Evidence for high-fidelity timing dependent synaptic plasticity of human motor cortex

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ABSTRACT

A single transcranial magnetic stimulation (TMS) pulse typically evokes a short series of spikes in corticospinal neurons (known as indirect (I)-waves) which are thought to arise from transynaptic input. Delivering a second pulse at inter-pulse intervals (IPIs) corresponding to the timing of these I-waves leads to a facilitation of the response, and if stimulus pairs are delivered repeatedly, a persistent LTP-like increase in excitability can occur. This has been demonstrated at an IPI of 1.5 ms, which corresponds to the first I-wave interval, in an intervention referred to as ITMS (I-wave TMS), and it has been argued that this may have similarities with timing dependent plasticity models. Consequently we hypothesised that if the second stimulus is delivered so as not to coincide with I-wave timing it should lead to LTD. We performed a cross-over study in ten subjects in which TMS doublets were timed to coincide (1.5 ms IPI, ITMS$_{1.5}$) or not coincide (2 ms IPI, ITMS$_{2}$) with I-wave firing. Single pulse motor evoked potential (MEP) amplitude, resting motor threshold (RMT) and short-interval cortical inhibition (SICI) were measured from the first dorsal interosseous (FDI) muscle. After ITMS$_{1.5}$ corticomotor excitability was increased by around ~170% for 15 minutes (p<0.05) and returned to baseline by 20 minutes. Increasing the IPI by just 500µs to 2 ms reversed the after-effect and MEP amplitude was significantly reduced (-35%, p<0.05) for 15 minutes before returning to baseline. This reduction was not associated with an increase in SICI, suggesting a reduction in excitatory transmission rather than an increase in inhibitory efficacy. RMT also remained unchanged suggesting that these changes were not due to changes in membrane excitability. Amplitude-matching ITMS$_{2}$ did not modulate excitability. The results are consistent with timing-dependent synaptic LTP/D-like effects, and suggest that there are plasticity mechanisms operating in the human motor cortex with a temporal resolution of the order of a few hundreds of microseconds.
INTRODUCTION

In the human, non-invasive brain stimulation such as transcranial magnetic stimulation (TMS) is increasingly being applied across a range of protocols to up- or down-regulate cortical synaptic plasticity, with the ultimate aim of improving functional impairment in neurological disorders. These TMS protocols have in turn been guided by synaptic plasticity mechanisms identified in experimental preparations such as activity-dependent (rate-related), patterned (theta burst) and timing-dependent (spike-timing dependent or associative) induction protocols that lead to long-term potentiation or depression (LTP/D) of synaptic transmission (for review see Thickbroom 2007).

We have previously described a TMS protocol that utilises the high temporal resolution of TMS to target the dynamics of synaptic transmission (Thickbroom et al. 2006). A single pulse of TMS typically sets in train a short volley of action potentials (APs; ~1.5 ms intervals) in principle cells of primary motor cortex that can be detected with epidural recordings (Boyd et al. 1986; Di Lazzaro et al. 2004). These APs are referred to as indirect (I)-waves, as they are thought to arise from repeated trans-synaptic bombardment of pyramidal cells through inter-neuronal networks (Patton and Amassian 1954; Ziemann and Rothwell 2000). I-waves can be studied non-invasively by using a paired-pulse TMS protocol known as short-intracortical facilitation (SICF) in which the response to the pair is facilitated if the second pulse coincides in time with an I-wave fired by the first pulse (Tokimura et al. 1996; Ziemann et al. 1998). This occurs phasically (depending on the number of I-waves generated) at 1.5 ms periodicity, but is not observed at intermediate intervals (e.g. 2 ms). The relevance to synaptic plasticity is that if pairs of pulses at a 1.5 ms interval facilitate trans-synaptic activation of pyramidal cells, then this interval is a candidate for more permanently modifying the efficacy of these synaptic inputs. It has been shown that 15-30 mins of 1.5 ms TMS doublets (delivered every 5 seconds) does result in a steady increase in corticomotor
excitability during the intervention, and that the increase in excitability (as determined from single pulse TMS and SICF) outlasts the intervention period (Benwell et al. 2006; Cash et al. 2009; Di Lazzaro et al. 2007; Hamada et al. 2007; Murray et al. 2011; Rodrigues et al. 2008; Silbert et al. 2011; Teo et al. 2012; Thickbroom et al. 2006).

