Motor training modulates intracortical inhibitory dynamics in motor cortex during movement preparation

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Running Head: GABA dynamics in motor training

Abstract

Background: The primary motor cortex (M1) has a vital role to play in the learning of novel motor skills. However, the physiological changes underpinning this learning, particularly in terms of dynamic changes during movement preparation, are incompletely understood. In particular, a substantial decrease in resting gamma-amino butyric acid (GABA) activity, i.e. a release of resting inhibition, is seen within M1 as a subject prepares to move. Although there is evidence that a decrease in resting inhibition occurs within M1 during motor learning it is not known whether the pre-movement "release" of GABAergic inhibition is modulated during skill acquisition.

Objective: Here, we investigated changes in pre-movement GABAergic inhibitory "release" during training on a motor skill task.

Methods: We studied $GABA_A$ activity using paired-pulse TMS (Short-Interval Intracortical Inhibition (SICI)) during training on a ballistic thumb abduction task, both at rest and at two time-points during movement preparation.

Results: Improvement in task performance was related to a later, steeper, release of inhibition during the movement preparation phase. Specifically, subjects who showed greater improvement in the task in the early stages of training showed a reduced level of GABAergic release immediately prior to movement compared with those who improved less. Later in training, subjects who performed better showed a reduction in GABAergic release early in movement preparation.

Conclusions: These findings suggest that motor training is associated with maintained inhibition in motor cortex during movement preparation.

Keywords

GABA

Motor cortex

Motor training

Movement preparation

Transcranial Magnetic Stimulation

Plasticity

Introduction

How we perform movements and, through practice, improve that performance is a fundamental question in neuroscience. The primary motor cortex (M1) acts as the major output module for voluntary movements, but also has an important role in the learning and consolidation of motor skills [1-3]. Animal studies have demonstrated the substantial reorganization of M1 as a consequence of motor learning [4,5], something that has been echoed in humans studies using TMS [6,7] and functional MRI [8,9]. However, the physiological processes that underpin this reorganization remain only partially understood.

Learning a novel motor skill has been suggested, at least in some tasks, to lead to a lasting increase in corticospinal excitability [10] with a concomitant reduction in resting intracortical inhibition [11]. The finding that inhibition is reduced after learning is consistent with Magnetic Resonance Spectroscopy (MRS) [12] and pharmacological [13] studies showing that decreases in GABA are associated with improvements in performance on motor learning tasks.

In addition, dynamic changes in GABAergic signaling in M1 have been linked to movement preparation, initiation and termination [14-16], where a significant release of resting inhibition occurs as a subject prepares to move. However, although much ground has been made unraveling the importance of GABA modulation for motor learning it is only understood at a broad temporal level, and changes in inhibitory dynamics, crucial for allowing movement to occur, are less understood. Here, we therefore aim to investigate how changes in inhibitory dynamics occur throughout the training on a motor task. To this end, we measured short interval intracortical inhibition (SICI), a paired pulse transcranial magnetic stimulation (ppTMS) approach that is sensitive to $GABA_A$-synaptic mediated inhibition [17,18] while participants performed a simple ballistic motor training task [19].

Materials and Methods

Participants

Nineteen healthy participants (age: 25.53 years ± 4.67 (20–40 years), 13 female, all right handed) gave full written informed consent to participate in the experiment in accordance with local ethics committee approval. Before the experiment commenced, each participant was screened for contraindications as laid out in established TMS guidelines [20].

Behavioural Task

Participants performed a ballistic thumb abduction training task (Muellbacher et al. 2001) that required the abduction of their left (non-dominant) thumb with maximal acceleration [21-23]. The behavioural task was separated into four blocks (figure 1A) with each training block, which contained no TMS, being interleaved with a TMS-block from which no performance data was acquired. All blocks were separated by a break of at least 3 minutes to minimize fatigue caused by repeated movements. All blocks containing TMS required participants to make movements at a rate of 0.25 Hz, whilst blocks containing no TMS had a faster rate of movement at 0.5 Hz [11]. The slower rate was imposed in the blocks containing TMS based on pilot experiments, to minimize the level of background muscle contraction that might result from repeating a ballistic movement in quick succession. Each block consisted of 120 trials, with a 30 second break between every 40 trials to avoid within block fatigue.

Participant's left arms were placed on a customized wooden board in the supine position. The left hand was chosen in an attempt to avoid ceiling effects that might be present in the dominant hand. The wrist, metacarpophalangeal and distal interphalangeal joints were fastened with Velcro straps to minimise the unintentional contribution of whole hand movement to the

ballistic acceleration, though the thumb was left free to move. The accelerometer was fastened to the distal phalanx of the thumb. Recording from the accelerometer was confined to one axis, which encompassed the vertical abduction of the thumb. This approach allows for good skill improvement by providing simplified feedback for the participant [11], but as it only measures performance in one axis, we are not able to comment on changes in accuracy of the movements.

The movement of the ballistic thumb abduction was paced using a ready-steady-go procedure, with each of three beeps (400Hz, 300ms duration) spaced at 500 ms intervals (figure 1B). Participants were instructed to move their thumbs at the onset of the third beep.

In all blocks except the baseline block, participants were instructed to move as fast as possible and were encouraged to try to increase their acceleration on every trial. Participants were given visual feedback about the acceleration of their movements on a trial-by-trial basis (see figure 1C). Feedback was presented as a scrolling bar chart with the magnitude of the current acceleration plotted after each trial. If the acceleration on the current trial was greater than on the previous trial, the bar was plotted in green, and if it was less the bar was plotted in red. If a movement was made too early or too late (i.e. movement outside a 300 ms window centered on one second after the first tone), no acceleration feedback was given. Instead, the message "too early" or "too late" respectively was presented. Additionally, participants were informed of their progress by displaying a moving average of acceleration values over the preceding 20 trials, indicated by a line plotted on screen over the locations of the 20 consequential trials.

In the baseline block, participants were told to move as closely as possible to the onset of the third tone, and feedback about the temporal accuracy of the movement was given by the experimenter, based on the onset of EMG activity, which was visible on a monitor out of the subject's view.

As we wanted to interrogate inhibition at different stages of movement preparation throughout training, TMS was delivered at three different time points relative to movement onset. In TMS blocks (figure 1A; Baseline, T+TMS1, T+TMS2, T+TMS3) there were 7 different trial types: (1) No TMS, (2) TMS at rest (which occurred 200 ms before the first tone, (3) TMS at 25% of pre-movement time (i.e. 25% of 1s = 250 ms after the first tone) and (4) TMS at 75% of pre-movement (i.e. 75% of 1s = 750 ms after the first tone). TMS was delivered as a single TMS pulse (spTMS) in 50% of cases and as paired TMS pulses (ppTMS) in 50% (see later). Within every block of 120 trials there were on average 17 trials of each condition. The trials were performed in a pseudo-random order; where each of the 7 trial types was presented in a random order before any were repeated.

