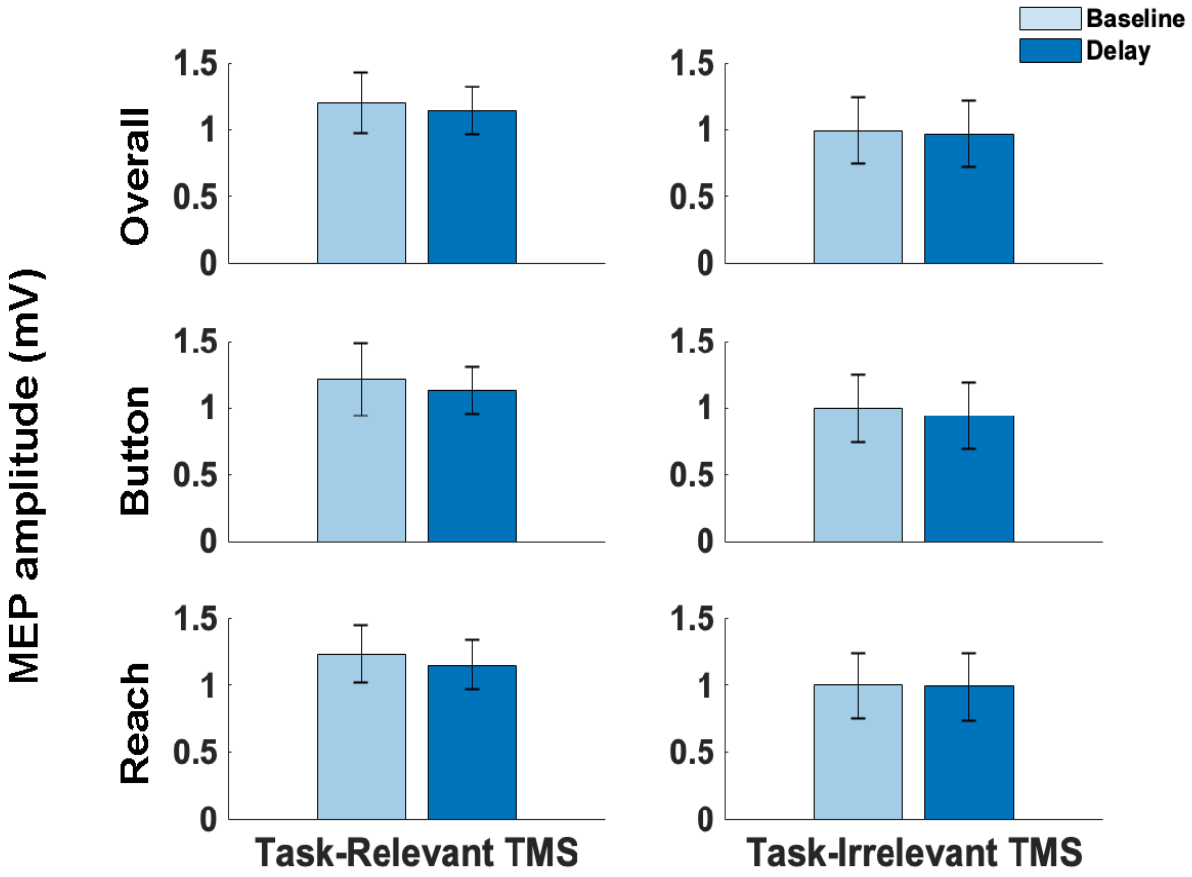

Figure 5.5 Mean MEP amplitudes elicited at baseline and delay in the right FDI (Task-Relevant) and the left FDI (Task-Irrelevant). A repeated measures ANOVA revealed no significant effects of TMS epoch (p = .4), response types (p = .6), or task-relevance (p = .6).

Pre-TMS EMG Activity

One potential explanation for the presence or absence of MEP modulation is pre-TMS EMG activity, measured as the root mean square of the 100ms window prior to TMS delivery. One would expect a higher pre-TMS EMG activity to induce a greater MEP amplitude by priming the corticospinal tract. When considering the Task-Relevant block, correlations between pre-TMS EMG activity and MEP amplitude were not observed in either right FDI (baseline, p=.5; delay, p=.3). The same was true for the Task-Irrelevant block, with no meaningful relationships observed in the left FDI (p*'s* = .8).

