Resistant Against De-depression: LTD-Like Plasticity in the Human Motor Cortex Induced by Spaced cTBS

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The long-term depression (LTD)-like changes in human primary motor cortex (M1) excitability induced by continuous theta burst stimulation (cTBS) are subject to reversal (i.e., de-depression) following behavioral engagement of M1, limiting its therapeutic potential under behaviorally relevant conditions. Experiments in animals suggest that the repeated, spaced application of stimulation trains may consolidate synaptic plasticity, making it resistant to reversal by physiological activity. Although there is evidence that repeated cTBS prolongs LTD-like M1 neuroplasticity in humans, whether these effects are resistant to de-depression has not been tested. We investigated whether the neuroplastic effects of paired cTBS trains were resistant to de-depression by a sustained, submaximal voluntary contraction of the hand muscles. In the absence of cTBS, voluntary contraction had no effect on motor evoked potentials (MEPs) recorded from the right first dorsal interosseous muscle. While the LTD-like MEP depression induced by a single cTBS was abolished by subsequent voluntary contraction, paired cTBS induced MEP depression that was resistant to reversal. This MEP depression was also resistant to reversal when an experimental de-depression protocol was used instead of a voluntary contraction. Our findings suggest that repeated cTBS applications consolidate LTD-like M1 neuroplasticity, which may have important implications for the clinical application of cTBS.

Keywords: consolidation, rTMS, theta burst stimulation, transcranial magnetic stimulation, voluntary contraction

Introduction

Neuronal networks within the human central nervous system undergo neuroplastic modulation throughout life in response to a variety of experiences. One technique which has been used to induce and study neuroplastic change in the human cortex in recent years is repetitive transcranial magnetic stimulation (rTMS). When applied to the human primary motor cortex (M1), rTMS can induce lasting changes in M1 excitability, which resemble long-term potentiation (LTP) and long-term depression (LTD) of synaptic plasticity as observed in animal models (Huang et al. 2007).

A feature of LTP and LTD induced in animal models is their susceptibility to reversal, either by subsequent physiological activity at the stimulated synaptic input (Xu et al. 1998; Zhou et al. 2003) or by delivery of a weak stimulation protocol following plasticity induction (Fuji et al. 1991; Huang et al. 1999; Chen et al. 2001). This reversal of LTP and LTD (termed depotentiation and de-depression, respectively) most likely acts as a safeguard preventing a consolidation of random activity (Zhou and Poo 2004). Similar depotentiation and de-depression phenomena have also been observed in humans, with behavioral engagement of the stimulated motor regions reversing rTMS-induced changes in M1 excitability (Huang et al. 2008).

As with the depotentiation and de-depression observed in animals, the reversal of rTMS-induced neuroplasticity may also be triggered by weak rTMS protocols which, when applied alone, do not change M1 excitability (Huang et al. 2010).

An important property of the LTP and LTD described in animal experiments is that they may become consolidated by applying repeated trains of electrical stimulation in a spaced manner (Bliss and Gardner-Medwin 1973; Trepel and Racine 1998). This consolidation is critical for the persistence of synaptic modifications under normal physiological conditions (Zhou et al. 2003) and is likely due to increases in de novo protein synthesis and gene transcription (Krug et al. 1984; Huang and Kandel 1994; Nguyen et al. 1994; Woo and Nguyen 2003). Similar to animal experiments showing long-lasting synaptic modifications with repeated induction protocols, the spaced application of rTMS has been shown to prolong the duration of induced neuroplasticity in the human cortex (Nyffeler et al. 2006, 2009; Goldsworthy et al. 2012a).

However, whether these longer lasting neuroplastic changes are also resistant to reversal has yet to be tested.

The capacity of rTMS to induce stable neuroplasticity under normal physiological conditions is critically important for its implementation as a therapeutic tool for treating disease. Therefore, the present study aimed to determine whether the repeated application of rTMS protocols could induce stable neuroplasticity resistant to reversal. We employed continuous theta burst stimulation (cTBS) which, when applied as a single train, induces LTD-like depression of M1 excitability lasting <1 h (Di Lazzaro et al. 2005; Huang et al. 2005, 2007). Two de-depression methods were used to test the stability of induced neuroplasticity: (1) Behavioral engagement of M1 during a sustained, submaximal voluntary contraction (sub-MVC) and (2) stimulation of M1 with a novel de-depression TBS protocol (Huang et al. 2010).

