Inhibitory Effect of Subthreshold TMS on the Long-Latency Response in the Flexor Carpi Radialis

Cody A. Helm[1], Fabrizio Sergi[1,2]

[1]Department of Biomedical Engineering, University of Delaware, Newark, DE, USA
[2]Department of Mechanical Engineering, University of Delaware, Newark, DE, USA

Correspondence: Fabrizio Sergi (fabs@udel.edu)

Abstract

The corticospinal pathway and several secondary motor pathways contribute to long-latency responses(LLRs). While evidence of the contribution of secondary motor pathways to LLRs is available, it is unknown if these pathways can produce LLRs independently from corticospinal input. Transcranial magnetic stimulation(TMS) can modulate corticospinal excitability during the LLR. *Subthreshold* TMS would be ideal for inhibition of corticospinal excitability as it would allow to study function of secondary motor pathways using functional imaging methods with low temporal resolution with minimal activation confound. However, the *subthreshold* TMS parameters that maximally inhibit corticospinal activity are unknown. In this study, twenty-four participants performed a protocol that combined surface electromyography(EMG), robot-evoked wrist perturbations, and *subthreshold* TMS applied to the motor cortex to study the effect of TMS intensity and latency on the LLR amplitude in the flexor carpi radialis. We tested two TMS intensities of 90% and 95% AMT and three different latencies, defined such that the motor evoked potential (MEP) peak would arrive at 0 ms, 20 ms, or 50 ms prior to perturbation onset (T_1, T_2, T_3, respectively). *Subthreshold* TMS significantly reduced the LLR amplitude when applied with T_2 ($p_{adj} = 0.0330$) and T_3 latencies ($p_{adj} = 0.0002$). Overall, our findings indicate that single-pulse, *subthreshold* TMS delivered to evoke an

MEP with timing comprised between 20 ms and 50 ms prior to perturbation onset significantly reduce the corticospinal component of the LLR. The outcome of this work can be used to inform functional neuroimaging protocols to study the causal role of secondary motor pathways on LLRs.

NEW & NOTEWORTHY This study for the first time shows the capability of subthreshold TMS to reduce the long-latency response amplitude in a forearm muscle. We evaluate the effect of the latency between subthreshold TMS and onset of a wrist perturbation using precisely timed TMS pulses. Insights from this work can be used to design neuroimaging protocols that aim to study the role of secondary motor pathways on motor function.

Keywords: Transcranial Magnetic Stimulation;Long-Latency Response;Wrist Perturbations;Flexor Carpi Radialis

---

INTRODUCTION

The human neuromuscular system incorporates feedback mechanisms to continuously update movements as we receive new sensory information from our environment. One feedback mechanism is the stretch response which is defined as a burst of EMG activity following an imposed limb displacement (1, 2). An important component of the stretch response is the long-latency response (LLR), which occurs from 50 ms to 100 ms following perturbation onset (3). The LLR occurs between the purely spinal short latency response and the delayed voluntary reaction, each occurring 20 ms to 50 ms and more than 100 ms following perturbation onset, respectively. Notably, the LLR has both spinal and supraspinal components (4, 5, 6).

Multiple motor pathways contribute to the generation of the LLR. A large body of evidence shows that the primary motor pathway, the corticospinal tract (CST), contributes to the LLR (4, 7, 5, 8, 9, 3, 6, 10, 1, 11, 12, 13, 14). Strong evidence for the involvement of the CST in the LLR stem from the association between motor cortex neural activity and the long-latency response (12, 6). Additionally, the reticulospinal tract (RST), a secondary motor pathway, has been suggested to contribute to the LLR (15, 16, 17, 18). Current evidence suggests that the RST is responsible for the increase in the LLR when participants are instructed to resist a perturbation rather than yield (3). Additionally, the StartReact experimental paradigm has shown to elicit a faster than normal planned voluntary EMG response (∼70 ms post perturbation onset) in the presence of a startling acoustic stimulus prior to a perturbation (19, 20). The decrease in the voluntary response latency is likely contributed by the RST as the only supraspinal pathway able to act with such a short response time (21, 19, 16). Furthermore, a joint perturbation and a startling acoustic stimuli induce a similar EMG recorded response, suggesting that both responses may

be generated by the same subcortical pathway (22). Additionally, using fMRI, neural correlates of the LLR were observed in the brainstem during wrist perturbations with the StretchfMRI paradigm (23). Theses studies provide the muscle-specific brainstem regions associated with the LLR and support the notion that the RST contributes to the LLR. While our understanding of the motor function of subcortical pathways like the RST is improving, due to limitations in the methods used in previous studies, it is still currently unknown if the RST can generate stretch reflex activity independently from the CST, or if the RST acts solely as a relay for descending CST signal. In the former case, the RST would be able to generate functional motor responses, stretch reflexes, also in absence of CST input. This would support the hypothesis that the RST can provide functional motor responses, regardless of CST input, suggesting its beneficial role in motor recovery in the presence of CST damage. Therefore, alternative methods need to be developed to study the isolated role of the RST on LLR formation, whereby a causal role of this pathway in producing LLR can be identified.

To study the direct role of the RST on the LLR, RST function needs to be decoupled from that of the CST. Transcranial magnetic stimulation (TMS) is a neuromodulation technique that can be used to decouple the function of different pathways contributing to motor function. TMS uses a non-invasive time-varying magnetic coil to excite neurons in the line of coil stimulation. When TMS is placed over the motor cortex, motor evoked potentials (MEPs) are measured via surface electromyography (EMG) of relevant muscles, with a time delay comprised between 10 ms and 30 ms. Following the MEP, there is a decrease in the EMG activity of the targeted muscle which has been referred to as the silent period (24, 25, 26, 27, 28, 29). This TMS-induced silent period has been suggested to last approximately 150 ms to 200 ms, with the first 80 ms of inhibition coming from spinal inhibition and the later part coming from longer lasting cortical inhibition via GABA receptors (29, 30, 31, 32). As such, TMS can modulate the excitability of the corticospinal tract, and thus modulate the contribution of the CST during a stretch-evoked motor response, which would allow to study the contribution of RST to LLRs during reduced CST excitability. With appropriately timed TMS pulses, we can overlay the cortical component of the silent period with the LLR to inhibit its contribution to the LLR.

The capability of TMS to inhibit the stretch-evoked long-latency response has been studied under a variety of TMS intensities, timing between evoked MEP and perturbation onset, and for a variety of tasks. The previous studies that use TMS in the attempt to partially inhibit LLRs are displayed in Fig. 1, broken down by the parameters of TMS stimulation (TMS amplitude and timing), and with an indication of what the measured effects have been.

When *suprathreshold* TMS elicits an MEP immediately after perturbation onset, there is a reduction in the LLR amplitude for a 'do not intervene' task (5). When timed to arrive prior to perturbation onset, *suprathreshold* TMS suppresses the LLR anticipatory modulation associated with a multi-joint dynamic mechanical environment (33). Furthermore, at the same TMS timing, *suprathreshold* TMS has been shown to decrease the LLR modulation associ-

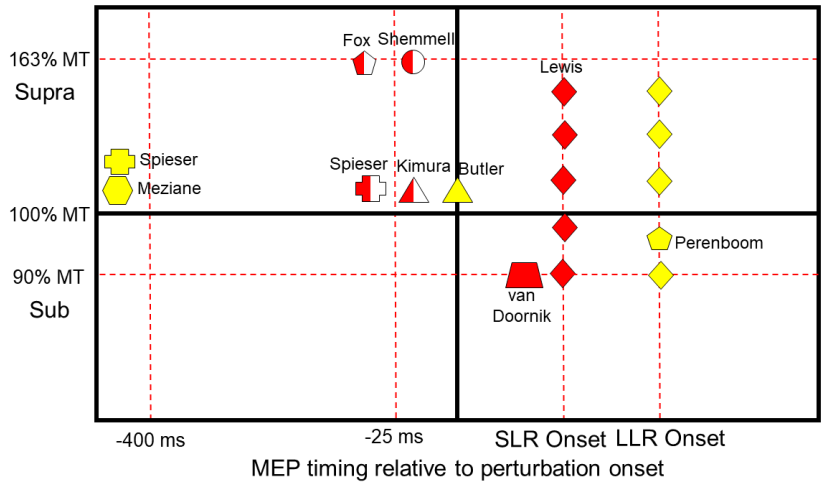

Figure 1: Schematic of previous LLR studies involving the use of *supra-* and *subtreshold* TMS. TMS intensity relative to motor threshold is labelled on th y-axis. The timing of the motor-evoked potential (MEP) relative to perturbation onset is labelled on the x-axis. Each symbol corresponds to a different study. Red indicates that the effect of TMS on the LLR was inhibitory. White indicates there was no effect of TMS on the LLR. Yellow indicates the study did not measure the effect of TMS on the LLR.

ated with a task-instruction (34). However, previous work also found that a strong *suprathreshold* TMS arriving prior to perturbation onset reduces LLR modulation associated with the environmental stiffness but does not reduce the LLR modulation associated with task instruction (35, 36). For *subthreshold TMS*, a reduction in the LLR amplitude has been demonstrated when TMS is applied such that the MEP arrives following perturbation onset (37, 5).

As far as we know, no studies have investigated the effect of *subthreshold TMS* on the LLR amplitude when the MEP is timed to arrive prior to perturbation onset in the FCR muscle. *subthreshold TMS* would be beneficial as it reduces the excitatory effect of TMS stimulation on the motor cortex regions, as would be observed with *suprathreshold TMS*, which further highlights the inhibitory effect of TMS. Additionally, *subthreshold TMS* timed such that the MEP arrives prior to perturbation onset would properly engage the cortical, and not spinal, component of the TMS-evoked silent period during the LLR. It remains unknown whether *subthreshold TMS* when the MEP is timed to arrive prior to perturbation onset can significantly reduce the LLR amplitude via the cortical component of the silent period. Additionally, the distinct *subthreshold TMS* stimulation intensity and latency that result in the maximal reduction of the cortical contribution, via the silent period, to the LLR of the FCR muscle are unknown.