Discussion

In this experiment, we set out to assess differences in preparatory inhibition between delayed reaching arm movements and finger button presses. MEPs were measured in task-relevant and task-irrelevant muscles during the preparatory delay period and compared with a resting inter-trial baseline. Our results did not support the presence of preparatory inhibition in either a button-press or a reaching response, whether MEPs were elicited from the task-relevant or -irrelevant FDI. These findings are at odds with previous studies that have utilized similar delayed response paradigms. However, several features of our experiment may explain why no MEP modulation was detected. We did observe an influence of TMS on reaction time, but only in specific muscles and specific task conditions. These findings extend our previous results with a similar task.

Lack of Preparatory Inhibition in Button Pressing

Given previous work, the null result for the button-press condition is puzzling. As mentioned, several TMS studies have observed preparatory inhibition in the FDI muscle prior to movement[1-4,6-9], although these studies do not involve a reaching response option. The inclusion of a reaching option may change the nature of the task such that preparatory inhibition is no longer detectable. While button and reach trials were analyzed separately, they were interspersed throughout the task, and thus the overall preparatory state may be different when compared to a paradigm that includes simple button-pressing only.

The effect of small variations in task design on preparatory inhibition has been explored. Quoilin et al.[10] measured MEP amplitudes in three different task variants. One involved abducting the finger 'in the air' without visual or tactile feedback, another involved abducting the finger 'in the air' with visual feedback of performance, and a third involved physical interaction with a keyboard. MEP suppression was found in all three variants, but to a considerably less degree in the keyboard variant, suggesting a dis-inhibitory effect of tactile feedback. Perhaps, in the current study, the tactile manipulation of the stylus elicited a similar dis-inhibitory effect.

Others have shown that MEP suppression is stronger when the task involves a choice between response hands[11]. While our proposed task involves a response choice (reach vs. button press), the choice concerns different effectors of one limb as opposed to homologous effectors on

both hands. This may have influenced our results in unexpected ways. In contrast, others have found evidence of preparatory inhibition during a delayed-response task, whether a choice was involved or not[4]. One key difference between the two previous studies, however, is in the feedback received. The former included visual feedback of performance while the latter did not, pointing to a potential effect of feedback on the intensity and range of MEP modulation.

Further, the added complexity of the reaching response in the present study may have washed out any observable modulation that was present. While previous evidence of preparatory inhibition has mostly involved finger abduction tasks, the current study involves a multi-joint movement of the limb. Additionally, in contrast to ballistic finger abductions in a general direction, the current task requires precise target acquisition. These two factors, contributing to the relative complexity of the task, may explain why no evidence of preparatory inhibition was observed. Prior work on CS excitability suggests that increasing task difficulty and complexity effects CS activity[12-14]. Alternatively, switching between two different types of responses in the same arm may influence CS excitability in unexpected ways. Given the complexity of our paradigm, we argue that any preparatory modulation measurable by TMS, if present, may have been overshadowed.

TMS Effects on EMG Onset Times

One surprising result was the effect of TMS on Reaction time. While TMS has been shown to hasten reaction time in a delayed-response task[3,4,6], we observed an additional effect of TMS administered at baseline. That is, TMS delivered at the onset of the preparatory cue was associated with a faster button-press in the targeted FDI (247ms) as well as a faster reach in the deltoid (249ms) when compared to trials without TMS (300ms). What's more, the effect was also present in the Task-Irrelevant block, but only in the deltoid.

There are several possible explanations for this pattern of results. The use of an anticipatory strategy, in which participants used the relatively unchanging interval between TMS administration and the imperative cue, may explain this finding. However, it is unlikely given the otherwise similar parameters in trials without TMS.

Alternatively, baseline TMS may have acted as an orienting signal, increasing participants' sensitivity to their arm position. Another potential explanation is that baseline TMS primed the corticospinal tract in a similar manner as delay TMS, thus facilitating forthcoming

activation of that pathway. Lastly, it is possible that baseline TMS eliminated any uncertainty regarding the subsequent administration of TMS closer to movement onset. After TMS was administered, participants knew that another pulse would not arrive, and thus could focus on the visual stimuli free of any distractions.

Limitations

A relatively small sample size may have limited our statistical power for certain analyses. Our estimated sample size was based on previous studies of preparatory inhibition; however, we did not account for potential task-related differences. While our task paradigm was novel, it shared considerable overlap with previous studies, and thus we accounted for a similar sample size.

Another limitation may lie in our mixed-response task design, in which button-press responses were interspersed with reach responses. A blocked design separating the two response types may have revealed a pattern of preparatory inhibition that unfolds over repeated trials. Perhaps switching between two responses on a trial-by-trial basis refreshes the CS pathway in a way that obscures a prevailing trend.