The remaining blocks (T1, T2 and T3) were regarded as "training-only blocks" and trials were completed without TMS application; here movement was unperturbed by TMS and thus feedback was more reliable.

Behavioural Data Analysis

All acceleration data were imported to Matlab for analysis. To investigate training, data from the training-only blocks (T1, T2 and T3) were analysed, as performance in these blocks was free from interference from the TMS pulses. For each trial, the maximal acceleration was calculated and any trials with a maximum acceleration less than 4.9m/s^2 were rejected. Additionally, if movements were made too early or too late, i.e the onset of the acceleration of the movement lay more than 300 ms before or after the expected movement time, they were also rejected. Together, this approach led to between 9.73 ± 1.91 (Mean ± Standard Deviation) and 10.37 ± 1.71 trials being removed per block of 120 trials across the experiment. There was

no statistical difference between the number of trials being removed per block (Repeated Measures ANOVA, main effect of Block ($F(3,51)=0.036$, $p=0.991$; Figure 2).

Transcranial Magnetic Stimulation (TMS), electromyography (EMG) and acceleration recording

All TMS data were acquired using a monophasic BiStim machine, connected to a figure-of-eight coil with an outer diameter of 70mm (Magstim Co., Whitland, Dyfield, UK). TMS was applied over the motor hotspot for the left adductor pollicis brevis (APB) muscle within the right primary motor cortex (M1), i.e. TMS was applied to the right hemisphere, contralateral to the moving (left) hand. EMG was recorded from the APB in a belly-tendon montage using ECG Neonatal electrodes (Covidien, US). Recordings were made using a D360 amplifier (Digitimer Ltd, UK), sampled at 2 kHz, and bandpass filtered at 20Hz – 1kHz. Data were imported online to MATLAB using a CED 1401 data acquisition device and the 'MATCED' interface (see CED contributed software).

TMS measures for active and 1mV motor threshold were obtained for each participant. Active motor threshold (aMT) was defined as the minimum stimulus intensity that produced a 200μV MEP in more than 5 out of 10 trials) during isometric contraction of the tested muscle at approximately 20% of maximum voluntary contraction (MVC). 1mV motor threshold (SI 1MV) was defined as the stimulus intensity required for eliciting an average peak-to-peak EMG response of 1mV, whilst the target muscle was at rest, across ten trials. Due to potential changes in motor cortex excitability throughout the experiment the 1mV threshold was interrogated at the beginning of each TMS block using 10 single TMS pulses and if the size of the EMG response was markedly (approximately 10%) larger or smaller, the stimulation intensity was altered until the elicited MEPs were again 1 mV in amplitude and this new MT_{1mV} was then used for the

duration of the TMS block. This occurred in <5% of cases, never more than once per subject and there were no systematic effects across the experiment. The stimulator intensity was never modulated by more than 2% in any case.

In each block, spTMS pulses were delivered at SI 1mV. All ppTMS measures were delivered according to a standard protocol for inducing SICI, with an interstimulus interval of 2.5ms, and the conditioning stimulus (CS) at 70% of aMT and the test stimulus (TS) at SI 1mV.

Acceleration recordings were made using a tri-axial accelerometer placed on the left thumb and pre-amplifier (Model ACL-300 and DataLINK DLK900, Biometrics Ltd, UK). Data were sampled at 1000 Hz and the signal recorded and stored using a CED 1401 and MATLAB using the 'MATCED' interface.

EMG Data pre-processing

EMG data were exported to Matlab and peak-to-peak amplitudes of TMS-evoked MEPs were extracted for every TMS trial. The trials were then split into ppTMS and spTMS trials. Outliers (Grubbs test, $p<0.005$) and trials with pre-contraction in the target APB muscle (absolute signal > 0.1 mV in the 100 ms preceding the pulse) were rejected, in line with previous studies using similar data [24-26]. Trials in which muscle activity onset was too close to the TMS pulse (movement time - TMS time < 0.05s) were removed to reduce potential ramping effects. Additionally, MEPs with amplitude below 0.1mV were rejected [26]. By rejecting small MEPs we hoped only to reject trials where the TMS pulse has failed to evoke an MEP. However, it is possible that very small MEPs elicited on paired pulse trials could resultant from strong inhibition, and rejecting those trials would bias the SICI effect. Thus, as a precaution, we examined trials directly before or after a paired pulse trial in which the MEP amplitude was <

188 0.1mV. If either of these trials also contained single pulse MEPs that fell below the 0.1mV
189 threshold the trial was rejected, otherwise it was retained.

190 Using the EMG data, the time between the TMS pulse and movement onset (M -T) was
191 established (figure 1D). We were interested in quantifying inhibition at three different time
192 points: rest, early pre-movement and late pre-movement. Thus, for each individual the paired
193 and single pulse trials were allocated to one of three time-points: rest (M-T > 1 s), 25% of pre-
194 movement (0.5 s < M-T < 0.9 s) or 75% of pre-movement (0.05 s < M-T < 0.5 s). For each time-
195 point, the average amplitude of the MEP from the paired pulse trials (ppMEP) was then
196 normalised by the single pulse MEP (spMEP) amplitude in the same condition to get a SICI
197 measure for each time-point in each block (i.e. average magnitude of paired pulse/average
198 magnitude of single pulse).

199 Calculation of degree of participant training-related improvements

200 We calculated two training measures for each participant: early-training (last 10 trials of T2
201 divided by 1st 10 trials of T1 [the first trials in which behavior was available]) and late training
202 (last 10 trials of T3 divided by 1st 10 trials of T1).

203 Calculation of time-point specific and pre-movement profile inhibitory change
204 measures

205 The primary goal of this experiment was to study changes in inhibition over time. We therefore
206 calculated two measures of SICI change for each participant for each time-point: early-change
207 (mean SICI in TMS 2 – mean SICI in TMS 1) and late change (mean SICI in TMS 3 – mean SICI in
208 TMS 1). Calculating the change in SICI measure in this way means that a positive value

represents a decrease in SICI between the blocks, whereas a negative change reflects an increase in SICI. Additionally, to investigate the dynamic release of inhibition we fitted a linear regression to SICI measures for each of the time-points for each block and took the gradient of the regression. We then compared the gradients for each participant between the training blocks: early-gradient change (slope in TMS 2 – slope in TMS 1) and late-gradient change (slope in TMS 3 – slope in TMS 1) to provide an indication of how the pre-movement inhibitory profile changed over training.