The temporal precision underlying this intervention has suggested a form of spike timing dependent plasticity (STDP) in which synaptic efficacy is up-regulated if an input contributes to the firing of the post-synaptic cell (Bi and Poo 1998), as in the present situation the TMS intervention is tuned to synaptic dynamics and AP triggering (Thickbroom 2007). However, a characteristic of timing-dependent models is that if an input is not appropriately timed so as to contribute to firing of the post-synaptic cell then LTD should ensue. To test the hypothesis, we measured corticomotor excitability and intracortical inhibition after a repetitive paired-pulse intervention at an inter-pulse interval of 2 ms, which falls between successive I-waves. We show that this intervention does decrease corticomotor excitability, and that this is not due to an increase in intracortical inhibition but rather is consistent with LTD-like effects. The results suggest that there are plasticity mechanisms in human motor cortex that operate with a sub-millisecond temporal resolution.

**METHODS**

**Subjects**

Eighteen healthy individuals participated in a series of studies (9 female, 23-32 years of age). All were right handed according to the Edinburgh Inventory (Oldfield 1971). Participants gave informed written consent and completed a safety questionnaire prior to the study which had the approval of the institutional Human Research Ethics Committee and conformed to the Declaration of Helsinki. Subjects were seated comfortably with arms resting on a cushion.
Electromyography (EMG)

Motor evoked potentials (MEPs) were recorded from surface electrodes placed in a belly-tendon arrangement over the first dorsal interosseous (FDI) muscle of the right hand. The EMG signal was amplified (x500), digitised (sample rate 10kHz; Labview 8.6, National Instruments), and stored on a computer. All measurements were taken at rest. EMG was monitored throughout the sessions, and EMG data for 100 ms prior to each TMS was stored and checked off-line.

TMS

Stimuli were delivered using a 7cm figure-of-eight coil connected to a dual-pulse stimulator (Bistim 2002, Magstim Co., UK). The coil was placed over the optimal site for the hand area of the left motor cortex to elicit MEPs in the right FDI, and orientated with the handle pointing backwards and ~45 degrees away from the sagittal plane. Single-pulse resting motor threshold (RMT) was defined as the lowest stimulator intensity required to elicit small MEPs (>50µV peak-to-peak amplitude) in at least half of 10 trials over the optimal scalp position.

Study 1. Paired-pulse Intervention Protocols

Two paired-pulse TMS interventions, with inter-pulse intervals (IPIs) of 2 ms and 1.5 ms, were compared (referred to as ITMS2 and ITMS1.5 respectively). Paired stimuli were delivered every 5 seconds for 15 minutes (180 stimuli). Both stimuli in the pair were of equal intensity and set for both interventions such that paired-pulse MEP amplitude was ~1 mV at an IPI of 1.5 ms, which was verified with a run of 12 paired-stimuli.

Ten healthy individuals were recruited for the crossover study (4 female; 23-29 years of age) comparing ITMS2 and ITMS1.5. The order of sessions was pseudorandomised and
counterbalanced between subjects, with gender also balanced and a minimum inter-
session period of 1 week.

In an additional experiment in 4 subjects, the stimulus intensity for the ITMS$_2$ protocol
was increased (maintaining $S1=S2$) so that the stimulus pair yielded a MEP of $\sim1mV$ and
was thus amplitude-matched to the ITMS$_{1.5}$ study. This protocol is referred to as ITMS$_2$*.