The objective of this study was to determine whether *subthreshold* TMS can significantly reduce the contribution of the CST to the LLR in a forearm flexor muscle, FCR, and to investigate the optimal TMS intensity and timing that would maximally inhibit the amplitude of LLRs evoked via controlled perturbation of the wrist. The outcomes of this study provide insight into the mechanisms by which *subthreshold* TMS results in inhibition of the LLR and the specific *subthreshold* TMS intensity and latency needed to overlay the cortical component of the EMG silent period with the LLR in the forearm. The knowledge from this study may be used to study the role of subcortical or spinal mechanisms in both the generation and the modulation of the long-latency response during reduced excitability of the corticospinal tract.

MATERIALS AND METHODS

Participants

24 subjects (16 male/8 female, age range: 19 – 39 years) performed the experimental protocol. The subjects were divided evenly into two groups (n=12), each exposed to different TMS modes. All of the subjects were right-handed and free from neurological disorders and did not have any contra-indications to MRI or TMS. The subjects provided informed written consent for the study. The study was approved by the Institutional Review Board of the University of Delaware (IRB no: 1545543-5 and -6) and was in accordance with the Declaration of Helsinki.

MRI Structural Scan

During a first visit, we acquired a high-resolution structural brain scan for each subject with a Siemens 3T MRI scanner (Center for Biomedical & Brain Imaging, University of Delaware). Subjects were screened with an MRI safety screening form to ensure they were safe to be scanned in the MRI. The subject laid in the supine position on the scanner bed and was instructed to relax and minimize movement during the scan. The high resolution T_1 MP-RAGE structural scan required a total of 30 minutes to perform with a 0.7x0.7x0.7mm^3 voxel resolution, TR = 2300 ms, TE = 3.2 ms and 160 slices with 256 x 256 px per image.

Experimental Setup

In this study, we combined a wrist robot, the MR-StretchWrist, used to elicit stretch reflexes in the forearm, with TMS, used to stimulate the motor cortex with precise timing, and surface EMG, used to record muscle activity in one forearm muscle. Details on these elements are provided below.

Surface Electromyography

Bi-polar surface electrodes were placed on the skin over the flexor carpi radialis (FCR) to record muscle activity. The location of the FCR muscle belly was determined by having the subject flex and relax their wrist and palpating down the forearm muscle. The skin was cleaned with a 70% Isopropyl alcohol wipe (Med Price) to remove any dirt and debris on the surface of the forearm. A thin layer of skin was abraded with Nuprep skin prep gel (Weaver and Company) to reduce contact impedance. The electrodes (standard multitrode Ag/AgCl bipolar electrodes from Brain Products, Munich, Germany) were firmly attached on the skin surface using double-sided adhesive collars (BIOPAC Systems, Inc.). The bi-polar electrodes were aligned with the direction of the muscle fibers. An additional electrode (ground electrode) was placed on the olecranon in the back of the elbow to serve as a ground. Each electrode was filled with a high-chloride abrasive electrolyte gel (EASYCAP) to further reduce contact impedance. Muscle activity was amplified using an 8-channel bipolar amplifier (MR Plus ExG, BrainVision, Munich, Germany). Muscle signal and external triggers were synced together using a Trigger and Sync Box (BrainVision). The EMG signal was filtered with a 60 Hz notch filter and visualized and recorded using BrainVision Recorder software. Proper electrode placement was confirmed by having the subject perform flexion and radial deviation contractions and observing a high signal to noise ratio in agonist muscles.

Robotic perturbations

We used the MR-StretchWrist to apply perturbations and evoke stretch reflexes on forearm muscles. The MR-StretchWrist is an MRI-compatible wrist robot previously developed by the Human Robotics Lab (23) at the University of Delaware uses a piezoelectric ultrasonic motor to apply velocity-controlled perturbations to the wrist joint and an incremental encoder to continuously measure wrist angle. The MR-StretchWrist incorporates a force/torque sensor to measure wrist torques applied by the subject. During a wrist perturbation, a velocity command of 150°/sec is sent to the MR-StretchWrist for 200 ms in the extension direction to elicit a stretch reflex response in wrist flexors, including the FCR muscle. Following perturbations, the wrist robot returns the wrist to the neutral posture at a slow velocity (35°/s) to avoid eliciting a stretch reflex.

Transcranial Magnetic Stimulation

A neuronavigation procedure was performed to position the TMS coil over the participant's forearm motor hotspot of the motor cortex. First, the participant's structural brain scan was loaded into the Neuronavigation software (Localite 3.3.0). With the Localite software, brain segmentation was performed to virtually extract a 3d rendering of the brain volume from the skull. We converted from subject space to Talairach space by manually defining the anterior commissure, posterior commissure, and cortical falx point on the brain scan. A target marker was placed on the forearm motor hotspot of the motor cortex as the

location that we aimed to stimulate with TMS. The target marker was defined visually by identifying the pre-central gyrus, and then moving laterally along the gyrus until reaching the hotspot for muscles responsible for movements of the distal upper extremity. An entry marker was placed on the surface of the skull that the TMS coil would be positioned. The entry marker was oriented approximately 45° from the mid-sagittal line for adequate target stimulation to occur (38).

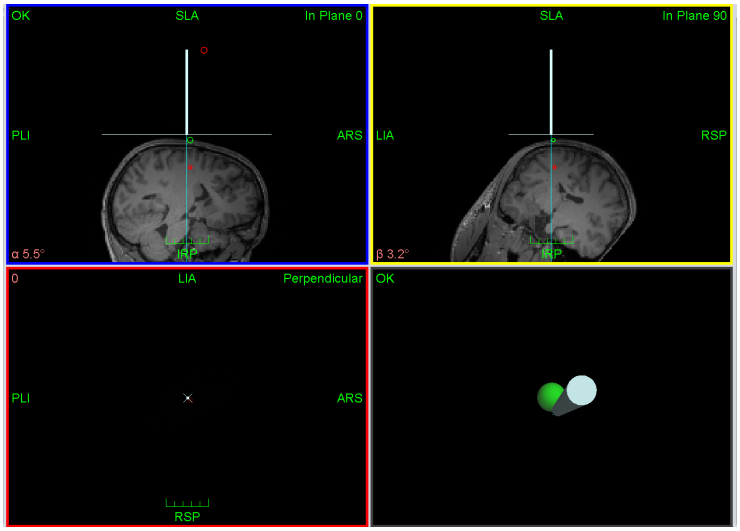

Figure 2: Localite Neuronavigation software displaying the position and orientation of the TMS coil (blue line) and the position of the entry marker (green circle) and target marker (red circle) for accurate coil placement. Blue box displays the sagittal plane and the yellow box displays the coronal plane. The bottom right box displays the plane looking down on the entry-target axis and the red box displays the plane perpendicular to the entry-target axis plane.

Next, we performed subject registration to register the physical features of the subject's head with the Neuronavigation system. We used motion capture tracking and reflective markers to register the position and orientation of the subject's head and TMS coil in 3D space. A figure-of-eight TMS coil (MCF-B65, Magventure) was calibrated to the camera which defined the position and orientation of the center of TMS coil relative to the coil's markers. We placed reflective markers on the subject's forehead via double-sided skin adhesive tape for subject registration. Subject registered included a 'landmark registration' and a 'surface registration'. For 'landmark registration', the 'left tragus', 'right tragus', and 'nasion point' were manually defined on the brain scan. A pointer was used to calibrate the features of the subject's head with the respective markers on the brain scan. During 'surface registration', a pointer was moved across the surface of the subject's head to collect a minimum of 200 points to

calibrate the surface of the subject's head with the TMS software. The landmark and surface registration steps were verified by comparing the positions of the landmarks and surfaces in real space with the respective locations on the virtual brain scan. A root mean squared error of less than 4.0 mm was considered acceptable for coil placement.

Following the Neuronavigation procedure, a chin support connected to the procedural chair was locked in place to support the subject's head and minimize head movement throughout the experiment. The TMS coil was placed over the forearm motor hotspot of the subject using the Neuronavigation system. Proper placement of the TMS coil was confirmed by sending three TMS pulses to the motor cortex (intensity: 80%) and observing whether motor evoked potentials were present. If there were no motor evoked potentials, the TMS coil was repositioned until motor evoked potentials were observed. The TMS stimulator (MagPro X100, Magventure) settings were set to biphasic waveform, twin pulse mode, and normal current direction for all the TMS pulse stimulations.

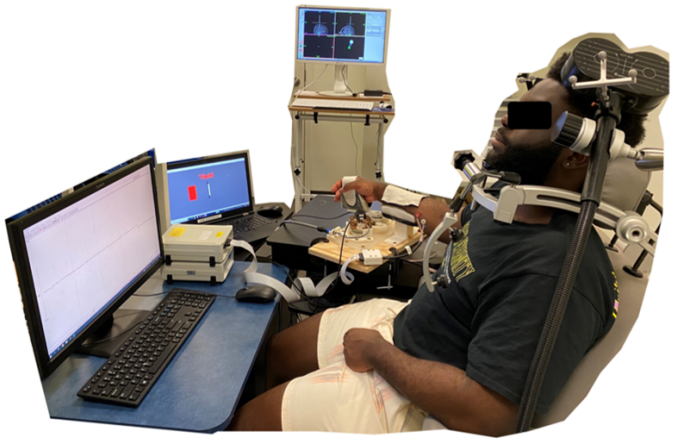

Figure 3: Laboratory experimental setup with the TMS system, MR-StretchWrist robot, and FCR surface EMG acquisition. The TMS coil is placed on the surface of the head and directed towards the forearm motor hotspot area. Accurate placement of the TMS coil is confirmed with the TMS neuronavigation software, Localite.