Materials and Methods

Subjects

A total of 30 healthy subjects (29 right-handed and 1 left-handed; 13 females) aged 19–49 [24.4 ± 5.7 years (mean age ± SD)] gave informed written consent to participate in this study (see Supplementary Table 1 for subjects’ characteristics, as well as their allocation to the different experiments). All participants were blinded to the purpose of the study and were screened for any contraindications to TMS prior to their...
involvement (Rossi et al. 2009). All experiments were performed in accordance with the 2008 Declaration of Helsinki and were approved by the University of Adelaide Human Research Ethics Committee and the ethics committee of the medical faculty of the Goethe-University of Frankfurt am Main.

Experiments
A schematic overview of the experiments included for this study is shown in Figure 1. Experiment 1 was performed on 10 subjects (7 females; 23.4 ± 3.6 years), and tested the effects of a voluntary contraction of the targeted hand muscle [i.e., the right first dorsal interosseous (FDI) muscle] on the excitability of M1 in the absence of prior intervention with cTBS (i.e., paired sham cTBS trains, separated by 10 min, were used). Experiment 2 was performed on 10 subjects (5 females; 25.7 ± 3.1 years); of whom, 7 had also participated in Experiment 1, and tested the effects of a voluntary contraction on the response to a single cTBS train (i.e., one sham cTBS train, followed 10 min later by one real cTBS train). Experiment 3 was performed on 10 subjects (6 females; 24.7 ± 4.0 years); of whom, 4 had also participated in Experiments 1 and 2, and tested the effects of a voluntary contraction on the response to paired cTBS (i.e., paired real cTBS trains, separated by 10 min). Each of Experiments 1–3 consisted of 2 sessions. In one session, subjects were instructed to keep their right hand completely relaxed for the entire postintervention recording period (i.e., FDI relaxed). In the other session, subjects were instructed to produce a submaximal isometric contraction of their right hand by pinching their right thumb and index finger (i.e., FDI contract). The contraction was sustained for 2 min at approximately 10% of maximal effort and was initiated 15 min following the first stimulation protocol. The method for setting the force of voluntary contraction is described in more detail below.

Experiment 4 was a control study performed on 8 subjects (2 females; 23.0 ± 3.5 years), and tested the impact of the time interval separating cTBS and the sub-MVC may have had on the depression response. Similar to Experiment 2, subjects for Experiment 4 received a single real cTBS protocol paired with a single sham; however, the order of sham/real cTBS delivery was reversed such that real cTBS was applied 10 min prior to sham. As with Experiments 1–3, subjects for Experiment 4 were required to attend 2 sessions: one FDI relaxed and the other FDI contract.

Experiment 5 was performed on 10 subjects (3 females; 26.4 ± 8.6 years); of whom, 5 had participated in Experiment 4, and was included to determine whether the response to paired cTBS was resistant to reversal by an experimental TBS paradigm designed to de-depress cTBS-induced LTD-like plasticity (Huang et al. 2010). For this experiment, subjects were required to attend 2 sessions. For one session, subjects received single cTBS (i.e., one sham cTBS train, followed 10 min later by one real cTBS train), while for the other session they received paired cTBS (i.e., 2 real cTBS trains, separated by 10 min).

Stimulation and Recording
Subjects were seated in a comfortable chair for each experimental session and were directed to keep their right hand and arm as relaxed as possible unless instructed otherwise. Surface electromyography was used to record muscle action potentials (MEPs) from the right FDI muscle using 2 Ag–AgCl electrodes arranged in a belly-tendon montage. Signals were sampled at a rate of 5 kHz (Cambridge Electrical Design 1401, Cambridge, UK), amplified with a gain of 1000, and band-pass filtered between 20 and 1000 Hz (Cambridge Electrical Design 1902 amplifier, Cambridge, UK) or 2000 Hz (Counterpoint Mk2 electromyograph, Dantec, Denmark). Samples were stored on a laboratory computer for later offline analysis.

