

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

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Running Head: GABA dynamics in motor training

24 Abstract

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

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

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

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

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

46	Keywords
47	GABA
48	Motor cortex
49	Motor training
50	Movement preparation
51	Transcranial Magnetic Stimulation
52	Plasticity

53 Introduction

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

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

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

76 Materials and Methods

77 Participants

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

82 Behavioural Task

83 Participants performed a ballistic thumb abduction training task (Muellbacher et al. 2001) that
84 required the abduction of their left (non-dominant) thumb with maximal acceleration [21-23].
85 The behavioural task was separated into four blocks (figure 1A) with each training block, which
86 contained no TMS, being interleaved with a TMS-block from which no performance data was
87 acquired. All blocks were separated by a break of at least 3 minutes to minimize fatigue caused
88 by repeated movements. All blocks containing TMS required participants to make movements
89 at a rate of 0.25 Hz, whilst blocks containing no TMS had a faster rate of movement at 0.5 Hz
90 [11]. The slower rate was imposed in the blocks containing TMS based on pilot experiments, to
91 minimize the level of background muscle contraction that might result from repeating a ballistic
92 movement in quick succession. Each block consisted of 120 trials, with a 30 second break
93 between every 40 trials to avoid within block fatigue.

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

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

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

106 In all blocks except the baseline block, participants were instructed to move as fast as possible
107 and were encouraged to try to increase their acceleration on every trial. Participants were given
108 visual feedback about the acceleration of their movements on a trial-by-trial basis (see figure
109 1C). Feedback was presented as a scrolling bar chart with the magnitude of the current
110 acceleration plotted after each trial. If the acceleration on the current trial was greater than on
111 the previous trial, the bar was plotted in green, and if it was less the bar was plotted in red. If a
112 movement was made too early or too late (i.e. movement outside a 300 ms window centered
113 on one second after the first tone), no acceleration feedback was given. Instead, the message
114 “too early” or “too late” respectively was presented. Additionally, participants were informed
115 of their progress by displaying a moving average of acceleration values over the preceding 20
116 trials, indicated by a line plotted on screen over the locations of the 20 consequential trials.

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

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

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

134 Behavioural Data Analysis

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

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

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

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

156 TMS measures for active and 1mV motor threshold were obtained for each participant. Active
157 motor threshold (aMT) was defined as the minimum stimulus intensity that produced a 200 μ V
158 MEP in more than 5 out of 10 trials) during isometric contraction of the tested muscle at
159 approximately 20% of maximum voluntary contraction (MVC). 1mV motor threshold (SI 1MV)
160 was defined as the stimulus intensity required for eliciting an average peak-to-peak EMG
161 response of 1mV, whilst the target muscle was at rest, across ten trials. Due to potential changes
162 in motor cortex excitability throughout the experiment the 1mV threshold was interrogated at
163 the beginning of each TMS block using 10 single TMS pulses and if the size of the EMG response
164 was markedly (approximately 10%) larger or smaller, the stimulation intensity was altered
165 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 ($0.5 \text{ s} < M-T < 0.9 \text{ s}$) or 75% of pre-movement ($0.05 \text{ s} < M-T < 0.5 \text{ s}$). 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).

229 The peak acceleration was then extracted for each trial from the training-only blocks (360
 230 accelerations per subject). These data were then grouped into bins of 10 trials and the mean
 231 acceleration for each bin calculated. Mean acceleration increased by 62.1% from the first bin of
 232 T1 to the final bin of T3 [T1: $15.21 \pm 1.571 \text{ m/s}^2$ (Mean \pm SE); T3: $24.66 \pm 3.847 \text{ m/s}^2$]. RM-
 233 ANOVA with TIME-BIN used as a factor found a significant main effect ($F(35,630) = 2.684$, $p <$
 234 0.001).

235 Cortical excitability remained stable over the course of the experiment

236 The mean sp-MEP amplitude for each subject was analysed using a repeated measures (RM)
 237 ANOVA with one factor of Block (Baseline, T+TMS1, T+TMS2, T+TMS3) and one factor of time-
 238 point (Rest, 25%, 75%). This showed no significant effect of either BLOCK ($F(3,51) = 2.089$,
 239 $p=0.11$), TIME-POINT ($F(1.19,20.3) = 0.824$, $p=0.45$) or BLOCK \times TIME-POINT ($F(6,102) =$
 240 0.522 , $p=0.79$), suggesting that test pulse amplitudes remained stable over the course of the
 241 experiment.

242 As described above, we carefully controlled for background EMG activity in our analyses, and
 243 removed trials where pre-contraction was observed. However, given pre-contraction even at
 244 very low EMG levels can significantly affect TMS amplitudes, we calculated the root mean
 245 square (RMS) of the EMG signal in the 100ms preceding each TMS pulse. A RM ANOVA with
 246 one factor of Block (Baseline, T+TMS1, T+TMS2, T+TMS3), one factor of time-point (Rest, 25%,
 247 75%) and one factor of pulse type (Single, Paired) showed no significant main effects or
 248 interactions (see supplementary information for details) suggesting that there were no
 249 differences in EMG activity.