Experimental Procedures

Active Motor Threshold & T_{MEP} Determination

Once it was confirmed that the TMS was placed over the forearm hotspot of the left motor cortex, we determined the subject specific active motor threshold (AMT) and T_{MEP} latency. The AMT was defined as the minimum TMS intensity that elicited at least 5 out of 10 motor evoked potentials (MEPs) when the muscle was in the target activation state. A MEP was recorded if the mea-

sured EMG response exceeded a threshold of 200 μV. The subject was cued to maintain a wrist flexion torque of 0.3 Nm as indicated by a visual display. The visual display was composed of a horizontal bar whose length was mapped to the measured wrist flexion torque, and whose color changed from gray to green if the measured torque was within 0.07 Nm from the target. Once participants matched the target torque, a train of TMS stimuli was applied. Ten single pulse stimuli were fired manually at each intensity, with an approximate inter-stimulus time of 5 s, and the number of MEPs that crossed the threshold were counted. The subject was cued to relax following the 10 stimuli. The TMS stimulus intensity started at 40% of the maximum stimulation output (MSO) of the TMS stimulator. If the number of MEPs recorded at a given amplitude was below 5, the intensity was increased by 10% until the number of MEPs was equal to or greater than 5. The stimulus intensity was then decreased by 5%, then changed by 2% until we found the lowest TMS intensity that yielded at least 5 MEPs. The measured value of AMT was used to define stimulation amplitudes in the later portion of the experiment. TMS timing were calculated for the later portions of the experiment based on the subject's T_{MEP} latency. The T_{MEP} latency was defined as the average delay between the time the TMS was fired and the absolute maximum or minimum of the measured EMG track. T_{MEP} was determined by pooling together the EMG responses elicited by supra-threshold stimuli at the subject's AMT.

Experimental Task

In the task, the MR-StretchWrist robot was used to elicit stretch reflexes in forearm muscles, TMS was used to stimulate the left motor cortex in proximity of the motor hotspot for muscles in the right forearm, and FCR muscle activity was recorded using sEMG. Perturbations were applied to the wrist joint in the extension direction to elicit a stretch response in the FCR muscle. To study the effect of *subthreshold* TMS intensity on the LLR amplitude, two *subthreshold* TMS intensities were employed in the study, with two levels (i.e., 90% AMT and 95% AMT). Each group was exposed to one TMS intensity, i.e. Group 1 was exposed to the 90% AMT intensity, and Group 2 was exposed to the 95% AMT intensity. Within each group, TMS pulses were applied with three different latencies ΔT relative to perturbation onset. The latencies were computed such that the MEP would peak T_L ms before perturbation onset (see Fig. 4). In our study, we investigated three levels for MEP latency T_L, with T_1=0 ms, T_2=20 ms, T_3=50 ms. Latencies were applied such that the ensuing silent window, supposed to be of approximately 150 ms of duration (26, 29), would completely overlap with the LLR portion of the stretch-evoked response.

In addition to the three TMS latency modes, we applied stimuli using two additional control mode, one (Pert Only), where perturbations were applied in absence of TMS, and one (Back Only), where no perturbations nor TMS were applied after the subject matched the torque target. There was a total of 20 trials per mode throughout the experiment for a total of 100 trials. The order of modes was randomized through the experiment at the individual trial level.

Prior to the experiment, participants were instructed to yield to the perturbation (when applied) as soon as they felt the robot began to perturb them. Each trial began by cueing the participant to maintain a wrist flexion torque of 0.3 Nm as indicated by a visual display, just like in the AMT session. Upon matching the torque target, the TMS stimulus would be applied at a random point normally distributed between 400 and 800 ms. Following perturbation, the robot would return the wrist to the neutral position at a low velocity, and the target bar for the next trial would appear after a delay ΔT calculated from a uniform distribution $\mathcal{U}_{[a,b]}$ with $a = 3$ s and $b = 7$ s.

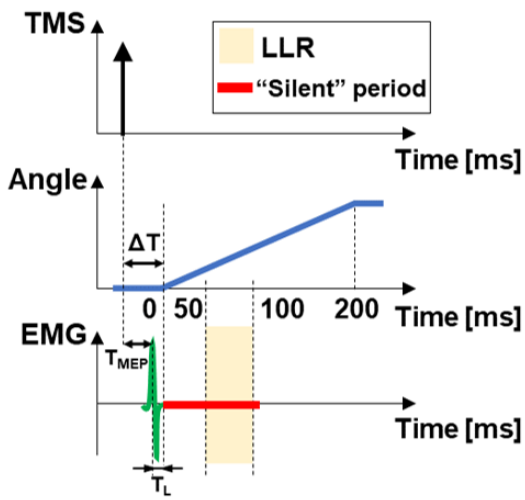

Figure 4: Experimental timing schematic showing the synchronization of TMS pulse application, wrist perturbation and expected FCR EMG response and silent period. T_L is the timing of the peak of the MEP relative to perturbation onset.

Data Analysis

EMG Analysis

Raw EMG data was acquired for the FCR muscle for all 100 experimental trials and digitized using the EMG amplifier. The digitized EMG data was filtered by applying a fourth order bandpass filter with a high pass frequency of 20 Hz and a low pass frequency of 250 Hz. Then, the EMG response was subject to full-waved rectification by taking the absolute value of the EMG. Finally, the rectified EMG response was filtered with a fourth order lowpass frequency of 60 Hz. Background EMG activity was defined as filtered EMG activity from 100-50 ms prior to perturbation onset. For a given trial, filtered EMG activity from 100 ms prior to pertubation onset to 150 ms post perturbation onset was subject to statistical analysis. The filtered EMG activity was normalized for

each trial by dividing it by the average background activity of all trials. Thus, the normalized EMG response in normalized units (n.u.) were obtained for each trial. For each trial, the normalized EMG response from 50-100ms was averaged to obtain the LLR amplitude (LLRa).

Statistical Analysis

Subject Specific Stimulation Parameters

We performed a t-test to compare the active motor threshold intensities and T_{MEP} between the two TMS intensity groups, as we wanted to verify that there were no significant differs between the active motor thresholds and the MEP latencies between the groups.

Group-level LLR analysis

We performed a linear mixed model to determine the effects of TMS intensity and latency on the amplitude of LLRs. Outliers were removed by subject using the Huber Method with an outlier threshold equal to 4 standard deviations from the mean, for each subject. In the model, the fixed effects were TMS mode (5 levels: T1, T2, T3, TMS off - Pert On, Background Only), TMS intensity (2 levels: 90% of AMT and 95% of AMT) and the interaction between TMS mode and intensity. The linear mixed model included random effects of subject and subject $\times$ TMS mode. The mixed model accounted for unequal variance between different TMS modes and subjects. In presence of a significant fixed effect, post-hoc analysis was performed using Dunnett's test for multiple comparisons with control to determine which TMS modes were significantly different from the control mode of TMS off - Pert On.

An additional analysis was performed using separate linear mixed models for each TMS intensity to determine the effect of TMS mode for each TMS intensity group (90% or 95% active motor threshold). The linear mixed model included fixed effects of TMS mode, and random effects of subject and subject $\times$ TMS mode. The mixed model accounted for unequal variance between different TMS modes and subject. In presence of a significant fixed effect, post-hoc analysis was performed using Dunnett's test for multiple comparisons with control to determine if any TMS mode was significantly different from the control mode (TMS off - Pert On). An alpha threshold value of 0.05 was set as the to determine statistical significance.

Subject-level LLR analysis

For each subject, we first used the Shapiro-Wilcoxon test to determine if the normalized LLRa dataset was normally distributed. Because the test highlighted that the LLRa data for each subject was non-normally distributed, nonparametric tests were used for statistical analysis.

Subject-level statistical analysis was performed using the Wilcoxon test, and post-hoc tests were performed using the Steel method, setting the TMS off –

Pert On mode as the control mode while controlling for multiple comparisons (4 per subject). As an outcome of this analysis, we counted the number of subjects that had a significant difference from control

All statistical analyses reported above were performed using JMP Pro Version 16.2.0 (SAS Institute Inc., Cary, NC, United States).

Time series analysis

A 1-D time series ANOVA was performed on the normalized FCR EMG for each subject from 100 ms before perturbation onset to 150 ms after perturbation onset using the SPM 1D software package (Pataky et al., 2015, 2016). While the 0D statistical tests focused on the effect of TMS timing and intensity on the LLRa over a specific time period, a 1D statistical tests allowed us to determine the effect of TMS timing and intensity on the normalized EMG response over the entire time period while accounting for multiple comparisons.

We performed a two-way model to test the effects of two factors, TMS mode, TMS intensity, and their interaction, on the normalized EMG response using the function `anova2onerm`. This test accounted for the random effect of subject ID and accounted for the repeated measures factor of TMS modes. Also, a 1-way rmANOVA was performed with all subjects to determine the effect of TMS mode for the entire stretch reflex response period. In each model, TMS mode had four levels: T_1, T_2, T_3, and PertOnly as the control mode. The measurements in the Back Only mode were not included in the analysis because the rmANOVA package currently assumes equal variances between conditions, and would lead to under-estimating the variance of the whole dataset in presence of EMG data measured in the Background Only mode (no stimulus response). Random factors in the rmANOVA model were subject ID, and the software automatically estimated terms for both subject and for the interaction between subject and TMS mode.

We performed two-sided paired t-tests for 1D post-hoc analysis to determine which TMS mode levels had a significantly different EMG response than the control. Post-hoc analysis was performed by comparing T_1, T_2, or T_3 with the control mode (PertOnly) to determine which TMS modes were significantly different at each time point (100ms prior to 150 ms post perturabation onset). The two-sided paired t-test were performed with the normalized FCR EMG response for each condition for all subjects. We used a bonferroni-corrected α value of 0.017 in the t-tests to accounted for the multiple comparisons that were performed. The averaged normalized FCR EMG response for all subjects was plotted for visualization purposes.

The one-way rmANOVA for the effect of TMS mode was repeated for each TMS intensity group. Again, TMS mode (levels: T_1, T_2, T_3, and PertOnly) was the fixed effect, with PertOnly as the control mode. Subject ID was used as a random effect and the software automatically estimated terms for both subject and for the interaction between subject and TMS mode. Again, post-hoc analysis was performed with two-sided paired t-tests to determine which TMS modes were significantly different from the control mode at each time point. We

used a bonferroni-corrected α value of 0.017 in the t-tests to accounted for the multiple comparisons that were performed.