Single-pulse TMS was used to test for changes in M1 excitability and was applied with a monophasic current waveform using a figure-of-eight magnetic coil (external wing diameter, 90 mm) connected to a Magstim 200 magnetic stimulator (Magstim, Whitchard, UK). The TMS coil was held tangential to the skull over the left M1 with the handle pointing posterolaterally at a 45° angle to the sagittal plane, such that the induced current direction across M1 was posterior–anterior. At the beginning of each experimental session, the optimal coil position for eliciting MEPs in the right FDI was identified using an intensity of stimulation just above threshold for producing a small motor response in the hand muscles. This position was marked on the subject’s scalp using a felt marker. The intensity of stimulation was then adjusted to evoke MEPs in the right FDI with the peak-to-peak amplitude of approximately 1 mV. This intensity was used for all subsequent MEP recordings.

TBS for Plasticity Induction
All TBS paradigms were applied with a biphasic current waveform [i.e., with an initial induced current direction (across M1) flowing posterior–anterior] using either Magstim Super Rapid (Magstim) or MagPro X100 (MagVenture, Farum, Denmark) magnetic stimulators. The same type of stimulator was used for each session of an experiment for each subject. The standard pattern of TBS was employed and consisted of bursts of 3 pulses at 50 Hz with bursts repeated at a frequency of 5 Hz (Huang et al. 2005). All cTBS protocols were applied for 40 s and consisted of 600 stimuli. While it is convention to set the intensity of cTBS relative to active motor threshold, there is evidence that the voluntary contraction required for active motor threshold measurement can influence the response to cTBS (Gentner et al. 2008; Goldsworthy et al. 2012a). Therefore, for this study, the intensity of cTBS was set to 70% of subjects’ resting motor threshold (RMT). RMT was assessed for each experimental session prior to cTBS application using the rTMS coil and was defined as the minimum stimulus intensity required to evoke an MEP from the relaxed right FDI muscle with the peak-to-peak amplitude of >50 μV in at least 5 of 10 consecutive trials. Sham cTBS was delivered to the same scalp site as real cTBS using a sham rTMS coil (either PN 3285-00, Magstim; or MCF-P-B65, MagVenture).

TBS for Plasticity Reversal
A shortened form of the intermittent TBS (iTBS) protocol was applied in both sessions of Experiment 5 to test the reversal of M1 neuroplasticity by externally generated network activity. The stimulation parameters were the same as those described by Huang et al. (2010) and consisted of the standard TBS pattern applied in 2 s trains repeated at 10 s intervals for a total of 150 pulses (i.e., iTBS150). The intensity of stimulation was the same as that used for cTBS.

Force of Voluntary Contraction
The force sustained during the 2-min isometric voluntary contraction in Experiments 1–4 was monitored for all subjects using a strain gauge, and visual feedback was provided to ensure constant force production during the contraction. The force of contraction was set to 0.45 kg for each subject, which, based on unpublished pilot data, we anticipated would roughly equate to 10% of maximal voluntary effort for most of the subjects. The duration and intensity of contraction were chosen to produce the greatest likelihood of de-depression while minimizing fatigue (Sogaard et al. 2006).

The rationale for setting the absolute force of voluntary contraction constant for each subject irrespective of their maximal effort was that we wanted to avoid subjects activating the targeted hand muscles (a requirement for assessing maximal voluntary effort) prior to cTBS application. A limitation of using this approach was that differences in the force of each subject’s contraction relative to their maximal effort may have introduced variability in the data. Therefore, to determine the extent to which this may have been a factor, each subject was required to perform 3 maximal voluntary contractions (MVCs) at the end of each TDI contract session (see Fig. 1). The MVC with greatest force was used to determine the intensity of each subject’s 2 min isometric contraction relative to their maximal voluntary effort.

Quantification of cTBS Effects
MEPs were recorded in blocks of 15 trials for all experiments. Within each experimental session, 3 blocks were recorded at baseline (B1, B2, and B3), and a total of 9 postintervention blocks were recorded following the first stimulation protocol. One postintervention block was...
Figure 1. Schematic overview of experimental design. Open rectangles designate blocks of 15 MEP trials measured with single-pulse TMS at baseline (B1–3) and postintervention (P1–9). All experiments were conducted with the FDI at rest. "FDI contract" as a condition only refers to the 2-min sub-MVC performed after the stimulation protocols and not during MEP measurement. Three maximal voluntary contractions (MVCs) were performed at the end of each FDI contract session for each subject.
recorded between stimulation protocols (i.e., P1), and another was recorded immediately following the second stimulation protocol and prior to the voluntary contraction (for Experiments 1–4) and iTBS150 (for Experiment 5) (i.e., P2). Seven blocks were recorded after the voluntary contraction/iTBS150, with recordings at 20 min (P3), 25 min (P4), 30 min (P5), 35 min (P6), 40 min (P7), 50 min (P8), and 60 min (P9) following the first stimulation protocol (see Fig. 1).