Future studies should include larger samples. To avoid potential effects of tactile feedback, future studies may include a 'reach in the air' task variant to match previous work. Lastly, we only measured MEPs in the FDI muscle. While the FDI served as a primary agonist for button-press trials, we did not have a direct comparison for reach trials, as no MEPs were measured from the deltoid. Future studies may target the deltoid directly for MEP measurements during reaching.

Conclusion

The results of the current study suggest that preparatory inhibition is either absent or washed-out during a delayed-response task involving a choice between a button-press and a forward reach. The lack of observable inhibition may have been the result of task-design elements, such as visual or tactile feedback, or the muscles from which MEPs were recorded. Whether action preparation is fundamentally different for reaching compared to simple finger or wrist movements is questionable, and further studies comparing the two task paradigms are needed. While our results fail to replicate previous observations of preparatory inhibition, this

innovative approach holds potential for further investigations into the connection between CS excitability and preparatory activity in reaching behaviors. This approach could be particularly valuable in clinical populations, including individuals with cerebellar-related dysfunction.

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CHAPTER VI
CONCLUSION

Planning and executing appropriate and desired actions is vital to living a healthy life. From the mundane activities of daily living to the ever-reaching boundaries of human performance, goal-directed behavior is pertinent to human flourishing. Consequently, motor deficits and psychological disorders that prevent or alter the manifestation of desired behavior can be debilitating for individuals. Because of this, goal-directed behavior remains an area of considerable interest to neurophysiology and motor psychology.

The neural mechanisms involved in motor behavior are complex and elusive, however, many methods have been developed in animals and humans to better understand these mechanisms. For example, investigations using direct neural recordings in non-human primates have revealed that individual cortical neurons are specifically tuned for direction, speed and extent of reaching movements[1,2]. Interestingly, this finding extends to neural activity that precedes movement[3]. Contrary to what one might expect, this neural preparatory activity is not simply a subthreshold version of movement-related activity, but rather a separate process predictive of the forthcoming response[4,5]. The intrigue of neural activity that is predictive of the parameters of movement, has drawn further investigations into the mechanisms of action preparation and its role in goal-directed behavior.

Transcranial Magnetic Stimulation (TMS) has been used to explore preparatory activity and its role in behavior. TMS offers a non-invasive method of probing the corticospinal (CS) pathway at precise timepoints relative to action. A relatively consistent finding from TMS studies in humans is the transient suppression of motor evoked potentials (MEPs) during the preparatory delay period of delayed response tasks. While there exists a small but growing body of work that has found facilitation or dis-inhibition, the conditions under which preparatory inhibition is observed (or not) are of critical importance to understanding its functional significance.

Preparatory Inhibition and its Putative Sources

Action preparation during a delay period is characterized by transient inhibition of the motor-output pathway of task-relevant muscles[6-13], as well as task-irrelevant muscles[7,9], referred

to as preparatory inhibition. While these findings suggest a reliable suppression in the motor system during action preparation, others have shown somewhat conflicting evidence[14,15]. Although few in number, these contrasting studies call into question the nature of preparatory inhibition and its role in healthy movement generation.

Many questions remain unanswered concerning the necessary conditions for producing and detecting preparatory inhibition. However, multiple studies indicate preparatory inhibition is sensitive to specific features of tasks and responses. These include task feedback[14], the complexity of a prepared response[16], the involvement of a choice[15], the muscle position on the body relative to the responding effector[10], and the time provided to prepare a response[11].

Additional important questions concern the functional significance of preparatory inhibition. Evidence suggests that the magnitude of inhibition correlates with response times[12,17], with greater inhibition corresponding to faster responses. These findings are important because they lend support to the idea that physiological inhibition facilitates the preparation of responses rather than suppresses motor output.

The potential sources for preparatory inhibition are uncertain. TMS-derived measures of intracortical inhibition suggest that while overall MEP amplitudes are suppressed this occurs in the context of a synchronous release of intracortical inhibition, evident in both a release of short-interval intracortical inhibition[8] and shorter cortical silent periods[18] – as described in Chapter 2. These combined pieces of evidence may implicate subcortical sources of preparatory inhibition.

One candidate subcortical source of corticospinal inhibition is input from cerebellar-thalamocortical pathways. Evidence from dual-coil TMS, with a conditioning pulse over the cerebellum followed by a test pulse over primary motor cortex at a latency of approximately 5-7ms, produces a reliable decrease in MEP amplitudes[19]. This phenomenon, referred to as cerebellar inhibition, suggests cerebellar inputs to M1 have a modulatory influence over corticospinal output.

Until now, investigations into action preparation using single-pulse TMS have been constrained to simple finger, wrist and ankle movements. By combining single-pulse TMS with a 2-dimensional reaching task, we have developed a novel paradigm that may further our understanding of preparatory mechanisms and the relationship between CS excitability and behavior, more broadly.