Corticospinal excitability was compared before and after the interventions with single-
pulse TMS. Intensity was set at baseline to generate a MEP of $\sim1mV$. Sets of 12 stimuli
were delivered (5 second intervals; total collection period 1 minute), the first two stimuli
were discarded to allow for a settle-in period, and peak-peak MEP amplitude for the
following 10 responses was averaged. Two baseline runs were performed (mean of the
two used to compute baseline amplitude), and measurements made at 2, 5, 10, 15 and 20
minutes post-intervention.

**Study 2. Short-intracortical Inhibition (SICI) protocol and RMT**

To establish whether the 2 ms intervention could have modified the level of inhibition, SICI
and RMT were measured in a separate session in nine subjects, five of whom had also
participated in the first crossover study (6F, 23-29 years of age). SICI was measured using
condition-test stimulus (CS, TS) pairs at an interval of 2 ms, as contamination by SICF is
minimal at this IPI (Peurala et al. 2008), and compared to the response to TS alone. TS
intensity was set to give a MEP of $\sim1mV$ amplitude at baseline, and CS intensity was set to
give 50% inhibition of the TS response in order to keep the measurement sensitive to change
and avoid floor or ceiling effects (Cash et al. 2010; McDonnell et al. 2006; Muller-Dahlhaus
et al. 2008). CS intensity was initially set to 65% RMT, as this has been shown to be optimal
for eliciting $\sim50\%$ SICI and is well below the threshold for evoking SICF (cf. Fig 3b Orth et
al. 2003; cf. Fig 2a Heide et al. 2006; Peurala et al. 2008), and then CS intensity was adjusted
slightly in each participant until the CS evoked 50% SICI (Cash et al. 2010; McDonnell et al. 2006; Muller-Dahlhaus et al. 2008). CS intensity was set relative to RMT rather than AMT to avoid potential metaplastic influences related to prior muscle activation (Gentner et al. 2008; Iezzi et al. 2008). In 6 participants, we adjusted CS intensity until maximum SICI was attained (which was 31±3% of unconditioned TS MEP amplitude), in order to confirm that the 50% SICI level was sensitive to both increases and decreases in SICI. Post-intervention, two TS intensities were used: (i) intensity matched - same intensity as prior to intervention (9 subjects) and (ii) amplitude matched (TS) - intensity adjusted to give 1 mV (6 subjects).

Figure 1 outlines the protocol, and the ordering of adjusted and unadjusted conditioned and unconditioned measurements and RMT.

**Data Analysis**

Results are expressed as mean ± SEM. An independent samples t-test was used to compare baseline TS amplitude between interventions to rule out inter-session differences or carry-over effects.

Baseline paired-pulse MEP amplitude at an IPI of 1.5 ms (mean of 12 stimuli) was compared between the two interventions using an independent samples t-test. The mean amplitude of the paired MEPs obtained each minute during an intervention (total of 12 MEPs per minute) was calculated for each subject, expressed as a percentage of the mean data for the first minute, and averaged across subjects. Significant change in paired MEP amplitude with time during an intervention was tested using a regression analysis of group data (Pearson product-moment correlation). A linear mixed model analysis was performed to determine the effect of intervention (ITMS$_2$, ITMS$_{1.5}$) on single pulse MEP amplitude over time (post-intervention) relative to baseline, allowing for a random-effects factor for the individual (subjects).
SICI was calculated from the amplitude of the conditioned MEP divided by that for TS alone, and expressed as a percentage. A higher value thus indicated less inhibition. SICI pre-and post intervention as well as RMT were compared using independent sample t-tests.

RESULTS

Paired-pulse Interventions: ITMS\textsubscript{1.5} and ITMS\textsubscript{2}

Pre-intervention baseline
Baseline MEP amplitude was 1.07±0.07 mV for the 2 ms intervention and 0.91±0.05 mV for the 1.5 ms intervention and these did not differ significantly. Baseline paired-pulse MEP amplitude (IPI 1.5 ms) was not significantly different between the two intervention sessions (1.00±0.04 mV vs. 1.03±0.06 mV). The mean stimulus intensity for the pulses in the pair to give these response amplitudes was 103±2\% of RMT. At this intensity, single-pulse MEP amplitude was 0.14±0.03 mV. The responses evoked by paired-pulse stimuli at 1.5 ms were significantly greater in amplitude than the sum of two single-pulse responses delivered separately (1.19±0.11 mV; p<0.001), but the paired response at 2 ms IPI was not (0.38±0.05 mV).