At least 2 days separated each experimental session. The order in which subjects attended each of the sessions for each experiment was randomized, and all experimental sessions were performed in the afternoon to control for time-of-day effects on neuroplasticity induction (Sale et al. 2007). Although the phase of menstrual cycle was not controlled for in this study, the random order and timing of experimental sessions make this unlikely to have significantly influenced our results. Background surface electromyography was monitored at all points during and between recording blocks to ensure complete relaxation of subjects’ right FDI muscle during periods, where a voluntary contraction was not required. Trials that contained background muscle activation during 100 ms prior to TMS application were excluded from analysis.

Data Analysis
All statistical analyses were performed with IBM SPSS Statistics 20 (IBM SPSS, Armonk, NY, USA) on normally distributed data (verified using the Kolmogorov–Smirnov test). Mean peak-to-peak MEP amplitudes were calculated for each recording block for each subject. Baseline data were analyzed using 2-way repeated-measures analysis of variance (ANOVA$_{BM}$) with CONDITION (Experiments 1–4: 2 levels—FDI relaxed and FDI contract; Experiment 5: 2 levels—single cTBS and paired cTBS) and BLOCK (3 levels—B1, B2, and B3) as within-subject factors to assess the stability of baseline MEP amplitudes between conditions and between baseline recording blocks for each experiment. The 3 blocks of baseline MEPs were averaged for each subject, and postintervention MEP amplitudes were expressed as a percentage of the average baseline for comparisons between experimental conditions.

For Experiments 1–4, the impact that the voluntary contraction had on MEP amplitudes was assessed using 2-way ANOVA$_{BM}$ with CONDITION (2 levels—FDI relaxed and FDI contract) and BLOCK (7 levels—P3, P4, P5, P6, P7, P8, and P9) as within-subject factors. One-way ANOVA$_{BM}$ were then performed on raw MEP data for each experimental condition separately with BLOCK (10 levels: the average baseline, as well as P1, P2, P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor. If a significant main effect of BLOCK was observed, post hoc comparisons were performed using paired t-tests to determine the time points at which MEP amplitudes were significantly different from baseline. Additional comparisons were performed on pooled MEP data using paired t-tests. Data were pooled into 2 time periods: An “early response” period representing the average response over the period 20–35 min following the first stimulation protocol (calculated as the average of P3, P4, P5, and P6) and a “late response” period representing the average response over the period 40–60 min following the first stimulation protocol (calculated as the average of P7, P8, and P9). The definitions of the early and late response time periods were based on the duration of MEP depression observed in Experiment 2 for the single cTBS, FDI relaxed condition (see Results).

Comparisons between the responses to no cTBS (Experiment 1) and single cTBS (Experiment 2) were performed only for those subjects that participated in both experiments (i.e., a total of 7 subjects) using 2-way ANOVA$_{BM}$ with STIMULATION TYPE (2 levels—no cTBS and single cTBS) and BLOCK (8 levels—P2, P3, P4, P5, P6, P7, P8, and P9) as within-subject factors. Likewise, the response to paired cTBS (Experiment 3) was compared with that for both no cTBS and single cTBS for subjects that participated in Experiments 1–3 (i.e., a total of 4 subjects).

Pearson correlation coefficient tests were performed on data from Experiment 2 to further characterize the impact that the voluntary contraction may have had on single cTBS-induced MEP depression. We looked to determine whether the de-depression of MEPs by a voluntary contraction for each subject was influenced by 2 factors: (1) The initial level of MEP depression prior to the voluntary contraction (i.e., the mean MEP amplitude at P2, expressed as a percentage of the average baseline) and (2) the relative intensity of the voluntary contraction (expressed for each subject as a percentage of their MVC). A measure of de-depression was calculated for each subject by subtracting the pooled MEP amplitudes recorded during the early response period for the FDI relaxed condition from that recorded during the same period for the FDI contract condition.