RESULTS

Subject Specific Stimulation Parameters

The AMT values were not found to be significantly different between groups ($T = 1.311$, $df = 22$, $p = 0.2034$, 90-95: 6.17 ± 4.7 %MSO). Average AMT was 71.8 ($\pm$12.6 std.) %MSO and 65.7 ($\pm$ 10.3 std.) %MSO for the 90% and 95% AMT groups, respectively. We also found no significant difference in the T_{MEP} latency values between the two groups ($T = 1.37$, $df = 22$, $p = 0.1845$, $90 - 95 : -3.83 \pm 2.80$ ms). The average T_{MEP} latency for 90% AMT was 26.8 ms with a standard deviation of 8.0 ms. The average T_{MEP} latency for 95% AMT was 30.6 ms with a standard deviation of 5.5 ms.

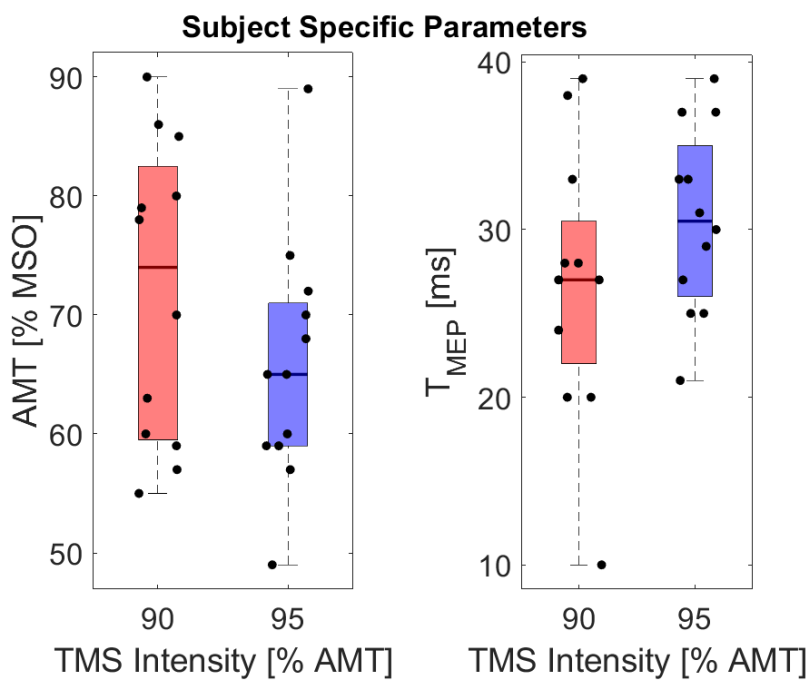

Figure 5: Average and standard deviation of active motor threshold and T_{MEP} latency measurements for each group. Solid black lines inside the boxplot indicate median, boxplot boundaries indicate 25% and 75% IQR and outer lines indicate min and max data points. AMT and T_{MEP} values for each subject are denoted with black dots.

Effect of TMS mode on LLRa

Group-level analysis

Based on the two-way linear mixed model, TMS mode had a significant effect on the normalized LLRa ($F = 16.85$, $df = 96.0$, $p_{adj} < 0.0001$). No significant effect was found for the effect of TMS intensity nor TMS intensity*mode ($F = 0.196$, $df = 22.0$, $p_{adj} = 0.6624$; $F = 0.228$, $df = 96.0$, $p_{adj} = 0.9218$, respectively). Post-hoc analysis performed using the Dunnett's method with control showed that T_2, T_3, and the Back Only modes were significantly different compared to the Pert Only mode (mean $\pm$ s.e.m. of the difference – T_2−PO: -0.694 ± 0.263 n.u., $p_{adj} = 0.0330$; T_3−PO: -1.09 ± 0.261 n.u., $p_{adj} = 0.0002$; BO−PO: -1.92 ± 0.254 n.u., $p_{adj} < 0.0001$), while T_1 was not (T_1−PO: -0.412 ± 0.264 n.u., $p_{adj} = 0.333$).

For the 90% AMT group, the linear mixed model showed that TMS mode had a significant effect on the normalized LLRa ($F = 11.5$, $df = 49.7$, $p_{adj} < 0.0001$). Post-hoc analysis performed using the Dunnett's method with control showed that the T_3 and the Back Only modes were significantly different compared to the Pert Only mode (T_3-PO: -1.18 ± 0.356 n.u., $p_{adj} = 0.0063$; BO-PO: -2.156 ± 0.343 n.u., $p_{adj} < 0.0001$), while T_1 and T_2 were not (T_1-PO: -0.483 ± 0.358 n.u., $p_{adj} = 0.4643$; T_2-PO: -0.817 ± 0.358 n.u., $p_{adj} = 0.0853$).

For the 95% AMT group, the linear mixed model showed that TMS mode had a significant effect on the normalized LLRa ($F = 6.1$, $df = 46.6$, $p_{adj} = 0.0005$). Post-hoc analysis using the Dunnett's method with control showed that the T_3 and the Back Only modes were significantly different compared to the Pert Only mode (T_3-PO: -1.01 ± 0.383 n.u., $p_{adj} = 0.0386$; BO-PO: -1.692 ± 0.375 n.u., $p_{adj} = 0.0002$, respectively), while T_1 and T_2 were not (T_1-PO: -0.342 ± 0.387 n.u., $p = 0.7881$; T_2-PO: -0.572 ± 0.385 n.u., $p = 0.3843$).

Fixed Effect	DFNum	DFDen	F Ratio	p-value
TMS Mode	4	96	16.85	<0.0001*
Intensity	1	22.0	0.196	0.6624
TMS Mode·Intensity	4	96	0.228	0.9218

Table 1: Linear Mixed Model results for the two-way ANOVA test.

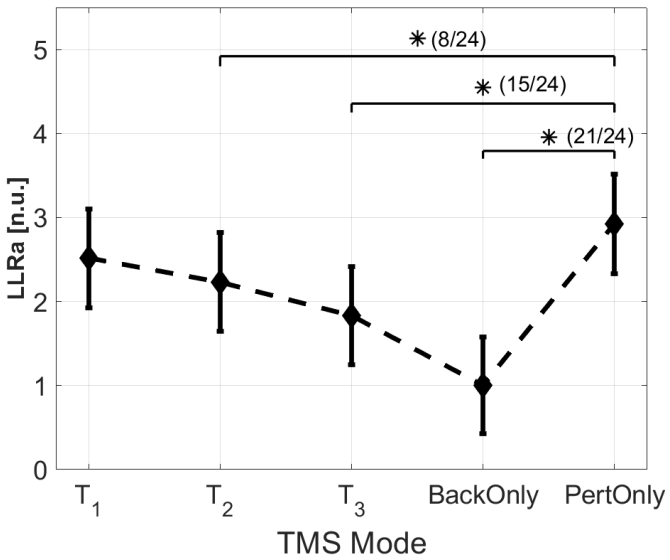

Figure 6: Effects of TMS mode the long latency response amplitude (LLRa) pooled in both groups. Asterisks denote significant effects at the group level, and numbers in parenthesis indicate the number of subjects for which that effect was detected.

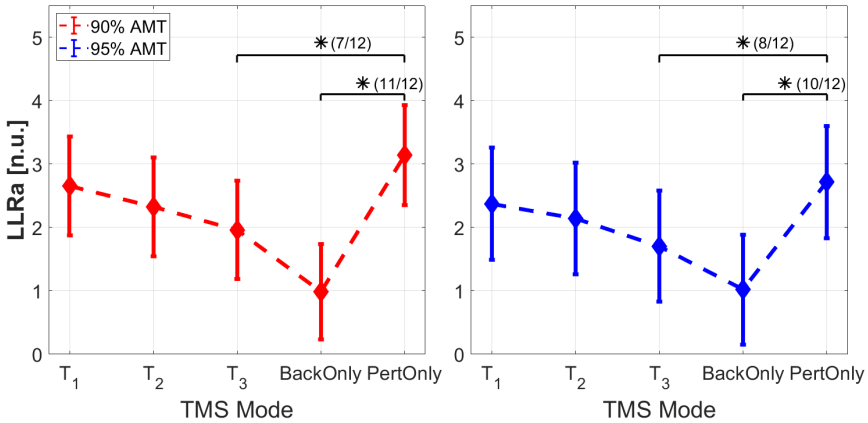

Figure 7: Effect of TMS mode on the long latency response amplitude (LLRa) broken down by TMS intensity (i.e., group). 90% AMT intensity group is in red and the 95% AMT intensity group is in blue. Asterisks denote significant effects within each group, and numbers in parenthesis indicate the number of subjects for which that effect was detected.

Subject-level analysis

From the Shapiro-Wilcoxon test for normality, the LLRa data was non-normally distributed for all subjects. For the 90% AMT intensity, a significant effect of TMS mode was found in 5 out of 12 subjects for T_1, 3 out of 12 subjects for T_2, and 7 out of 12 subjects for T_3 compared to the Pert Only mode. For the 95% AMT intensity, a significant effect of TMS mode was found in 4 out of 12 subjects for T_1, 5 out of 12 subjects for T_2, and 8 out of 12 subjects for T_3 compared to the Pert Only mode. For the Back Only mode, 11 out of 12 subjects in the 90% AMT group and 10 out of 12 subjects in 95% AMT group had a significant effect compared to Pert Only mode. In subjects without a significant difference from background, the evoked muscle stretches might not have induced a large enough LLR.