Statistical Analysis

Data were tested for normality. All statistical analyses were performed using repeated-measures ANOVA, using SPSS and MATLAB, with *post-hoc* t-tests as appropriate. Standard linear regression was used to assess the relationship between SICI and training and the slopes of the resultant fits were compared using ANCOVA. When sphericity assumptions were violated, results are reported with a Greenhouse-Geiser correction.

Results

Participants' performance improved across the motor task

Firstly, to check how accurate movements were within the bins, the movement time relative to TMS pulse was extracted for each trial for each time-point across TMS blocks for each participant. Within each time-point the movement time relative to TMS pulse was closely centered around times selected to be representative of rest, early pre-movement (0.25s) and late pre-movement (0.75s; Figure 3A; Table 1).

The peak acceleration was then extracted for each trial from the training-only blocks (360 accelerations per subject). These data were then grouped into bins of 10 trials and the mean acceleration for each bin calculated. Mean acceleration increased by 62.1% from the first bin of T1 to the final bin of T3 [T1: 15.21 ± 1.571 m/s^2 (Mean ± SE); T3: 24.66 ± 3.847m/s^2]. RM-ANOVA with TIME-BIN used as a factor found a significant main effect ($F(35,630) = 2.684$, $p < 0.001$).

Cortical excitability remained stable over the course of the experiment

The mean sp-MEP amplitude for each subject was analysed using a repeated measures (RM) ANOVA with one factor of Block (Baseline, T+TMS1, T+TMS2, T+TMS3) and one factor of time-point (Rest, 25%, 75%). This showed no significant effect of either BLOCK ($F(3,51) = 2.089$, $p=0.11$), TIME-POINT ($F(1.19,20.3)= 0.824$, $p=0.45$) or BLOCK x TIME-POINT ($F(6,102) = 0.522$, $p=0.79$), suggesting that test pulse amplitudes remained stable over the course of the experiment.

As described above, we carefully controlled for background EMG activity in our analyses, and removed trials where pre-contraction was observed. However, given pre-contraction even at very low EMG levels can significantly affect TMS amplitudes, we calculated the root mean square (RMS) of the EMG signal in the 100ms preceding each TMS pulse. A RM ANOVA with one factor of Block (Baseline, T+TMS1, T+TMS2, T+TMS3), one factor of time-point (Rest, 25%, 75%) and one factor of pulse type (Single, Paired) showed no significant main effects or interactions (see supplementary information for details) suggesting that there were no differences in EMG activity.

Intra-cortical inhibition at baseline

First we wanted to verify that our SICI paradigm led to significant inhibition at baseline. Multiple t-tests controlling for false discovery rate [27] indicated that for each time-point and block the SICI measure was less than 1, demonstrating significantly lower MEP amplitude when a paired pulse was delivered relative to a single pulse, consistent with the successful application of the SICI protocol (all $p < 0.05$, with control for FDR; figure 3E).

Release of inhibition prior to movement

Average single and paired pulse amplitudes for each block and condition are shown in figure 3A. We first wished to investigate whether we observed the previously reported release of inhibition during movement preparation. A RM-ANOVA with one factor of Block (Baseline, T+TMS1, T+TMS2, T+TMS3) and one factor of time-point (Rest, 25%, 75%) on SICI revealed a main effect of time-point ($F(2,34) = 3.48$, $p = 0.042$, $\rho^2 = 0.170$) but no effect of Block ($F(3,51) = 1.91$, $p > 0.1$, $\rho^2 = 0.101$), and no Block by Time-point interaction ($F(6,102) = 1.015$, $p > 0.1$, $\rho^2 = 0.056$). Given the main effect of time-point we went on to explore differences between the three time-points. Post-hoc t-tests indicated that, as would be predicted, there was significantly more inhibition at both the rest and 25% pre-movement time-points than at the 75% pre-movement time-point across the duration of the experiment ($t(17) = 2.373$, $p = 0.03$ and $t(17) = 2.367$, $p = 0.03$ respectively; figure 3D).

Baseline SICI does not predict subsequent response to training

The degree of resting inhibition during the baseline block was not linked to baseline acceleration, nor was it predictive of subsequent change in SICI, or to the degree of training-

related improvement in performance (all linear regressions with $p > 0.1$). Additionally, we used the first 10 trials from the T1 Block to calculate a "baseline" performance measure on the task. There was no significant relationship between this measure of initial performance and measures of either early ($r=0.04$, $p=0.85$) or late ($r=0.05$, $p=0.83$) learning.

Dynamics of inhibitory release relate to motor training

We then went on to explore whether the dynamics of the inhibitory release could inform us about change in GABAergic processing during training. There was no change in the slope of inhibitory release across the blocks on (RM-ANOVA $F(3,51) = 0.382$, $p > 0.7$). However, when we related the change in the slope of inhibitory release to the degree of training-related behavioural improvements we observed a close relationship, such that participants who showed the greatest training-related improvements were those in whom the GABAergic release became less pronounced, whereas participants who showed the least training-related improvements had more pronounced GABAergic release. This relationship held for both early and late training (early-earning/early change in inhibition slope: $R^2 = 0.4319$, $F(1,17) = 12.163$, $p = 0.003$; late-training/late change in inhibition slope: R-squared = 0.2181, $F(1,18) = 4.7411$, $p = 0.0438$); Figure 4).

Change in Inhibition at 75% pre-movement was related to the early stages of training

To further explore the relationship between changes in inhibitory dynamics and training we first considered the relationship between SICI and training for the early-training time period (Figure 6). For each time-point separately, the change in SICI was plotted against response to training for each subject, and a simple linear regression was fitted to the data. Consistent with the association demonstrated above, there was a significant relationship between response to

training and change in SICI at 75% of pre-movement ($R^2 = 0.564$, $F(1,17) = 20.67$, $p < 0.001$) but not at rest ($R^2 \approx 0$, $F(1,17) = 0.004$, $p > 0.9$) or at 25% of pre-movement ($R^2 = 0.194$, $F(1,17) = 3.84$, $p = 0.064$), such that participants who exhibited greater increase in inhibition at the late pre-movement time-point were also those who showed greater response to training during the early stages.

To further examine this relationship between response to training and inhibitory release we performed an ANCOVA with early-training as a covariate, early-change in SICI as the dependent variable and SICI measures grouped by TIMEPOINT. This revealed a significant interaction of early-training and TIMEPOINT ($F(2,48) = 4.13$, $p = 0.022$), indicating a difference in the rate of change of the SICI relative to early-training for each TIMEPOINT. Post-hoc pairwise comparison indicated that there was a significant difference between the effect of the covariate between the rest and 75% change in SICI groups (Tukey-Kramer HSD; $p = 0.016$) but indicated no difference between 75% and 25% change in SICI (Tukey-Kramer HSD, $p > 0.1$) or 25% and rest change in SICI (Tukey-Kramer HSD, $p > 0.1$).