Reaching and Visuomotor Adaptation

Goal-directed reaching is a fundamental skill that enables humans to interact with each other and our environment. As a highly investigated behavior in the fields of Motor Physiology, Psychology, and Neuroscience, reaching has generated an extensive body of literature, encompassing various research methodologies. However, reaching has not yet been employed in investigations of preparatory inhibition using single-pulse TMS. We argue that reaching provides an optimal direction to extend the preparatory inhibition literature to more complex movements.

Visual perturbations have been used to elicit a temporary adaptation, which reflects a process of implicit motor learning[20]. Several studies have employed adaptation to investigate the effects of motor learning on the CS pathway. This literature has shown that CS excitability is enhanced following visuomotor adaptation involving finger[21] and wrist movements[22]. Similar investigations that involve motor learning without adaptation have also found an association between the degree of learning and increased CS excitability[22-26].

Since the cerebellum is strongly implicated in mediating visuomotor adaptation[27] especially for reaching[28,29], our investigations indirectly probe cerebellar influence on CS pathway during preparation. Previous work suggests cerebellar-thalamocortical projections reduce M1 activity[30,31], and conditioning pulses of TMS over the cerebellum reduce MEP amplitudes elicited from M1[19]. However, after adaptation in a reaching task, this cerebellar-mediated inhibition is reduced[32,33]. This effect has been shown in the primary agonist of a trained behavior as well as a task-irrelevant muscle[34]. This body of work, however, did not examine the CS pathway excitability during the preparatory state, i.e. the current topic of interest. The inclusion of adaptation in the current study was an attempt to probe cerebellar-mediated effects of motor learning on the preparation of actions.

Specific Contributions of Our Experiments

CSP Results

In the first experiment described (Ch. II), we found evidence of both inhibition - seen as reduced MEPs - and a release of inhibition - seen as shorter CSPs. These opposing processes point to a more nuanced model of action preparation, one in which both cortical and subcortical influences modulate the motor output pathway independent and in opposition to one another.

These findings are consistent with previous studies showing a release of short interval intracortical inhibition during the preparation of actions[8] and suggest potential involvement of subcortical sources of preparatory inhibition. One candidate source is cerebellar input via the thalamus to M1, and this motivated our follow-up investigations of preparatory inhibition in the context of reaching.

MEP Results

In our first two studies using the novel paradigm (described in Ch. IV and V), we did not observe evidence of preparatory inhibition. In Experiment one (Ch. IV), there was no effect of TMS epoch or visual feedback block on MEP amplitude. Nor was there an effect of adaptation on CS excitability, as MEPs measured in the Exposure block were similar to those in the rest. While these findings suggest a lack of preparatory inhibition during reaching behaviors, it is unlikely that the preparatory mechanisms that govern reaching are fundamentally different than those that govern finger and wrist movements. It is likely that the added complexity of multi-joint target acquisition introduced a level of noise in the motor system that overshadowed any existing CS modulation.

Similarly, in the follow-up study (Ch. V), there was no effect of TMS epoch, Response type or Task-Relevance on MEP amplitude. While the lack of preparatory inhibition during reaching trials aligns with the results of the preceding study (Ch. IV), the lack of preparatory inhibition during button trials is more surprising. Given that CS modulation seems to be sensitive to task-related elements such as feedback and response mode, it is possible that our task paradigm is not suited to detect trial-to-trial changes in CS excitability.

Task Complexity & Corticospinal Excitability

While Reaching is a popular behavioral task in neurophysiology, the present study represents the first investigations with single-pulse TMS during preparation of reaching. While we expected to observe preparatory inhibition, as others have found using more simple tasks, it is plausible that the added complexity of multi-joint target acquisition somehow washed out any observable phenomenon of inhibition. Corticospinal (CS) excitability has been shown to increase with greater task complexity during unimanual actions[35-37] - as evidenced by larger MEPs. Importantly, this effect of task complexity has also been seen during the preparatory period[38,39],

which has direct implications for our results. While previous work examining the influence of complexity on preparatory inhibition showed greater preparatory inhibition was associated with more complex manual responses[16], this work was still confined to manual button-pressing. Perhaps in the realm of reaching, there is simply too much variation associated with the multi-joint synergies necessary to perform the behavior to observe trial-to-trial modulation in CS excitability.

Now, if it is the case that the additional complexity of reaching obscured preparatory inhibition, that still does not account for the null result in the button-press condition in our follow-up study (Ch. V). It could be that the potential option of a reaching response, on any given trial, may have altered the preparatory state of button trials, as well. If response types were not interspersed, and instead blocked, perhaps this would not be the case.