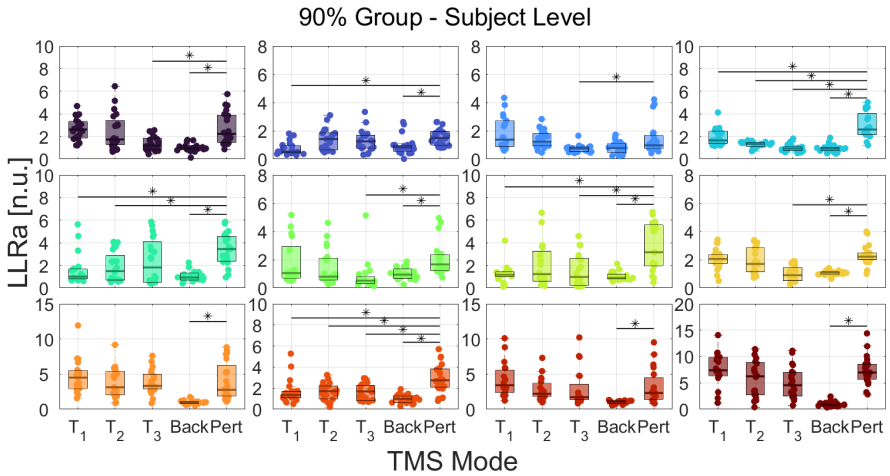

Figure 8: Subject-level results of the effect of TMS mode on the normalized long-latency response amplitude (LLRa) for the 90% AMT intensity group. Each panel represents the response from one subject. In each boxplot, the median is indicated via a horizontal line, the box extends to the 25th and 75th precentiles, and the whiskers extend to the most extreme datapoint not considered outliers (more than 2.7 standard deviations away from the mean for normally distributed data). Subject measurements of normalized LLRa for each trial are plotted as dots. Conditions that are significantly different from the Pert condition are indicated with an asterisk. The significance threshold is 0.05, corrected using the Steel's method.

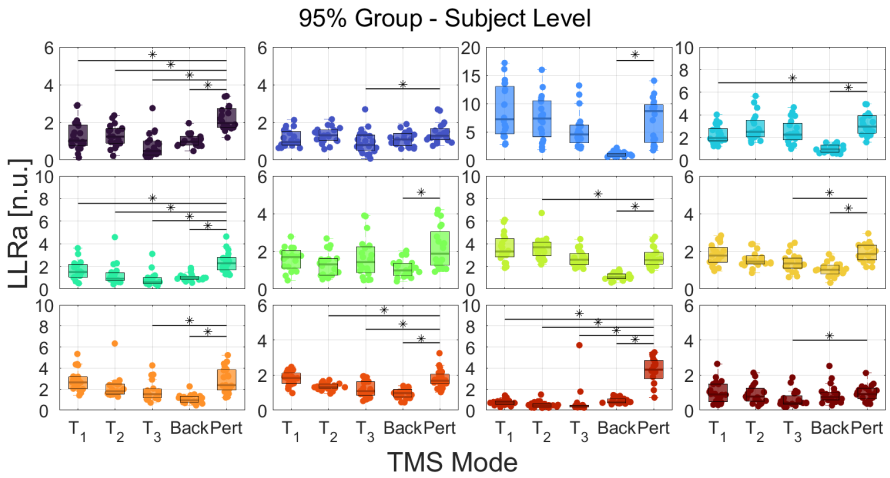

Figure 9: Subject-level results of the effect of TMS mode on the normalized long-latency response amplitude (LLRa) for the 95% AMT intensity group.

Time series analysis

The two-way repeated measures ANOVA revealed that TMS mode had a significant effect on the normalized EMG response prior to perturbation onset, immediately before and after perturbation onset, and during the LLR period. Specifically, there were significant intervals observed from -74.0ms to -38.2ms, -30.7ms to 13.7ms, and 64.1ms to 105.6ms relative to perturbation onset (local peaks at t=-59.0 ms, F=20.5; t=-25.1 ms, F=13.8; t=4.7 ms, F=14.7;and t=81.5 ms, F=20.5, respectively). There were no significant intervals from the time series analysis for the factors of TMS intensity (I) and the interaction between TMS intensity and TMS mode (I*T). Because the TMS stimuli were delivered at different times relative to perturbation onset in the different conditions, post-hoc analysis was performed on TMS modes, individually, to further break down the levels that resulted in significant differences.

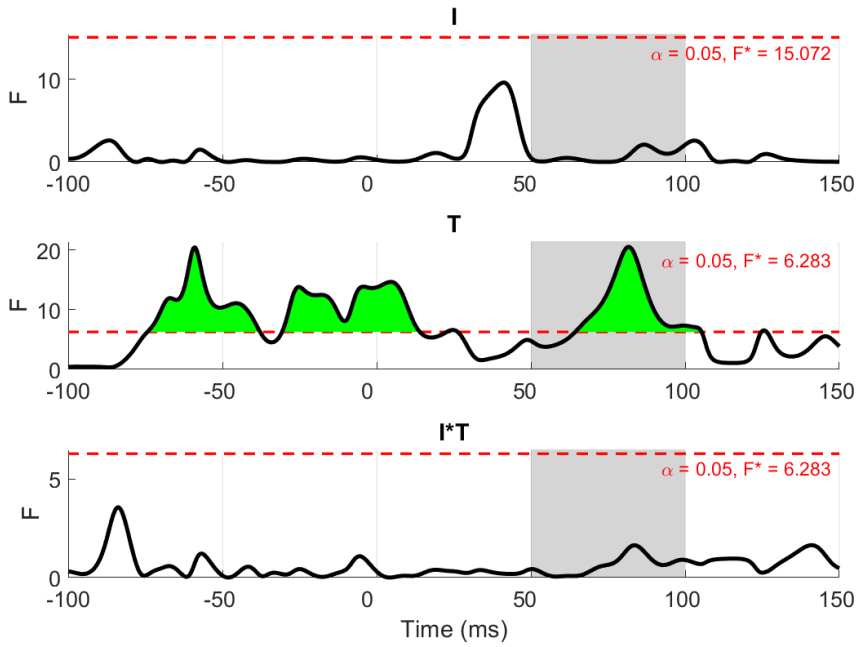

Figure 10: Time series Analysis - Two-way ANOVA for the effect of TMS Intensity (I), TMS mode (T), and their interaction (I*T) on the normalized EMG response from -100ms to 150ms relative to perturbation onset for all subjects. Alpha value is equal to 0.05. The F ratio threshold is indicated by the horizontal red line and the threshold value is given at the top right of each plot.

In T_1 TMS mode, the EMG response significantly increased prior to the LLR period from -23.8ms to 29.8ms (local peak at t=-7.8 ms, T=12.0), reflecting the excitatory effect of TMS stimulation. The EMG response significantly decreased during the LLR period from 79.4ms to 86.8ms (local peak at t=83.0 ms, T=-4.1) and during voluntary response from 134.4 ms to 150.0 ms (local peak at t=150.0 ms, T=-4.1). The T_2 TMS mode significantly increased the EMG response prior to the LLR period from -52.8ms to 10.8ms (local peak at t=-26.8 ms, T=12.2). In T_2 TMS mode, EMG response significantly decreased during the LLR period from 53.8 ms to 92.0 ms (local peak at t=82.0 ms, T=-6.51) and during the voluntary response from 97.4 ms to 104.8 ms (local peak at t=101.0 ms, T=-4.60). In the T_3 TMS mode, the EMG response was significantly increased prior to perturbation onset, from -78.0 ms to -16.0 ms (local peak at t=-58.2 ms, T=13.1). The EMG response was significantly decreased during and after the LLR period, from 34.2 ms to 150 ms (local peaks at t=82.6 ms, T=-11.71).

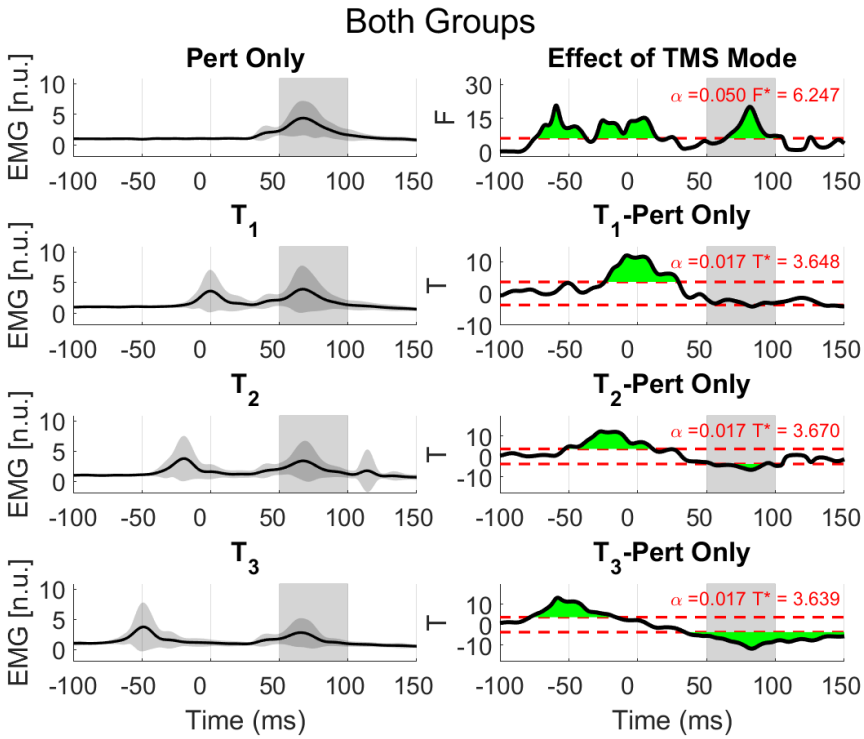

Figure 11: Time series Analysis - One-way rmANOVA for the effect of TMS mode with post-hoc tests from -100 ms to 150 ms relative to perturbation onset for all subjects. Group mean normalized EMG responses for each TMS mode are displayed on the left panels. The F scores for the effect of TMS mode are displayed on the top right panel for each time point. T test scores resulting from post-hoc time series pairwise comparisons between each TMS mode and the control mode (PertOnly) are displayed on the right bottom three panels.

For the 90% AMT intensity, from the one-way rmANOVA for the effect of TMS mode, TMS mode had a significant effect on the normalized EMG response prior to perturbation onset, during the LLR period, and during the voluntary response period. Specifically, TMS mode had a significant effect from -61.2 ms to -56.2 ms, 75.7 ms to 87.3 ms, and 134.0 ms to 148.8 ms relative to perturbation onset (local peaks at t=-59.0 ms, F=10.2; t=81.7 ms, F=11.9; t=143.2 ms, F=10.0, respectively).