Change in Inhibition at 25% pre-movement was related to the late stages of training

Next we explored relationship between SICI and training for the late-training time period (Figure 7). Similarly to above, the change in SICI was plotted against response to training for each subject, and a regression was fitted to the data. Consistent with the previous findings, a significant relationship was found between late-training and 25% pre-movement change in SICI ($R^2 = 0.392$, $F(1,18) = 10.97$, $p < 0.005$) but not at rest or 75% pre-movement ($R^2 = 0.130$, $F(1,18) = 2.55$, $p > 0.1$ and $R^2 = 0.142$, $F(1,18) = 2.81$, $p > 0.1$), such that participants who exhibited a greater increase in inhibition at the early pre-movement time-point were also those who showed a greater response to training.

316 In a similar approach for that used for early-training above, we conducted an analysis of co-
317 variance (ANCOVA) to assess the differential effect of the late-training on each of the groups,
318 with late-training as a covariate, late-change in SICI as the dependent variable and SICI
319 measures grouped by timepoint. This approach revealed a significant interaction between
320 group and the late-training covariate ($F(2,51) = 6.17$, $p < 0.005$). Post-hoc pairwise
321 comparisons revealed a significant difference between the effect of the covariate on rest and
322 25% pre-movement groups (Tukey-Kramer HSD, $p < 0.005$), but no difference between rest
323 and 75% pre-movement (Tukey-Kramer HSD, $p = 0.061$) or 25% and 75% pre-movement
324 (Tukey-Kramer HSD, $p = 0.51$).

325 There was no relationship between change in inhibition at rest and change in inhibition at
326 either pre-movement timepoint (early SICI change rest/early SICI change 25%: $R^2 = 0.025$,
327 $F(1,17) = 0.405$, $p > 0.5$; early SICI change rest/early SICI change 75%: $R^2 = 0.042$, $F(1,17) =$
328 0.700, $p > 0.4$; late SICI change rest/late SICI change 25%: $R^2 = 0.003$, $F(1,18) = 0.047$, $P > 0.8$;
329 late SICI change rest/ late SICI change 75%: $R^2 = 0.035$, $F(1,18) = 0.618$, $p > 0.443$).

330 Discussion

331 This study was designed with two main aims: to investigate the dynamic changes in inhibition
332 within the primary motor cortex as participants prepare to move, and to explore whether these
333 movement preparation-related dynamic shifts in GABA signaling were modulated by training
334 of a simple thumb abduction task.

335 In line with previous findings [16,28], we showed a significant release of GABAergic inhibition
336 within the muscle representation of M1 as a subject prepared to move that muscle [29]
337 Participants then performed a simple motor training task. Although motor training induced no

overall change in the degree of inhibition at any of our time-points, the change in the individual's inhibitory release across the course of the experiment was related to the degree of training-related behavioural improvement the subject demonstrated. We did not find that inhibitory release at baseline was predictive for subsequent behavioural improvement, but this may be because subjects could not be given feedback during the baseline block, altering the task demands.

At earlier stages of training, greater training-related behavioural improvements correlated with an increase in the level of late pre-movement SICI. This effect was significantly different to the effect of training on change in SICI found at rest. However, at later stages of training greater improvements in abduction acceleration correlated with an increase in early pre-movement changes in SICI. This relationship was significantly different to that of training and rest SICI as measured at this stage.

Taken together these findings demonstrate a changing profile of pre-movement inhibitory dynamics, as assessed in healthy humans using TMS. This dynamic change in inhibition correlates with the degree of training-related behavioural improvement achieved by an individual. As participants trained on the task the period before movement during which this inhibition was maintained increased – early in training, successful performance was related to greater inhibition at the later pre-movement timepoint, whereas later in training it was related to greater inhibition at the early pre-movement timepoint.

It is important to note that our measure of behaviour reflects maximum acceleration alone, and does not include metrics such as number of rejected trials, or accuracy of movements. This metric was chosen as we believe it gives the best reflection of the motor aspects of the task, which were of primary interest

here. We also note that the TMS, although performed in separate blocks to the behaviour, may have some influence on the learning of the task.

Relating Disinhibition to training

We have demonstrated a relationship between changes in the dynamics of inhibition and training. Considering the nature of the task participants had to undergo, a potentially successful strategy to increase performance would be to effectively inhibit the target muscle until the go command was issued. It would seem plausible that successful and focal inhibition would allow for the greatest coordinated contribution of muscular activity to generate the consequential maximal ballistic thumb movement. In line with this hypothesis, we see that participants who exhibit greater training-related improvements tend to display greater increases in pre-movement SICI at early and late training stages. Indeed, startle response experiments suggest a reduction in preparation time when information indicating the onset of an upcoming movement is precise, that is when subjects knew when to accurately initiate movement [30]. Thus as individuals successfully train on the task it may be that preparation is more precisely deployed, which is reflected by the changes in release of inhibition seen here.

Resting inhibition and learning

Previous studies investigating changes in inhibition during training have demonstrated a training-related decrease in resting inhibition, either as measured using TMS [11,31,32] or MRS [12]. Additionally, in chronic stroke patients, a model for long-term plasticity, ppTMS measures have demonstrated deficient levels of inhibition at rest [28,33-35]. In addition, studies utilizing non-invasive stimulation techniques to alter the level of GABA in M1 have shown a relationship

between learning and the degree of change of GABA, as assessed by MRS [36]. However, other studies have failed to see a change in SICI as a result of motor training [37].

In the present study we did not observe a decrease in SICI at rest relative to the degree of training-related behavioral improvements. This may be resultant of a difference in the type of 'rest' recordings that can be taken. Here, "rest" was defined as a period prior to an initial cue signaling the onset of a movement to occur one second later and TMS pulses were delivered prior to the defined pre-movement period. However, individuals were still under task constraints and requirements meaning that their levels of attention and preparedness may be elevated; a kind of 'active-rest' [38,39]. In many other studies investigating inhibition, using both TMS and MRS, rest recordings are taken when participants are not under any task requirements and attention or alertness is not required.

Pre-movement Release of Inhibition

Several studies have shown disinhibition in M1 in the lead up to the onset of the movement [14,16,28]. We have also demonstrated a similar disinhibition, however the observed decrease in inhibition was more modest than that reported previously, where facilitation at points very proximal to movement onset has been demonstrated. We do not see the previously reported increases in MEP amplitude in the late stages of movement preparation. This is an important factor to consider as an increased MEP amplitude in response to the TS alone can modulate SICI measurements, making them difficult to interpret [40]. However, while we do not see significant changes in MEP amplitude either across the duration of the experiment, or across the three time-points, we cannot entirely rule out that small effects that do not reach statistical significance may modulate our effects. The same concerns might hold as regards the intensity of the CS. While modulation of the CS intensity is very difficult to achieve and not routinely

done in studies of this type, changes in underlying cortical excitability will influence the effects of the CS, which in turn will have significant effects on the SICI measure [41].