For Experiment 5, the impact of iTBS150 on single and paired cTBS-induced changes in MEP amplitudes was assessed by comparing these data (expressed as a percentage of the average baseline) with that for the FDI relaxed conditions of Experiment 2 (for single cTBS) and Experiment 3 (for paired cTBS) using 2-way mixed-design ANOVA$_{BM}$ with CONDITION (2 levels—FDI relaxed (i.e., no iTBS150) and iTBS150) as the between-subject factor and BLOCK (7 levels—P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor. Separate 1-way ANOVA$_{BM}$ were then performed on raw MEP data with BLOCK (10 levels: the average baseline, as well as P1, P2, P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor, and contingent on a significant main effect of BLOCK, post hoc analyses were performed using paired t-tests. Additional comparisons were performed on MEP data pooled into early and late response time periods using independent-samples t-tests.

Where necessary, the degrees of freedom for ANOVA$_{BM}$ were adjusted using the Huynh-Feldt correction for nonsphericity, and multiple comparisons were corrected for using the false discovery rate procedure (FDRP; Curran-Everett 2000). All statistical analyses were 2-tailed, and unless indicated otherwise, all data represent group means ± standard deviation. Statistical significance was accepted for P-values of ≤0.05.

Results

Experiment 1—No cTBS
Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 1 were 0.94 ± 0.29 and 0.93 ± 0.18 mV, respectively. There was no difference in baseline MEP amplitudes between conditions (F1,9 = 0.08, P = 0.79), nor was there a difference between baseline recording blocks (F2,18 = 1.27, P = 0.31).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 1 was 10.1 ± 3.6% of MVC. Analysis of the postintervention MEP recording blocks that followed the contraction showed no significant main effect of BLOCK (F6,54 = 0.73, P = 0.63), CONDITION (F1,9 = 0.08, P = 0.78), and no interaction between the 2 factors (CONDITION $\times$ BLOCK: F6,54 = 0.29, P = 0.92) (Fig. 2A). There were also no differences between the FDI relaxed and FDI contract conditions when MEP data were pooled into early (FDI relaxed: 95.1 ± 12.9% of average baseline and FDI contract: 98.4 ± 24.1% of average baseline; paired t9 = −0.42, P = 0.69) and late (FDI relaxed: 95.8 ± 22.3% of average baseline and FDI contract: 97.3 ± 25.7% of average baseline; paired t9 = −0.13, P = 0.90) response periods following the contraction (Fig. 2B).

Experiment 2—Single cTBS
Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 2 were 0.91 ± 0.20 and 0.90 ± 0.14 mV, respectively. There was no difference in baseline MEP amplitudes between conditions (F1,9 = 0.03, P = 0.88), nor was there a difference between baseline recording blocks (F2,18 = 0.09, P = 0.92). In the subset of 7 subjects that participated in both Experiments 1 and 2, there was no difference between baseline MEP amplitudes for the FDI relaxed...
conditions of each experiment ($F_{1,6} = 0.67, P = 0.45$). However, analysis of postintervention MEPs revealed a significant main effect of STIMULATION TYPE ($F_{1,6} = 9.92, P = 0.02$), and this was due to a depression of MEP amplitudes compared with baseline for the single cTBS, FDI relaxed condition of Experiment 2 (BLOCK: $F_{7,43} = 3.30, P = 0.006$).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 2 was 9.5 ± 3.4% of MVC. The intensity of contraction did not differ from that used for the no cTBS, FDI contract condition of Experiment 1 in the 7 subjects that participated in both experiments (paired $t_{6} = 0.22, P = 0.84$). Analysis of the postintervention MEP recording blocks that followed the contraction revealed significant differences between conditions ($F_{1,9} = 5.22, P = 0.05$) and also between recording blocks ($F_{6,54} = 3.96, P = 0.002$), although there was no interaction between the 2 factors ($F_{6,54} = 0.79, P = 0.58$). Separate 1-way ANOVA were performed to explore the main effect of condition. There was a significant difference between recording blocks for the single cTBS, FDI relaxed condition of Experiment 2 ($F_{5,65} = 5.72, P < 0.001$), and this was due to depression of MEP amplitudes at P2, P3, P4, P5, and P6 compared with baseline ($P \leq 0.02$ for all; FDRP correction). However, there was no change in MEP amplitudes from baseline values during the postintervention recording period for the single cTBS, FDI contract condition ($F_{3,31} = 1.51, P = 0.23$) (Fig. 2).