From the time series post-hoc analysis, the normalized EMG response significantly increased in T_1 prior to the LLR period, from -23.4 ms to 27.4 ms (local peak at t=-8.2 ms, T=8.6) and significantly decreased during voluntary response from 136.6 ms to 145.0 ms (local peak at t=139.8 ms, T=-3.9). In the T_2 mode, EMG response was significantly increased prior to the LLR period from -41.2 ms to 6.2 ms (local peak at t=-27.4ms, T=8.61). EMG responses

significantly decreased during and after the LLR period from 70.2 ms to 86.6 ms and 134.2 ms to 147.4 ms (local peaks t=76.4 ms, T=-4.6; t=141.4 ms, T=-4.4, respectively). In the T_3 mode, the EMG response significantly increased prior to perturbation onset from -74.4ms to -14.0ms (local peak at t=-58.6 ms, T=9.5) and significantly decreased during the LLR period, from 54.6 ms to 150.0 ms (local peak at t=82.2 ms, T=-9.3).

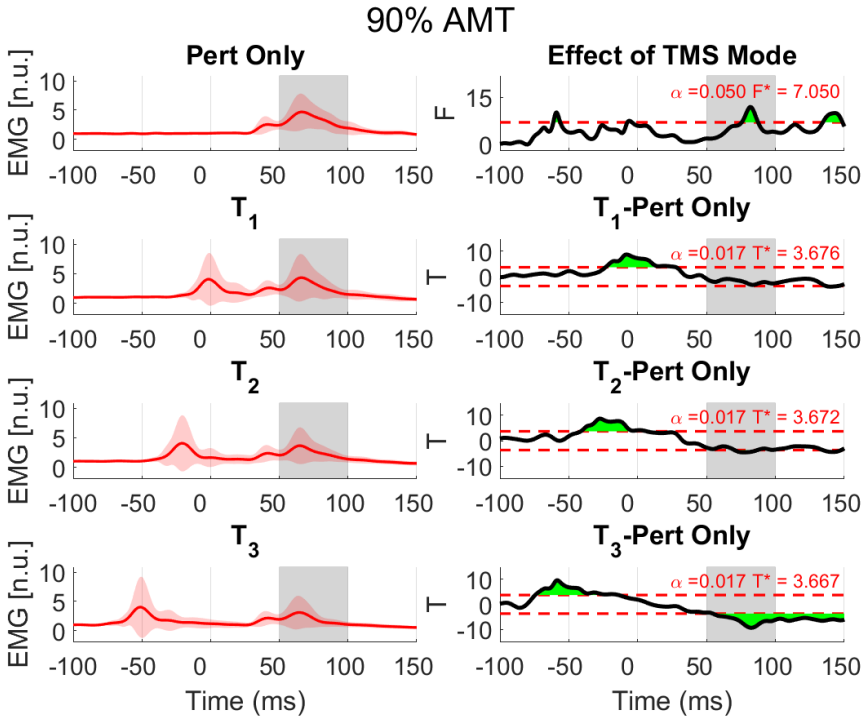

Figure 12: Time series Analysis - One-way rmANOVA for the effect of TMS mode in the 90% AMT group with post-hoc tests from -100ms to 150ms relative to perturbation onset. Group mean normalized EMG response for each TMS mode are displayed on the left panels. The F scores for the effect of TMS mode are displayed on the top right panel for each time point. T test scores resulting from post-hoc time series pairwise comparisons between each TMS mode and the control mode (PertOnly) are displayed on the right bottom three panels.

For the 95% AMT intensity, from the one-way rmANOVA, TMS mode had a significant effect on the normalized EMG response prior to perturbation onset and during the LLR period. Specifically, TMS mode had a significant effect from -67.6 ms to -46.0 ms, -27.8 ms to -15.0 ms, and -5.4 ms to 7.6 ms relative to perturbation onset (local peaks at t=-54.0 ms, F=17.3; t=-24.0 ms, F=12.6; t=-0.9 ms, F=10.4, respectively). EMG response was significantly modulated during the LLR period, 75.8 ms to 90.0 ms (local peak at t=79.9 ms, F=8.95).

From the time series post-hoc analysis, the normalized EMG response significantly increased in T_1 prior to the LLR period, from -20.2 ms to 27.8 ms (local peak at t=-1.0 ms, T=11.8). In the T_2 mode, EMG response was significantly increased prior to the LLR period from -54.6 ms to -45.0 ms and -41.8 ms to 8.6 ms (local peak at t=-50.8ms, T=5.4; t=-21.8ms, T=11.2, respectively). In the T_2 mode, EMG response was significantly decreased during the LLR period from 58.8 ms to 65.5 ms and 77.6 ms to 89.4 ms, and during the voluntary response from 103 ms to 103.4 ms (local peak at t=62.4 ms, T=-4.3; t=83.6 ms, T=-5.6; t=103.2 ms, T=-3.7, respectively). In the T_3 mode, the EMG response significantly increased prior to perturbation onset from -83.6 ms to -76.4 ms and -69.2 ms to -24.0 ms (local peaks at t=-80.4 ms, T=4.2; t=-53.4 ms, T=13.5), and significantly decreased during the LLR period, from 35.2 ms to 130.6ms (local peak at t=83.2 ms, T=-7.4).

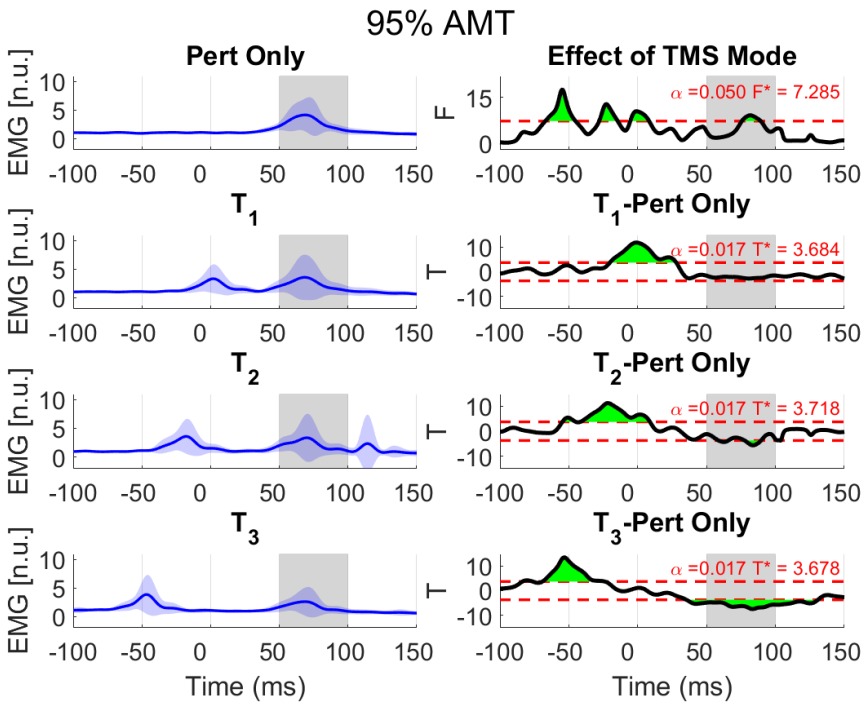

Figure 13: Time series Analysis - One-way rmANOVA for the effect of TMS mode in the 95% AMT group with post-hoc tests from -100ms to 150ms relative to perturbation onset. Group mean normalized EMG response for TMS mode plotted on the left. The F scores for each time point of the effect of TMS mode are displayed plotted on the top right. Post-hoc time series analysis results for each TMS mode compared to the control mode (PertOnly) plotted on the right bottom three panels.

DISCUSSION

The primary objective of this study was to determine whether *subthreshold* TMS stimulus can significantly reduce the LLRa in the FCR muscle via the silent period and identify the *subthreshold* stimulus intensity and latency that resulted in the greatest reduction of the LLRa. We used surface EMG to record the LLR in the FCR muscle at different levels of TMS intensity and TMS latency relative to perturbation onset. We applied *subthreshold* single pulse TMS to the left primary motor cortex to modulate corticospinal excitability during the LLR. We used a wrist robot, the MR-StretchWrist, to elicit velocity-controlled perturbations and evoke stretch responses in the FCR muscle. We found a significant effect of TMS on the perturbation-evoked LLR, indicating that changes in the LLR were associated with *subthreshold* TMS application. Specifically, we found that TMS timing had a significant effect on the LLRa, indicating that the ability of TMS to reduce the LLR is dependent on when the TMS pulse is applied relative to the onset of a perturbation. We found no significant effect of TMS intensity on the LLRa, suggesting that the two chosen *subthreshold* TMS intensities (90% and 95% of the active motor threshold - AMT) result in a similar cortical inhibition when applied at a specific timing prior to perturbation onset. Via a 1D time series analysis, we found a sustained effect of TMS on the LLR, suggesting that the inhibition is based on processing delays and silent period duration. Thus, the findings of this study highlight the inhibitory effect of *subthreshold* TMS when applied prior to perturbation onset on the LLR in the FCR muscle. Knowledge in this study is crucial in understanding how TMS can be used to decouple the contribution of cortical and subcortical contributions to the long-latency response in the forearm and provide insight for the design of *subthreshold* TMS protocols integrated with functional neuroimaging.