Previous studies demonstrating pre-movement disinhibition using TMS have utilized a reaction time based task, where pulses are delivered at points relative to an individual's reaction time to a go-cue [16,28]. This kind of response is potentially reflexive and arguably action preparation has occurred before the go-cue has been presented. Indeed, EEG studies that have using a fixed, predictable movement onset time demonstrate a rising negative movement related potential (MRP) [42]. However, in instances where the movement onset cue is reactive MRPs are absent, suggesting that either the upcoming movement has either been prepared well in advance or that a reflexive – rather than planned - method of movement initiation is adopted. That this aspect of our experimental design differs from previous studies where a reaction time-based task has been used may explain apparent discrepancies in results [16,28].

Conclusion

This study was performed to explore changes in pre-movement inhibitory dynamics in response to a motor training task. We demonstrated that increased training-related behavioural improvements were associated with maintenance or even increase in pre-movement inhibition. These data suggest that maintaining pre-movement inhibition may be a potentially successful strategy to better co-ordinate muscle activity, to perform the required action.

Grants

This study was supported by a Sir Henry Dale Fellowship, funded by the Wellcome Trust and the Royal Society, to CJS (102584/Z/13/Z), and the European Research Council (ERC), to SB and JH (ActSelectContext, 260424).

Figure Captions

Figure 1

(A) The experimental protocol and the time course of the blocks to be completed by the participants. (B) Schematic representation of all possible trials and the timings of the TMS pulses relevant to the cue stimuli. (C) An example of the feedback a participant received during the all blocks (except the baseline block). Only the most recently plotted bar was filled with color, with red representing a decrease in peak acceleration relative to the previous trial, and green representing an increase. The green and red lines represent the average of the previous 20 trials and were plotted above the upcoming feedback for the next 20 trials. The subject was given feedback about responses that occurred prematurely or too late by text reading 'Too early' or 'Too late' respectively. d) Example data from a single trial from one participant. The top, grey, trace shows the acceleration recording during a TMS trial where the pulses were delivered at rest. The bottom black trace shows the recorded EMG on the same trial. The onset of movement/EMG activity and peak of the thumb abduction are indicted by the dotted lines labeled M (movement/EMG onset) and P (peak thumb abduction). M-T indicates the time between the TMS pulse (T) and movement onset (M), which was used for allocating trials to rest, early pre-movement and late pre-movement conditions.

Figure 2

Total number of trials rejected from each block across all criteria. Each block consisted of 120 trials.

Figure 3

(A) Average time between the TMS pulse and movement onset for each condition across participants. The horizontal width indicates the standard deviation between participants. (B) Average ballistic thumb abduction acceleration. Each point represents the mean of 10 trials across participants and the error bars depict the standard error between participants.

Figure 4

(a) Each point represents an individual participant with change in inhibitory slope between T + TMS 1 and T + TMS 2 plotted against early learning. **b)** Each point represents an individual participant with change in inhibitory slope between T + TMS 1 and T + TMS 3 plotted against early learning. Each of the datasets are fitted with a linear regression.

Figure 5

(a), (b) and (c) Average MEPs for single and paired pulses recorded at rest, 25% pre-movement and 75% pre-movement respectively. The solid grey lines represent the average single pulse and the dotted black line represents the average paired pulse. Within *a), b)* and *c)* the four panels represent MEPs collected in each TMS block (starting with baseline at top left and moving clockwise) **d)** The average SICI measure for participants across all TMS blocks for rest, 25% of pre-movement and 75% of pre -movement. **e)** Shows the average SICI measure broken down into individual TMS blocks with each of the bars within each block representing the different rest/pre-movement times.

Figure 6

In the *top left/right and bottom left panel* each point represents an individual participant with their change in SICI from *T + TMS 1 to T + TMS 2* for rest, 25% of pre-movement and 75% of pre-movement plotted against their early-learning, respectively. Each of the datasets is fitted with a linear regression. The *bottom right panel* depicts each of the regression fits overlaid to allow for better visualization and visual comparison.

Figure 7

In the *top left/right and bottom left panel* each point represents an individual participant with their change in SICI from *T + TMS 1 to T + TMS 3* for rest, 25% of pre-movement and 75% of pre-movement plotted against their late learning, respectively. Each of the datasets is fitted with a linear regression. The *bottom right panel* depicts each of the regression fits overlaid to allow for better visualization and visual comparison.

Tables

Table 1: Average movement time relative to TMS pulse in each block

	Rest (s)	25% of pre-movement (s)	75% of pre-movement (s)
Baseline	1.222 ± 0.050	0.736 ± 0.031	0.292 ± 0.070
T + TMS 1	1.194 ± 0.041	0.726 ± 0.046	0.268 ± 0.058
T + TMS 2	1.177 ± 0.046	0.707 ± 0.047	0.263 ± 0.069
T + TMS 3	1.170 ± 0.054	0.712 ± 0.045	0.258 ± 0.068