During Intervention
Paired-pulse MEP amplitude during ITMS\textsubscript{2} decreased significantly, from 0.38±0.05 mV (first minute) to 0.26±0.06 mV (last minute; p<0.01). In contrast, MEP amplitude during ITMS\textsubscript{1.5} increased from 1.12±0.13 mV to 1.40±0.46 mV (p<0.05).
The linear mixed models analysis revealed a significant effect of intervention (p<0.001). Following ITMS\textsubscript{2}, MEP amplitude was significantly reduced at 2, 5, 10 and 15 minutes, to 0.68±0.09, 0.65±0.08, 0.69±0.13 and 0.75±0.10 mV respectively compared to the baseline MEP of 1.07±0.07 mV (p<0.05, Figure 2). The mean decrease over this 15-min post-intervention period was 36% and the maximum decrease in MEP amplitude in individual subjects ranged from 22% to 64%. MEP amplitude was not significantly different to baseline at 20 minutes post-intervention (0.82±0.12 mV).

Following ITMS\textsubscript{1.5}, MEP amplitude was significantly increased for 15 minutes, from pre-intervention baseline of 0.91 mV, to 1.54±0.18; 1.37±0.13; 1.41±0.13; 1.54±0.25 at 2, 5, 10 and 15 minutes respectively (p<0.05; Figure 2). The mean increase over this period was 164% and the maximal increase post-intervention in individual subjects ranged from 144% to 309%. MEP amplitude was not significantly different to baseline at 20 minutes (1.20±0.12 mV).

**Amplitude-matched Intervention (ITMS\textsubscript{2}*)**

Adjusted paired-pulse MEP amplitude (2ms IPI) was 1.19±0.14mV. Baseline MEP amplitude (single pulse) was 1.12±0.14mV, and was not significantly different post-intervention (linear mixed-model analysis; p=0.79). MEP amplitude at 2, 5, 10, 15 and 20 minutes post-intervention was 0.90±0.11, 1.21±0.28, 1.01±0.21, 0.90±0.25 and 0.90±0.10mV respectively.

At the stimulus intensity used for ITMS\textsubscript{2}*, the ratio of MEP amplitude for paired-pulse TMS (2ms IPI) to single-pulse TMS (at the same intensity) was 108%. 
SICI and RMT

At baseline, TS MEP amplitude was 0.96±0.07 mV and SICI was 48±2% (conditioned MEP amplitude 0.46±0.03 mV). Following ITMS2 for this session, TS amplitude was again reduced significantly (0.48±0.05 mV; 51±6% of baseline; p<0.001). Post-intervention, with TS unadjusted (intensity-matched condition) SICI was 108±17% (conditioned MEP amplitude 0.57±0.08 mV), which was reduced in strength significantly compared to baseline (p<0.005). Post-intervention intensity-adjustment (TS) gave a MEP amplitude of 0.95±0.09 mV, which was not significantly different to baseline TS (0.96 ± 0.07 mV), indicating successful adjustment. SICI measured with TS was 55±12% (conditioned MEP amplitude 0.51±0.06 mV), which was not significantly different to baseline SICI. RMT was unchanged pre- and post-intervention (56.25±2.99% vs. 56.88 ± 3.44% MSO).