Effect of TMS Latency on the Long-Latency Response

In our study, we found that *subthreshold* TMS can significantly reduce the LLR in the FCR when TMS is applied prior to perturbation onset. Specifically, we found that *subthreshold* single-pulse TMS applied such that the MEP peak would arrive at the muscle at either 20 or 50 ms prior to perturbation onset significantly reduced the LLRa compared to the perturbation only control. However, there was not a significant reduction in the LLRa when the MEP peak was timed to arrive at the muscle at 0 ms prior to perturbation onset. Our data support the idea that the ability of *subthreshold* single-pulse TMS to inhibit the LLR is time-dependent. Previous studies have shown that *suprathreshold* TMS stimulus significant reduces the LLR when applied 50 ms prior to perturbation onset in both the biceps brachii and the extensor carpi radialis (35, 36). Additionally, when TMS is applied such that the MEP arrives during the SLR onset, both *subthreshold* and *suprathreshold* TMS result in reduction in the LLRa (5, 37). This evidence indicates that as long as there is a sufficient delay between the silent period onset and the LLR onset, both *subthreshold* and

suprathreshold TMS can significantly reduce the LLRa. This time-dependent effect of TMS can be explained by the neuromuscular delays following TMS application. There is an initial inhibition attributed to the motoneuron after-hyperpolarization of spinal origin, 50 ms to 80 ms following MEP offset (29). The later component of the silent period, 80 ms to roughly 150 ms following MEP offset, is suggested to be due to activation of GABA inhibitors which reduce the excitability of cortical motor pathways (29, 26, 39). This may explain why the LLRa reduction increased with latency and why it was greatest in the T_3 condition. In previous studies, TMS reduced the LLRa with roughly a 38.3% reduction in the biceps brachii and 32% reduction in the ECR when applied 50 ms prior to perturbation onset (35, 36). These findings align with our T_2 condition where the MEP arrives 20 ms prior to perturbation onset and results in a 36.3% LLR reduction. In our findings, *subthreshold* TMS reduced the LLRa to 21.8% for T_1, 36.3% for T_2, and 57.0% for T_3 compared to the control condition. This suggests there is a minimum latency required between the MEP peak and the LLR for significant reduction to occur following *subthreshold* TMS stimulation. In addition, *subthreshold* TMS applied too early would led to the extinction of the silent period prior to the LLR. All this taken together, there exists a specific TMS latency window needed to overlay the cortical component of the silent period with the LLR to induce significant inhibition. From this study, it has been shown that *subthreshold* TMS applied such that the MEP arrives at the muscle 50 ms prior to perturbation onset results in the greatest reduction of the LLRa in the FCR muscle.

Effect of TMS Intensity on the Long-Latency Response

In this study, we found that both *subthreshold* TMS intensities of 90% and 95% AMT resulted in a similar reduction of the LLR when applied prior to perturbation onset. From our findings, we found an LLR reduction of 21.5% for T_1, 36.3% for T_2, and 57% for T_3 which aligns with the degree of inhibition observed in previous studies (35, 36, 33, 34). When applied 50 ms prior to perturbation onset, *suprathreshold* TMS reduces the LLR amplitude by 38.3% in the biceps brachii and 32% in the ECR for a stiff, do not intervene condition (35, 36). Furthermore, *suprathreshold* TMS applied at the same timing reduces the LLR modulation by 60% in the pectoralis and 31.5% in the FCR. (33, 34). When TMS is applied during the SLR onset, there is a 15% reduction in the LLR for *suprathreshold* TMS and a 43% reduction for *suprathreshold* TMS (5). However, it is possible that this inhibition is likely due to spinal inhibition lasting approximately 50-80 ms post MEP offset.

In our study, since the difference in TMS intensites was 5% AMT, the difference may not have been large enough to cause a significant change in the degree of inhibition in the LLRa. It is suggested that higher *subthreshold* TMS intensities would activate more inhibitory motor neurons and result in a longer silent period duration compared to lower *subthreshold* TMS intensities (40, 41, 42, 43). This may explain why with higher TMS intensities the silent period duration increases and thus the resulting LLR reduction would be greater over a speci-

fied time period. Future research needs to be done to determine if higher TMS intensities lead to a greater reduction in LLR when applied at the same time prior to perturbation onset.

In this study, the interaction of TMS intensity and latency did not significantly affect the LLRa, suggesting that the effect of TMS timing on the LLRa is not affected by the TMS intensity applied or vice versa. It is important to note that both of the TMS intensities were *subthreshold* and were elicited prior to perturbation onset. Therefore, it is important to consider both the TMS stimulus intensity and the TMS timing in regards to the effects of TMS on the LLR. By specifically targeting the motor cortex with TMS, we were successfully able to modulate the excitability of the corticospinal tract on muscle activity in the FCR. From the results of this study, we advise stimulating the motor cortex at either 90% or 95% AMT intensity at a timing such that MEP peak arrives at the muscle 50 ms prior to perturbation onset to significantly reduce the CST contribution to the LLR via the cortical silent period.

Effect of *Subthreshold* TMS on the Entire EMG Response

We conducted a 1D time series analysis to determine the effect of *subthreshold* TMS on the FCR EMG response starting at a time during background contraction through the entire LLR period. First, there was an increase in the EMG response prior to perturbation onset associated with the TMS-induced MEP. This is due to the fact that the *subthreshold* TMS stimulus intensity was close to the AMT thus it is likely that an observable MEP will be elicited in some cases. In all of the TMS conditions, there was not a significant effect of TMS during the SLR period from 20 ms to 50 ms following perturbation onset which aligns with the findings from previous studies (37, 35, 5). Additionally, this supports the idea that the inhibitory effect of *subthreshold* TMS is not purely of spinal origin. With regards to the LLR period, there was a significant decrease in the EMG response throughout the LLR and voluntary response period and this decrease was sustained until a gradual return of EMG activity. This result shows that the inhibition was present throughout the LLR period rather than only at a specific instance.

Previous studies also show a sustained inhibition in the LLR following TMS stimulation. There was a noticeable sustained decreased in EMG response throughout the LLR period following TMS compared to the control in the FCR muscle (5). Following *suprathreshold* TMS applied 50 ms prior to perturbation onset, there is a sustained EMG inhibition during the LLR period that returns approximately 20 ms into the voluntary response period (36). *Subthreshold* TMS results in sustained EMG reduction only during the LLR period and diminishes during the voluntary response onset in the tibialis anterior (37). In aggreement with our findings, these studies show that the LLR inhibition due to *subthreshold* TMS is sustained throughout the LLR period.

Significance for Future Work

From our results, the LLRa never completely diminishes under any *subthreshold* TMS latencies, suggesting continued supraspinal contributions during corticospinal inhibition. It has been shown that the LLR has spinal, subcortical, and cortical components (5, 6). This suggests that the LLRa did not completely diminish during cortical inhibition because of the sustained contribution of spinal and subcortical circuits (5). This suggests that subcortical circuits, such as the reticulospinal tract, may be capable of contributing to LLRs independently from cortical input. This finding suggests the RST may contribute to functional motor behavior during conditions of reduced corticospinal signal, possibly including those resulting from such lesions responsible for upper motor neuron syndrome. However, the clinical implications of these findings need to be confirmed in a population with corticospinal impairment. Additionally, our findings support the idea that LLRs may serve as an effective way to elicit motor responses in the reticulospinal tract. Future directions of this work involve performing this TMS protocol with functional neuroimaging to quantify the cortical and subcortical brain areas associated with *subthreshold* TMS, wrist perturbations, and LLR inhibition.

Study Limitations

In this study, there are some limitations that should be considered in the discussion of the results. One limitation of this study is that subjects may have moved during the experimental procedure relative to the start of the experiment. If the subject moved >4 mm or rotated >15°, movement may result in stimulating the forearm motor hot spot with a lower TMS intensity than desired result in lower cortical inhibition. Alternatively, head movements could result in a better location for stimulating the forearm motor hot spot resulting in a higher TMS intensity than desired. We tracked head movement throughout the experiment and the active motor threshold determination procedure. If the subject moved >4 mm in any direction or rotated >15° in any plane relative to the starting position and orientation, the TMS coil was re-localized to be placed over the forearm motor hotspot and the AMT was confirmed. In order to minimize subject head movement, subjects were given a chin support to support the weight of their head and keep it in position during the experiment.

Another limitation in our study is the possibility that no stretch responses occurred in the forearm during wrist perturbations or the stretch responses were not larger enough compared to background contraction activity. This could be because subjects might not have been activating their FCR muscle enough during background contraction or the velocity of the perturbation was not large enough to induce a high stretch response in that muscle. On the other hand, it is possible that some subjects could have resisted some perturbations. When resisting a perturbation, there will be a higher long-latency response then when yielding to a perturbation. Ideally, this would not have occurred in all perturbations but may have occurred in some. This would result in the

inhibitory effect of TMS not being appropriately quantified in the long-latency response amplitude. To try to minimize the potential for participants to resist perturbations, they were cued to yield to the perturbation before the start of each trial.

A third limitation in our study is the participant's body energy levels could have had an effect on how excitable their corticospinal tract was to TMS. For example, participants who drink coffee in the morning would be more sensitive to TMS and have an AMT lower than they normally would without caffeine in their system. Alternatively, if participants did not get sufficient sleep or were in a fatigued state, their AMT might be higher than usual on that specific day. To account for this, we instructed participants to avoid drinking coffee or working out during the day of the experimental session. No participant verbally reported of having drunk coffee or working out prior to their data collection session.

Conclusions

The objective of this study was to determine the TMS parameters, intensity and latency relative to perturbation onset, that significantly reduced the long-latency response amplitude in the FCR muscle when applied to the motor cortex. We found that the ability of *subthreshold* TMS to reduce the LLRa depends on the latency when *subthreshold* TMS is applied prior to perturbation onset. Furthermore, during the LLR period following TMS, there remains a positive LLRa suggesting the involvement of subcortical brain regions or spinal circuitry in generation of the LLR during reduced excitability of the corticospinal tract. Further studies need to be done to determine the location of the cortical component of the silent period induced by TMS during the LLR. Additionally, future work needs to be conducted to determine the role of subcortical brain regions in the contribution to the LLR during the silent period. Knowledge from these studies could provide further information on how TMS modulates corticospinal excitability and how subcortical brain regions contribute to functional motor output during reduced corticospinal excitability. The findings of these studies would provide crucial information on the role of secondary motor pathways such as the reticulospinal tract on the generation of motor commands during reduced cortical input.

ACKNOWLEDGEMENTS

This research was funded by Delaware CTR ACCEL and the Delaware INBRE program.