EMG Reaction Time Results

One surprising result observed in both experiments was the effect of TMS on Reaction time. While delay-period TMS has been shown to hasten reaction time in a delayed-response task[9], we observed an additional effect of TMS administered at the baseline epoch. That is, TMS delivered at the end of the inter-trial interval, but before the preparatory cue, was associated with faster deltoid onset during reaching in both of our reaching studies, as well as faster FDI onset during button-pressing in our follow-up study, when compared to trials without TMS. What's more, the effect was also present in the Task-Irrelevant block of the follow-up study, but only in the deltoid. While it is not surprising for delay-period TMS to affect response times, since it is delivered during preparation of movement and thus primes the CS tract, it is not clear why baseline-TMS would have this effect. One might argue that participants took advantage of an anticipatory strategy, using the relatively fixed intervals between the preparatory cue and the imperative cue to time their responses. However, the intervals between the visual cues were similar for all trials, and thus this strategy would have been equally viable for trials without TMS. It is possible that magnetic stimulation of M1 provides the motor system with a more visceral marker of movement-related timing than does visual stimulation. We know that proprioception is perceived faster than visual information, and so perhaps TMS stimulation acts as a proxy proprioceptive marker for the timing of forthcoming responses.

Broader Impacts, Limitations, and Future Directions

While several TMS studies have found evidence of preparatory inhibition, and a number of models have been put forth to explain its functional relevance to behavior, its functional significance remains somewhat of a mystery. The described experiments represent a novel direction for TMS investigations of preparatory inhibition, while also providing a framework for future investigations. By combining reaching behavior, as well as adaptation, with single-pulse TMS methodology, we were able to probe CS excitability during the preparation of a complex behavior while exploring any potential cerebellar-mediated effects on the CS pathway. This was an ambitious attempt to form a bridge between several desperate bodies of work, and potentially reveal a common mechanism.

A number of limitations are of concern. These include the relatively small sample sizes in both experiments, which may have limited power for statistical analyses. While we estimated our sample size based on similar studies of preparatory inhibition, we did not account for the potential influence of certain task differences that may have increased noise in our MEP measures. Additionally, while we observed robust statistical effects for our EMG onset measures, we may not have had sufficient sensitivity to detect more subtle MEP effects, and this may have been particularly true across behavioral task blocks.

Future studies may include larger sample sizes. In addition, when comparing two response types, it may be beneficial to use a blocked design, as separating the two response types may reveal a pattern of preparatory inhibition that requires successive trials. Moreover, to avoid potential effects of tactile feedback, future studies may include a 'reach in the air' task variant to match previous work[14]. Lastly, we only measured MEPs in the FDI muscle. While the FDI served as a primary agonist for button-press trials, it was only partially involved in reaches. For a more direct investigation of reaching preparation, future studies may target the deltoid for MEP measurements during reaching.

One limitation of TMS and the motor-evoked potential is a lack of specificity. As a summary analog for momentary CS activity, the MEP contains limited resolution regarding the up-stream neural mechanisms it attempts to capture. Because of this, it is difficult to ascertain whether a null result is evidence of the absence of inhibition, or evidence of a secondary process overshadowing existing inhibitory mechanisms.

Final Remarks

More work is required to interrogate the reliability, scale, and relevance of preparatory inhibition. Questions still remain whether MEP suppression is confined to the preparation of simple, ballistic finger and wrist movements, or whether it scales to multi-joint limb movements. The present set of studies - the first of their kind to our knowledge - would suggest that preparatory inhibition does not scale to reaching movements. This also calls into question the purported functional relevance of preparatory inhibition as a global mechanism of the motor system. Given the novelty of this paradigm, more work is necessary to draw definitive conclusions about the factors that influence preparatory inhibition and whether it is even detectable in the context of reaching.

The observed TMS effects on reaction time present a novel direction for understanding the relationship between stimulation of motor cortex in advance of behavior and response-relevant muscles. These data are interesting in that they suggest TMS administration may alter the speed of contralateral limb movements as well as ipsilateral reaching movements. Future studies may help to understand whether this is a product of a specific physiological process within the motor system, a less specific attentional orienting response, or a mixture of the two.

In summary, the present findings add to a small but growing body of work that challenges the current understanding of preparatory inhibition. Future work will be able to unravel which task-related parameters are relevant to observing preparatory inhibition and why. Lastly, future work will establish whether reaching shares a common or distinct preparatory mechanism to finger and wrist movements.

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