250 Intra-cortical inhibition at baseline

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

256 Release of inhibition prior to movement

257 Average single and paired pulse amplitudes for each block and condition are shown in figure
 258 3A. We first wished to investigate whether we observed the previously reported release of
 259 inhibition during movement preparation. A RM-ANOVA with one factor of Block (Baseline,
 260 T+TMS1, T+TMS2, T+TMS3) and one factor of time-point (Rest, 25%, 75%) on SICI revealed a
 261 main effect of time-point ($F(2,34) = 3.48$, $p = 0.042$, $\rho^2 = 0.170$) but no effect of Block ($F(3,51)$
 262 $= 1.91$, $p > 0.1$, $\rho^2 = 0.101$), and no Block by Time-point interaction ($F(6,102) = 1.015$, $p > 0.1$,
 263 $\rho^2 = 0.056$). Given the main effect of time-point we went on to explore differences between the
 264 three time-points. Post-hoc t-tests indicated that, as would be predicted, there was significantly
 265 more inhibition at both the rest and 25% pre-movement time-points than at the 75% pre-
 266 movement time-point across the duration of the experiment ($t(17) = 2.373$, $p = 0.03$ and $t(17)$
 267 $= 2.367$, $p = 0.03$ respectively; figure 3D).

268 Baseline SICI does not predict subsequent response to training

269 The degree of resting inhibition during the baseline block was not linked to baseline
 270 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-training/early change in inhibition slope: $R^2 = 0.4319$, $F(1,17) = 12.163$, $p = 0.003$; late-training/late change in inhibition slope: $R\text{-squared} = 0.2181$, $F(1,18) = 4.7411$, $p = 0.0438$); 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

293 training and change in SICI at 75% of pre-movement ($R^2 = 0.564$, $F(1,17) = 20.67$, $p < 0.001$)
 294 but not at rest ($R^2 \approx 0$, $F(1,17) = 0.004$, $p > 0.9$) or at 25% of pre-movement ($R^2 = 0.194$, $F(1,17)$
 295 $= 3.84$, $p = 0.064$), such that participants who exhibited greater increase in inhibition at the late
 296 pre-movement time-point were also those who showed greater response to training during the
 297 early stages.

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

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

308 Next we explored relationship between SICI and training for the late-training time period
 309 (Figure 7). Similarly to above, the change in SICI was plotted against response to training for
 310 each subject, and a regression was fitted to the data. Consistent with the previous findings, a
 311 significant relationship was found between late-training and 25% pre-movement change in
 312 SICI ($R^2 = 0.392$, $F(1,18) = 10.97$, $p < 0.005$) but not at rest or 75% pre-movement ($R^2 = 0.130$,
 313 $F(1,18) = 2.55$, $p > 0.1$ and $R^2 = 0.142$, $F(1,18) = 2.81$, $p > 0.1$), such that participants who
 314 exhibited a greater increase in inhibition at the early pre-movement time-point were also those
 315 who showed a greater response to training.

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

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

Discussion

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

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

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

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

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

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

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

362 Relating Disinhibition to training

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

375 Resting inhibition and learning

376 Previous studies investigating changes in inhibition during training have demonstrated a
377 training-related decrease in resting inhibition, either as measured using TMS [11,31,32] or MRS
378 [12]. Additionally, in chronic stroke patients, a model for long-term plasticity, ppTMS measures
379 have demonstrated deficient levels of inhibition at rest [28,33-35]. In addition, studies utilizing
380 non-invasive stimulation techniques to alter the level of GABA in M1 have shown a relationship

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

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

392 Pre-movement Release of Inhibition

393 Several studies have shown disinhibition in M1 in the lead up to the onset of the movement
394 [14,16,28]. We have also demonstrated a similar disinhibition, however the observed decrease
395 in inhibition was more modest than that reported previously, where facilitation at points very
396 proximal to movement onset has been demonstrated. We do not see the previously reported
397 increases in MEP amplitude in the late stages of movement preparation. This is an important
398 factor to consider as an increased MEP amplitude in response to the TS alone can modulate SICI
399 measurements, making them difficult to interpret [40]. However, while we do not see
400 significant changes in MEP amplitude either across the duration of the experiment, or across
401 the three time-points, we cannot entirely rule out that small effects that do not reach statistical
402 significance may modulate our effects. The same concerns might hold as regards the intensity
403 of the CS. While modulation of the CS intensity is very difficult to achieve and not routinely

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

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

416 Conclusion

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

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427 Figure Captions

428 Figure 1

429 (A) The experimental protocol and the time course of the blocks to be completed by the
 430 participants. (B) Schematic representation of all possible trials and the timings of the TMS
 431 pulses relevant to the cue stimuli. (C) An example of the feedback a participant received during
 432 the all blocks (except the baseline block). Only the most recently plotted bar was filled with
 433 color, with red representing a decrease in peak acceleration relative to the previous trial, and
 434 green representing an increase. The green and red lines represent the average of the previous
 435 20 trials and were plotted above the upcoming feedback for the next 20 trials. The subject was
 436 given feedback about responses that occurred prematurely or too late by text reading 'Too
 437 early' or 'Too late' respectively. d) Example data from a single trial from one participant. The
 438 top, grey, trace shows the acceleration recording during a TMS trial where the pulses were
 439 delivered at rest. The bottom black trace shows the recorded EMG on the same trial. The onset
 440 of movement/EMG activity and peak of the thumb abduction are indicted by the dotted lines
 441 labeled M (movement/EMG onset) and P (peak thumb abduction). M-T indicates the time
 442 between the TMS pulse (T) and movement onset (M), which was used for allocating trials to
 443 rest, early pre-movement and late pre-movement conditions.

444 Figure 2

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

446 Figure 3

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

451 Figure 4

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

456 Figure 5

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

465 Figure 6

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

471 Figure 7

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

477 Tables

478 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

479

480 Conflicts of Interest

481 The authors declare no conflicts of interest

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