References

[1] Peter B.C. Matthews. The human stretch reflex and the motor cortex. *Trends in Neurosciences*, 14(3):87–91, 1991.

[2] T. Sinkjaer, E. Toft, S. Andreassen, and B. C. Hornemann. Muscle stiffness in human ankle dorsiflexors: Intrinsic and reflex components. *Journal of Neurophysiology*, 60(3):1110–1121, 1988.

[3] Isaac L. Kurtzer. Long-latency reflexes account for limb biomechanics through several supraspinal pathways. *Frontiers in Integrative Neuroscience*, 8(JAN):1–19, 2015.

[4] J. Andrew Pruszynski and Stephen H. Scott. Optimal feedback control and the long-latency stretch response. *Experimental Brain Research*, 218(3):341–359, 2012.

[5] Gwyn N. Lewis, Melody A. Polych, and Winston D. Byblow. Proposed cortical and sub-cortical contributions to the long-latency stretch reflex in the forearm. *Experimental Brain Research*, 156(1):72–79, 2004.

[6] J. Andrew Pruszynski, Isaac Kurtzer, and Stephen H. Scott. The long-latency reflex is composed of at least two functionally independent processes. *Journal of Neurophysiology*, 106(1):449–459, 2011.

[7] E. Palmer and P. Ashby. Evidence that a long latency stretch reflex in humans is transcortical. *The Journal of Physiology*, 449(1):429–440, 1992.

[8] CGM Meskers MJL Perenboom, M Van de Ruit, JH De Groot, AC Schouten. Evidence for sustained cortical involvement in peripheral stretch reflex during the full long latency reflex period. *Neuroscience*, 584:214–218, 2015.

[9] N. Mrachacz-Kersting, M. J. Grey, and T. Sinkjær. Evidence for a supraspinal contribution to the human quadriceps long-latency stretch reflex. *Experimental Brain Research*, 168(4):529–540, 2006.

[10] Isaac L. Kurtzer, J. Andrew Pruszynski, and Stephen H. Scott. Long-Latency Reflexes of the Human Arm Reflect an Internal Model of Limb Dynamics. *Current Biology*, 18(6):449–453, 2008.

[11] C. Capaday, R. Forget, R. Fraser, and Y. Lamarre. Evidence for a contribution of the motor cortex to the long-latency stretch reflex of the human thumb. *The Journal of Physiology*, 440(1):243–255, 1991.

[12] P. D. Cheney and E. E. Fetz. Corticomotoneuronal cells contribute to long-latency stretch reflexes in the rhesus monkey. *The Journal of Physiology*, 349(1):249–272, 1984.

[13] Vaughan G. Macefield, John C. Rothwell, and Brian L. Day. The contribution of transcortical pathways to long-latency stretch and tactile reflexes in human hand muscles. *Experimental Brain Research*, 108(1):147–154, 1996.

[14] N Petersen, L O D Christensen, H Morita, T Sinkjòr, J Nielsen, Physiologisches Institut, and Christian-albrechts-universit Kiel. Tjp0512-0267. pages 267–276, 1998.

[15] Robert A. Davidoff. Skeletal muscle tone and the misunderstood stretch reflex. *Neurology*, 42(5):951–963, 1992.

[16] Vengateswaran J. Ravichandran, Claire F. Honeycutt, Jonathan Shemmell, and Eric J. Perreault. Instruction-dependent modulation of the long-latency stretch reflex is associated with indicators of startle. *Experimental Brain Research*, 230(1):59–69, 2013.

[17] Jonathan Shemmell, Matthew A. Krutky, and Eric J. Perreault. Stretch sensitive reflexes as an adaptive mechanism for maintaining limb stability. *Clinical Neurophysiology*, 121(10):1680–1689, 2010.

[18] Yonas Akalu, Ashlyn K. Frazer, Glyn Howatson, Alan J. Pearce, Ummatul Siddique, Mohamad Rostami, Jamie Tallent, and Dawson J. Kidgell. Identifying the role of the reticulospinal tract for strength and motor recovery: A scoping review of nonhuman and human studies. *Physiological Reports*, 11(14):1–26, 2023.

[19] Claire Fletcher Honeycutt, Michael Kharouta, and Eric Jon Perreault. Evidence for reticulospinal contributions to coordinated finger movements in humans. *Journal of Neurophysiology*, 110(7):1476–1483, 2013.

[20] Christopher J. Forgaard, Ian M. Franks, Kimberly Bennett, Dana Maslovat, and Romeo Chua. Mechanical perturbations can elicit triggered reactions in the absence of a startle response. *Experimental Brain Research*, 236(2):365–379, 2018.

[21] Christopher J. Forgaard, Ian M. Franks, Dana Maslovat, Laurence Chin, and Romeo Chua. Voluntary reaction time and long-latency reflex modulation. *Journal of Neurophysiology*, 114(6):3386–3399, 2015.

[22] J.C. Rothwell. The startle reflex, voluntary movement, and the reticulospinal tract. *Supplements to Clinical Neurophysiology*, 58:223–231, 2006.

[23] Andrea Zonnino, Andria J. Farrens, David Ress, and Fabrizio Sergi. Measurement of stretch-evoked brainstem function using fMRI. *Scientific Reports*, 11(1):1–21, 2021.

[24] Ulf Ziemann, Johannes Netz, Andrea Szelényi, and Volker Hömberg. Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neuroscience Letters*, 156(1-2):167–171, 1993.

[25] Sergiu Groppa, Boris H. Schlaak, Alexander Münchau, Nicole Werner-Petroll, Janin Dünnweber, Tobias Bäumer, Bart F.L. van Nuenen, and Hartwig R. Siebner. The human dorsal premotor cortex facilitates the excitability of ipsilateral primary motor cortex via a short latency cortico-cortical route. *Human Brain Mapping*, 33(2):419–430, 2012.

[26] K. E. Hupfeld, C. W. Swanson, B. W. Fling, and R. D. Seidler. TMS-induced silent periods: A review of methods and call for consistency. *Journal of Neuroscience Methods*, 346(June):108950, 2020.

[27] M Inghillerj, A Berardelli, G Cruccu, and M Manfredi. Silent Period Evoked By Transcranial Stimulation of. *Journal of Physiology*, 466:521–534, 1992.

[28] B. W. Connors, R. C. Malenka, and L. R. Silva. Two inhibitory postsynaptic potentials, and GABAA and GABAB receptor-mediated responses in neocortex of rat and cat. *The Journal of Physiology*, 406(1):443–468, 1988.

[29] Jakob Škarabot, Ricardo N.O. Mesquita, Callum G. Brownstein, and Paul Ansdell. Myths and Methodologies: How loud is the story told by the transcranial magnetic stimulation-evoked silent period? *Experimental Physiology*, 104(5):635–642, 2019.

[30] K. Krnjevij, Mirjana Randiot, and D W Straughant. An Inhibitory Process in the Cerebral Cortex. *Journal of Physiology*, 184:16–48, 1966.

[31] Michelle N. McDonnell, Yuri Orekhov, and Ulf Ziemann. The role of GABAB receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research*, 173(1):86–93, 2006.

[32] Nicolas Lang, Elke Sueske, Alkomiet Hasan, Walter Paulus, and Frithjof Tergau. Pregabalin exerts oppositional effects on different inhibitory circuits in human motor cortex: A double-blind, placebo-controlled transcranial magnetic stimulation study. *Epilepsia*, 47(5):813–819, 2006.

[33] Toshitaka Kimura, Patrick Haggard, and Hiroaki Gomi. Transcranial magnetic stimulation over sensorimotor cortex disrupts anticipatory reflex gain modulation for skilled action. *Journal of Neuroscience*, 26(36):9272–9281, 2006.

[34] L. Spieser, S. Aubert, and M. Bonnard. Involvement of SMAp in the intention-related long latency stretch reflex modulation: A TMS study. *Neuroscience*, 246:329–341, 2013.

[35] Jonathan Shemmell, Hi An Je, and Eric J. Perreault. The differential role of motor cortex in stretch reflex modulation induced by changes in environmental mechanics and verbal instruction. *Journal of Neuroscience*, 29(42):13255–13263, 2009.

[36] Jonathan Fox and Jonathan Shemmell. The ipsilateral motor cortex does not contribute to long-latency stretch reflex amplitude at the wrist. *Brain and Behavior*, 4(1):60–69, 2014.

[37] Johan Van Doornik, Yoshihisa Masakado, Thomas Sinkjaer, and Jens Bo Nielsen. The suppression of the long-latency stretch reflex in the human tibialis anterior muscle by transcranial magnetic stimulation. *Experimental Brain Research*, 157(3):403–406, 2004.

[38] A. Pascual-Leone, L. G. Cohen, J. P. Brasil-Neto, and M. Hallett. Non-invasive differentiation of motor cortical representation of hand muscles by mapping of optimal current directions. *Electroencephalography and Clinical Neurophysiology/ Evoked Potentials*, 93(1):42–48, 1994.

[39] S. A. Wilson, R. J. Lockwood, G. W. Thickbroom, and F. L. Mastaglia. The muscle silent period following transcranial magnetic cortical stimulation. *Journal of the Neurological Sciences*, 114(2):216–222, 1993.

[40] King Hee Ho, Kannan Nithi, and Kerry R. Mills. Covariation between human intrinsic hand muscles of the silent periods and compound muscle action potentials evoked by magnetic brain stimulation: Evidence for common inhibitory connections. *Experimental Brain Research*, 122(4):433–440, 1998.

[41] Janet L. Taylor, Gabrielle M. Allen, Jane E. Butler, and S. C. Gandevia. Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimulation. *Experimental Brain Research*, 117(3):472–478, 1997.

[42] V. Di Lazzaro, D. Restuccia, A. Oliviero, P. Profice, L. Ferrara, A. Insola, P. Mazzone, P. Tonali, and J. C. Rothwell. Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Experimental Brain Research*, 119(2):265–268, 1998.

[43] Ulf Ziemann, John C. Rothwell, and Michael C. Ridding. Interaction between intracortical inhibition and facilitation in human motor cortex. *Journal of Physiology*, 496(3):873–881, 1996.