Discussion

Repetitive paired-pulse stimulation of the motor cortex at an IPI of 1.5 ms using the ITMS protocol transiently increased corticomotor excitability by a mean of ~170% for ~15 minutes. Increasing the IPI by just 500µs reversed this after-effect, from that of potentiation to depression, with a comparable time-course and effect size. There was no associated increase in SICI or RMT, suggesting that a reduction in excitatory transmission rather than a change in inhibitory efficacy or membrane excitability was responsible for the depression. The results are consistent with timing-dependent LTP/D-like effects, and provide evidence that there are plasticity mechanisms operating in the human motor cortex that have a temporal resolution of the order of just a few hundred microseconds.
Temporal coupling between synaptic events is at the foundation of synaptic plasticity models that exhibit the properties of associativity, specificity and cooperativity, all of which depend on temporal contiguity (Malenka 2003). With ITMS$_{1.5}$, temporal contiguity is provided by the association of S2 with the I-wave events triggered by S1 that follow a 1.5ms periodicity (Thickbroom 2007). However, the specificity of the intervention to 1.5 ms timing has not previously been demonstrated. There remained the possibility that the temporal closeness of the stimulation pair may be important, but the timing not critical, and that some form of more broadly-tuned activity-dependent plasticity may be in operation (Bliss and Lomo 1973). Thus the shift to an IPI of 2ms was a decisive test of the timing hypothesis, as the increase of 500µs would be expected to have little influence on an activity model, but would be important for a temporal contiguity model. Indeed, our hypothesis was that mistiming I-wave facilitation would lead to depression of excitability, and this was confirmed. We therefore propose that ITMS represents a class of intervention that depends on mechanisms of high-fidelity timing-dependent plasticity.

It remains to be determined which mechanisms might exist to support these temporal resolutions. Most activity-dependent and spike-timing dependent models can not readily explain the present dynamics, however phase-dependent plasticity (PDP) offers a plausible mechanism for the current findings. With PDP, the direction of synaptic modification is determined by the level of membrane potential (Vm) at the time of synaptic input, and if Vm oscillates according to cortical rhythms, then LTP or LTD can be induced depending on whether synaptic input coincides with Vm peaks or troughs respectively (Wespatak et al. 2004). It has been suggested that I-wave dynamics may be a consequence of EPSPs from excitatory pyramidal neurons in cortical layers II/III and V alternating with IPSPs from inhibitory GABA-ergic interneurons (Di Lazzaro et al. 2011; Ziemann and Rothwell 2000).
These circuits could comprise the minimal architecture to induce $V_m$ oscillations at I-wave intervals in pyramidal neurons even if the neuron does not fire on every oscillation. With this model, the second TMS pulse in a pair that is delivered at an I-wave interval (1.5ms) is likely to coincide with a $V_m$ peak, whereas at the longer interval of 2ms it is likely to coincide with a $V_m$ trough, and a PDP model would predict potentiation and depression of synaptic strength respectively. PDP has been demonstrated at theta (Holscher et al. 1997; Huerta and Lisman 1995; 1993; Hyman et al. 2003; Pavlides et al. 1988), beta and gamma frequencies (Wespatat et al. 2004). Higher-frequency oscillations in the sigma band (~600Hz) have been linked to I-wave dynamics (Curio 2000), and the present results suggest that PDP may also operate at sigma frequency.