Conflicts of Interest

The authors declare no conflicts of interest

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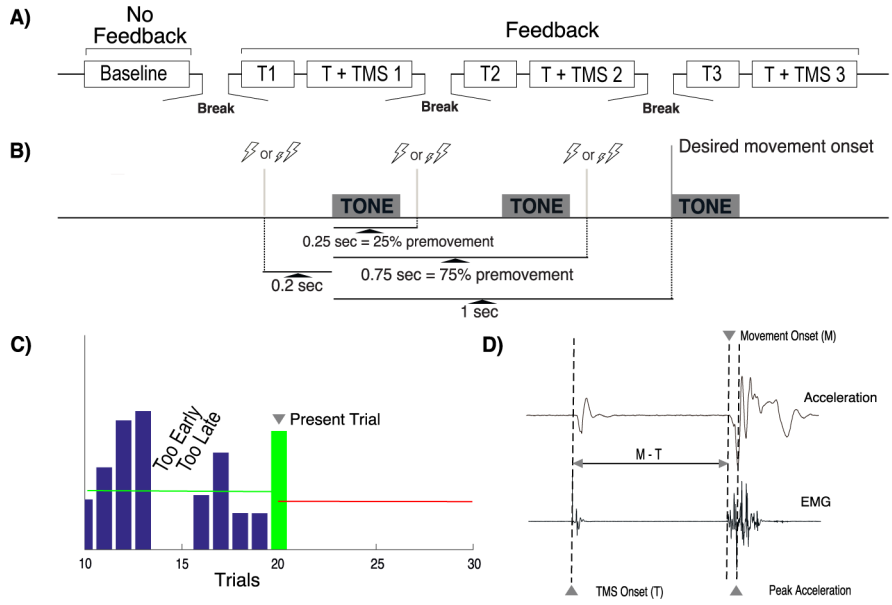
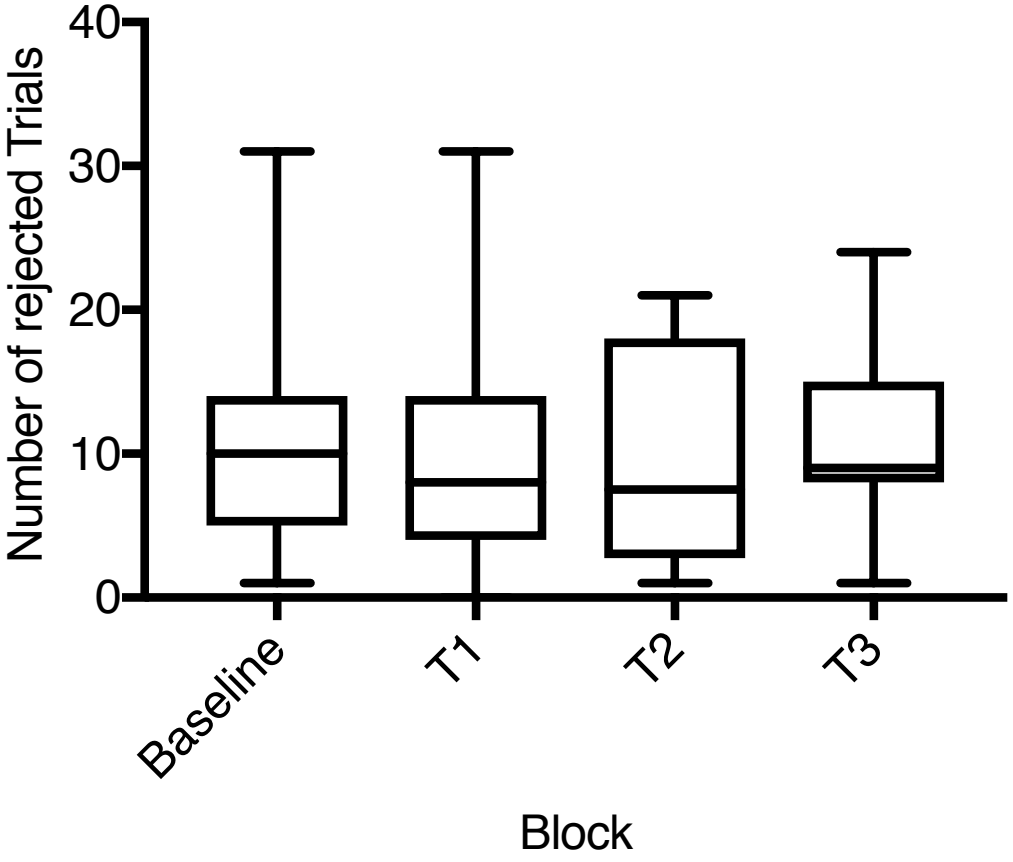
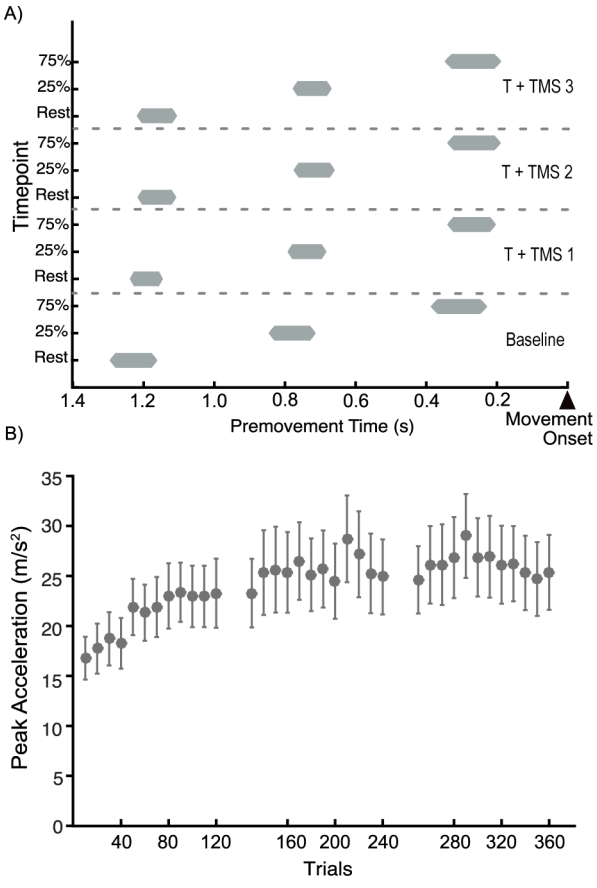
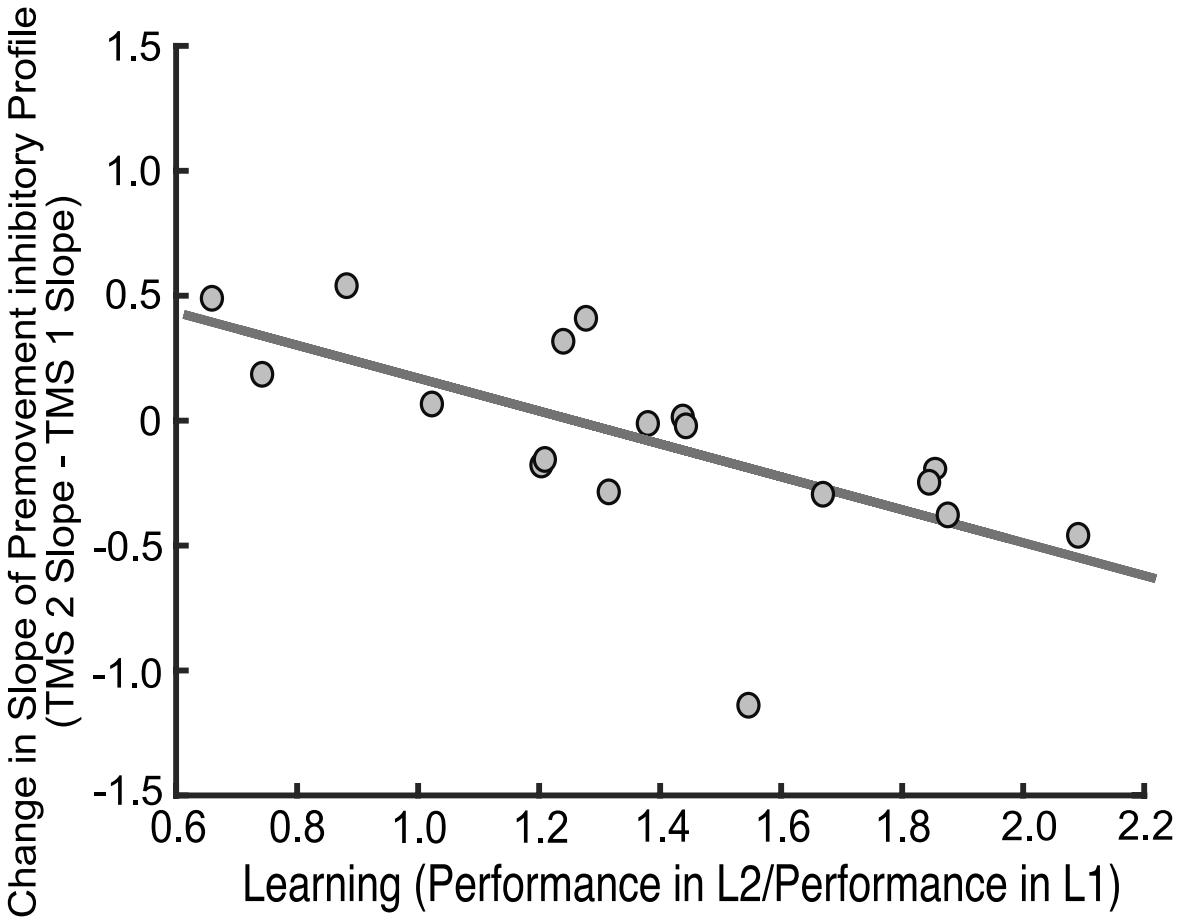

A)
No Feedback
Feedback
Baseline
T1
T + TMS 1
T2
T + TMS 2
T3
T + TMS 3
Break
Break
Break
B)
or
or
or
Desired movement onset
TONE
TONE
TONE
0.25 sec = 25% premovement
0.2 sec
0.75 sec = 75% premovement
1 sec
C)
Too Early
Too Late
Present Trial
10
15
20
25
30
Trials
D)
Movement Onset (M)
Acceleration
M - T
EMG
TMS Onset (T)
Peak Acceleration

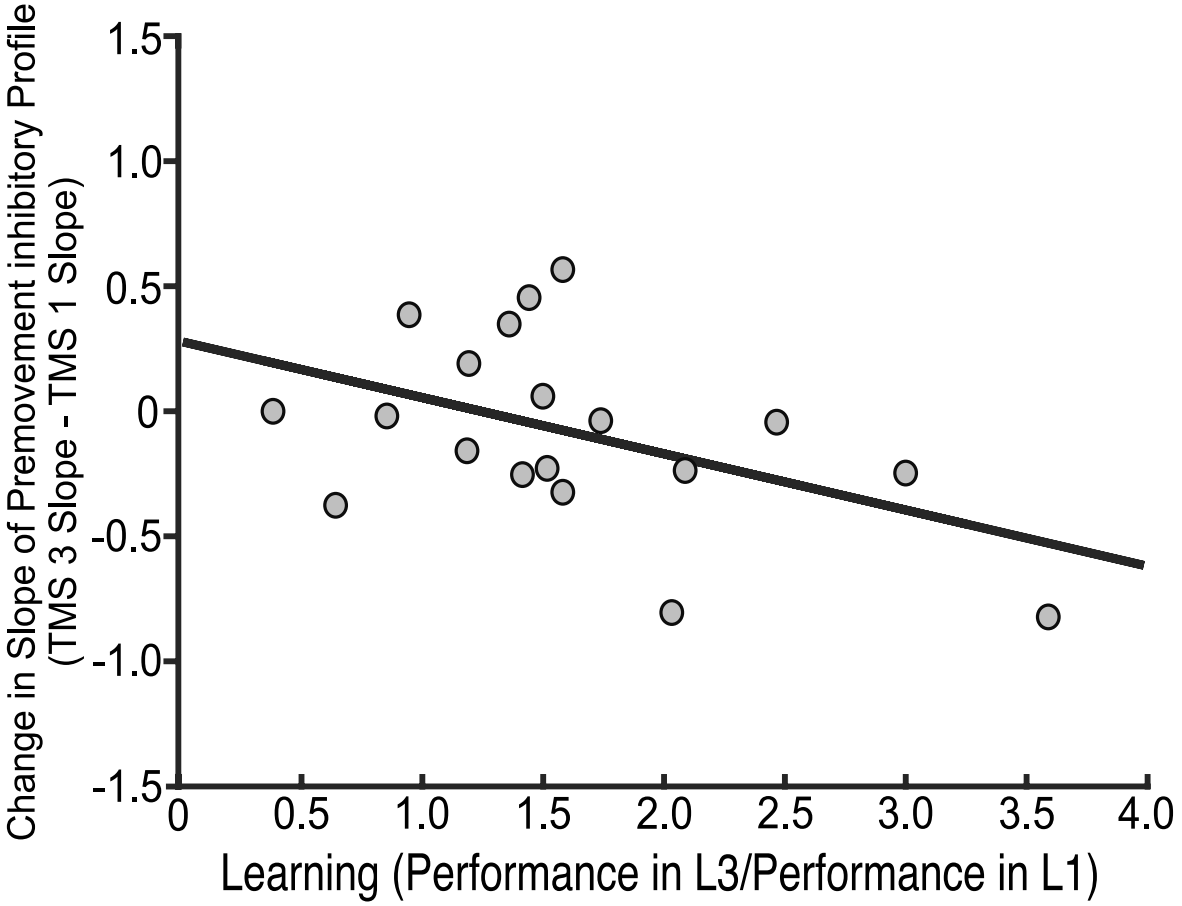

Number of rejected Trials
40
30
20
10
0
Baseline
T1
T2
T3
Block

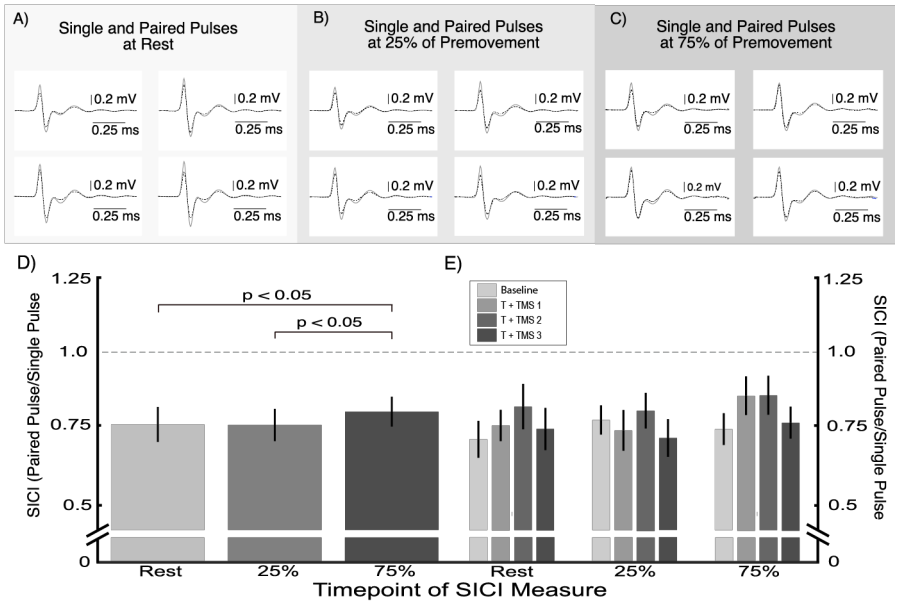

A)
75%
25%
Rest
T + TMS 3
75%
25%
Rest
T + TMS 2
75%
25%
Rest
T + TMS 1
75%
25%
Rest
Baseline
Timepoint
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Premovement Time (s)
Movement Onset
B)
Peak Acceleration (m/s²)
35
30
25
20
15
10
5
0
40
80
120
160
200
240
280
320
360
Trials

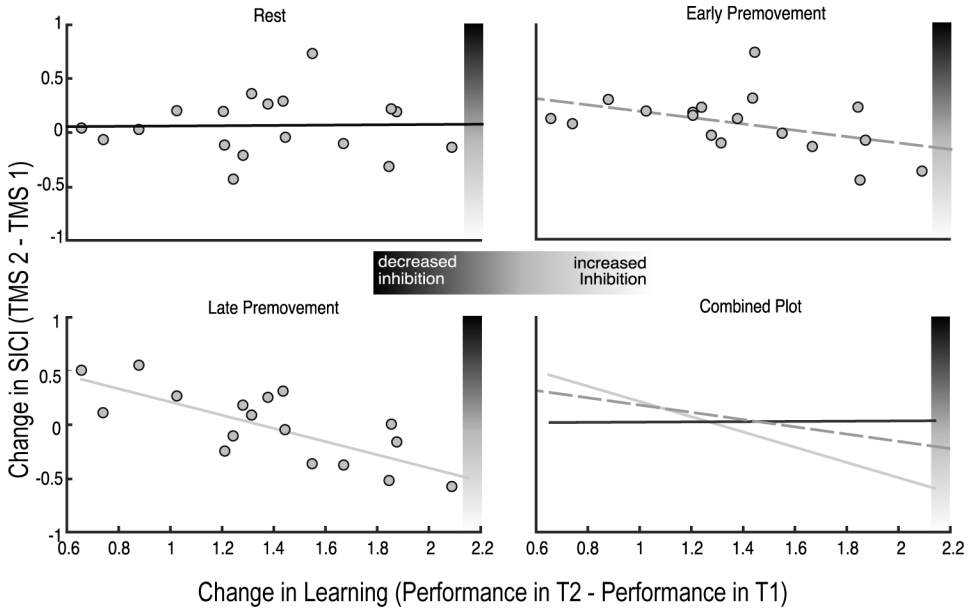
A)

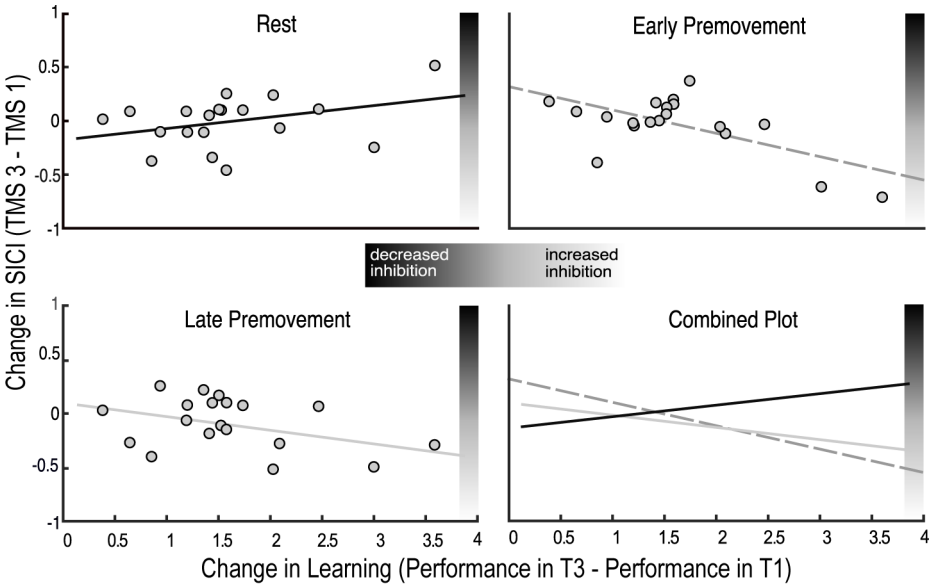

Change in Slope of Premovement inhibitory Profile
(TMS 2 Slope - TMS 1 Slope)
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Learning (Performance in L2/Performance in L1)

B)


Change in Slope of Premovement inhibitory Profile
(TMS 3 Slope - TMS 1 Slope)
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Learning (Performance in L3/Performance in L1)


A)
Single and Paired Pulses at Rest
B)
Single and Paired Pulses at 25% of Premovement
C)
Single and Paired Pulses at 75% of Premovement
0.2 mV
0.25 ms
D)
E)
p < 0.05
p < 0.05
Baseline
T + TMS 1
T + TMS 2
T + TMS 3
SICI (Paired Pulse/Single Pulse
1.25
1.0
0.75
0.5
0
Rest
25%
75%
Timepoint of SICI Measure


Rest
Early Premovement
Late Premovement
Combined Plot
decreased inhibition
increased Inhibition
Change in SICI (TMS 2 - TMS 1)
Change in Learning (Performance in T2 - Performance in T1)
1
0.5
0
-0.5
-1
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2


Rest
Early Premovement
Late Premovement
Combined Plot
decreased inhibition
increased inhibition
Change in SICI (TMS 3 - TMS 1)
Change in Learning (Performance in T3 - Performance in T1)
1
0.5
0
-0.5
-1
0
0.5
1
1.5
2
2.5
3
3.5
4