A feature of the present generalisation of ITMS has been the uncovering of a bidirectional effect on corticomotor excitability, in keeping with a number of previous TMS protocols that operate according to activity, patterned or lower-resolution timing-dependent mechanisms (Chen et al. 1997; Hamada et al. 2008; Huang et al. 2005; Pascual-Leone et al. 1994; Stefan et al. 2000; Wolters et al. 2003). Synaptic plasticity mechanisms need to be bi-directional for reasons of functionality and stability. The demonstration of bi-directionality with ITMS emphasises the relevance of the TMS induction protocol to the underlying physiology of the plasticity mechanism being targeted, and increases the potential clinical utility of the TMS approach. The new LTD-like protocol (ITMS$_2$) could be applicable across a range of disorders where a reduction in excitability may be desirable, such as epilepsy, chronic pain, spasticity and other hyperexcitability disorders, as well as the non-lesioned hemisphere after stroke, which can be hyperexcitable (Hallett 2007).
A possible confound to the LTD-like interpretation of ITMS2 is that a 2 ms IPI is commonly used to evoke SICI, and therefore the reduction in MEP amplitude may not have been a correlate of LTD of excitatory-excitatory synapses, but rather a potentiation of inhibitory-excitatory synapses that mediate GABAergic inhibition. SICI is measured using a sub-threshold conditioning stimulus (CS) followed 2-3 ms later by a supra-threshold test stimulus (TS). The CS activates GABAergic networks which inhibit the response to TS, and the ratio of the amplitude of the MEP for the conditioned TS over that for TS alone is used as an index of the strength of GABAergic inhibition. A previous TMS intervention, designed to target SICI networks by using low-intensity stimulation as described by Kujirai et al. (1993) to elicit SICI, showed that 25 mins of very low-intensity (80% AMT) paired stimulation (3 ms IPI, 500 pairs) can increase both RMT and SICI (Khedr et al. 2004), and argued that this results from an increase in the effectiveness of inhibition. Our ITMS2 protocol differs from this in that we used pairs of higher intensity stimulation (at or above RMT). However, to be certain that ITMS2 did not facilitate inhibition, we also measured SICI and RMT after this intervention and demonstrated no change in the adjusted TS condition, while the unadjusted TS data suggested that there could have been, perhaps paradoxically, a reduction in SICI. It is not entirely certain whether the adjusted or unadjusted data are more representative of the real level of post-intervention inhibition, however a recent modelling paper has suggested that a decrease in excitability can manifest as a decrease in SICI if no adjustment is made to TS intensity (Thickbroom 2010). This could happen if the depressive effect of the intervention is due to a reduction in late I-waves (e.g. I3) which are also the I-waves targeted by CS in SICI protocols. As a result, these I-waves are not available to be conditioned by CS, and SICI appears reduced. Adjusting TS to match pre-intervention TS-MEP amplitude should restore later I-waves such that they are again available to be conditioned. Under such circumstances SICI was not significantly different to pre-interventional baseline. In either case there was no
evidence for an increase in inhibition after the intervention that could have explained a
decrease in excitability after ITMS$_2$. A further consideration is that ITMS$_2$ may have
increased SICI but that there was also an increase in excitability that precisely counter-
balanced this change. However, this seems unlikely as TS MEP amplitude would be expected
to remain constant after the intervention under the influence of a balanced increase in
inhibition and excitability (Butefisch et al. 2003), rather than be reduced as observed here.

For the main experiments, stimulus intensity was kept the same for both ITMS$_{1.5}$ and ITMS$_2$
so that the only parameter adjusted was the pulse interval. The intention was to explore time-
based plasticity mechanisms. However, we also performed an additional experiment in which
we amplitude-matched the two interventions by increasing ITMS$_2$ intensity, and found that
the adjusted ITMS$_2^*$ intervention had no significant effect on cortico-motor excitability.
These higher intensities may have altered the interaction between S1 and S2, and we
observed that there was little difference between paired-pulse and single-pulse MEP
amplitude at baseline, suggesting that paired-pulse interaction was in fact minimal. This
differed to the unadjusted ITMS$_2$ condition, in which there was a summation effect of the two
pulses but not a facilitation. Thus the adjusted intervention could be thought of as similar to a
period of single-pulse stimulation, which is known not to modulate excitability (Benwell et
al. 2006; Murase et al. 2005). We did not perform the inverse adjustment, namely reducing
ITMS$_{1.5}$ intensity to amplitude-match that of ITMS$_2$, however it seems likely that at the
correspondingly very low stimulus intensity the interaction between pulses would be reduced
and the intervention would not be as effective. This highlights the importance of selecting a
TMS intensity that activates target networks sufficiently but without saturation.
We have previously provided evidence that ITMS\textsubscript{1.5} acts at the cortical level (Thickbroom et al. 2006), and it seems unlikely that the subtle change in timing for ITMS\textsubscript{2} would cause a switch to a spinal mechanism, however we can not rule this out based on the present data. Given the short intervals between successive stimuli in each doublet, there may have been some refractoriness following the first stimulus. However this seems unlikely given the high-frequency discharge capability of the networks involved, that an IPI at the lesser interval of 1.5 ms is in fact facilitatory and leads to SICF (Tokimura et al. 1996; Ziemann et al. 1998) and increased excitability following ITMS\textsubscript{1.5}. Finally, while SICI was not altered by ITMS\textsubscript{2}, it remains possible that such networks are activated during the intervention and could have downstream effects, or that some inhibitory networks not associated with SICI could likewise have been recruited. However, as SICI is the main inhibitory phenomenon that is active shortly after stimulation it was considered to the most relevant measure of inhibition to explore after ITMS\textsubscript{2}.

We conclude that paired-pulse ITMS has the capability to evoke either potentiation or depression through a change in the pulse interval that is determined according to I-wave dynamics. The results suggest that a reduction in excitatory transmission rather than a change in inhibitory efficacy or membrane excitability was responsible for the depression. The I-wave guided paired-pulse intervention complements the present battery of non-invasive brain stimulation interventions, and extends their temporal resolution to a new domain. The results suggest that timing considerations are at the basis of this intervention, and predict that plasticity mechanisms operating in human motor cortex in vivo can have a temporal resolution of the order of a few hundreds of microseconds.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1: Experimental protocols (see methods). Experiment 1: ITMS was delivered with matched intensity at IPI 1.5 ms or 2 ms (ITMS$_{1.5}$, ITMS$_{2}$) in the main experiments, or with matched amplitude ITMS$_{2}^*$ in a supplementary study. Experiment 2: Study of the effect of ITMS$_{2}$ on SICI and RMT. SICI was compared pre- and post-intervention TS unadjusted and adjusted for excitability changes.

Figure 2: Comparison of the percentage change (compared to baseline) in single pulse MEP amplitude 2-20 minutes after (A) ITMS delivered at equal intensity: IPI 1.5 ms (white circles, ITMS$_{1.5}$) and IPI 2 ms (black circles, ITMS$_{2}$); or (B) at IPI 2ms with intensity increased to match 1.5ms IPI MEP amplitude (ITMS$_{2}^*$). Asterisks denote significant difference from baseline (p<0.05).

Figure 3: Data from Study 2 pre- and post ITMS$_{2}$ with TS unadjusted or adjusted for excitability changes. A) SICI, B) RMT C) comparison of MEP amplitude changes for TS (black) and CS.TS (grey) and D) MEP data from one subject (average of ten MEPs) for TS and for conditioned TS. Asterisks denote significant differences (p<0.005).
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FIG 1.

**Setup & Baseline**

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<td>1) RMT</td>
<td>1) Baseline TS MEPs</td>
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<td>2) TS intensity to give 1mV</td>
<td>2) Baseline SICI (EXP 2)</td>
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<td>3) CS intensity for 50% SICI (EXP 2)</td>
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<td>4) Paired-pulse Intensity to give 1mV at IPI 1.5ms (ITMS&lt;sub&gt;1.5&lt;/sub&gt;, ITMS&lt;sub&gt;2&lt;/sub&gt;), or 1mV at IPI 2ms (ITMS&lt;sub&gt;2&lt;/sub&gt;*).</td>
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**Post-intervention**

**Experiment 1**

- TS
- CS.TS
- RMT

**Experiment 2**

- Adjusted TS

# Adjusted TS

2 4 6 8 10 12 14 16 18 20min
FIG 2.

A

MEP (% baseline)

TIME (mins post-Intervention)

ITMS  ○  1.5ms  ●  2ms

B

MEP (% baseline)

TIME (mins post-Intervention)
FIG 3.

**A**

SICI (%)  

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**B**

RMT (%MSO)  

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**C**

MEP (mV)  

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<td>0.2</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**D**

TEST MEP  

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|       | 20ms | 0.2mV |

- **PRE**  
- **POST TS**  
- **POST TS Adjusted**