Based on the time course of the response to single cTBS for the FDI relaxed condition, postcontraction MEPs were pooled into an early response period (i.e., the P3, P4, P5, and P6 recording blocks, corresponding to postcontraction time points significantly affected by single cTBS in the FDI relaxed condition) and a late response period (i.e., the P7, P8, and P9 recording blocks, corresponding to postcontraction time points that did not differ from baseline for either condition). Comparisons of pooled MEP data revealed a significant difference between conditions for the early response period.
following the contraction (paired $t_{(9)} = -2.40, P = 0.04$), with depression observed for the FDI relaxed condition (65.2 ± 14.3% of average baseline), but not for the FDI contract condition (96.6 ± 32.7% of average baseline). There was no difference between the FDI relaxed and FDI contract conditions for the late response period (FDI relaxed: 92.8 ± 19.9% of average baseline and FDI contract: 107.0 ± 27.1% of average baseline; paired $t_{(9)} = -1.32, P = 0.22$) (Fig. 2D).

**Experiment 3—**Paired cTBS

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 3 were 0.88 ± 0.19 and 0.92 ± 0.15 mV, respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,9} = 0.73, P = 0.42$), nor was there a difference between baseline recording blocks ($F_{2,18} = 0.49, P = 0.62$). Data from Experiment 3 were compared with that from Experiments 1 and 2 in the subset of 4 subjects that participated in all 3 experiments. Baseline MEPs did not differ between the FDI relaxed and FDI contract conditions of Experiment 3 revealed that although there was no depression observed for the FDI relaxed condition from that recorded during the same period for the FDI contract condition (i.e., values >0 indicate MEP depression). There tended to be less MEP depression in subjects that responded to single cTBS with greater MEP depression (shown as a smaller mean depression) than those that responded to paired cTBS (FDI relaxed condition from that recorded during the same period for the FDI contract condition (i.e., values >0 indicate MEP depression). (A) There tended to be less MEP depression in subjects that responded to single cTBS with greater MEP depression (shown as a smaller mean depression) than those that responded to paired cTBS (FDI relaxed condition from that recorded during the same period for the FDI contract condition (i.e., values >0 indicate MEP depression).

**Factors Affecting MEP Depression Following Contraction**

Correlation analyses on single cTBS data for Experiment 2 indicated no significant relationship between normalized MEP amplitudes recorded during the FDI contract session at P2 (i.e., just prior to the contraction) and the level of MEP depression observed during the early response period following the contraction (r = 0.53, P = 0.12). Subjects that performed a higher intensity contraction (expressed as a percentage of their MVC) showed a greater MEP depression (r = 0.90, P = 0.001, excluding an outlier (open circle)).

**Experiment 4—Single cTBS (Control)**

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 4 were 0.92 ± 0.21 and
0.86 ± 0.22 mV, respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,5} = 0.90, P = 0.38$), nor was there a difference between baseline recording blocks ($F_{2,14} = 0.28, P = 0.76$).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 4 was 9.3 ± 2.1% of MVC. This did not differ from the contraction intensities used for Experiments 1 (independent $l_{(16)} = -0.56, P = 0.59$), 2 (independent $l_{(16)} = -0.14, P = 0.89$), and 3 (independent $l_{(16)} = -1.12, P = 0.28$).

Analysis of the postintervention MEP recording blocks that followed the contraction revealed significant differences between conditions ($F_{1,7} = 13.4, P = 0.008$) and recording blocks ($F_{6,42} = 2.76, P = 0.02$), as well as a significant interaction between the 2 factors ($F_{6,42} = 3.17, P = 0.01$). There was a significant depression of postintervention MEP amplitudes for the FDI relaxed condition ($F_{2,52} = 3.12, P = 0.007$), with depression of MEPs recorded at P1, P2, P3, P4, and P5 compared with baseline ($P < 0.02$ for all; FDRP correction). A significant main effect of BLOCK was also observed for the FDI contract condition ($F_{9,63} = 3.79, P = 0.001$), and this was due to depression of MEPs compared with baseline at P1 ($P = 0.006$). There was a trend towards MEP facilitation (compared with baseline levels) at recording blocks P5 and P7, although these did not reach statistical significance ($P ≥ 0.08$ for both) (Fig. 4A).

Comparisons of pooled MEP data revealed a significant difference between each of the conditions for the early response period following the contraction (paired $l_{(7)} = -4.67$, $P = 0.002$), with depression observed for the FDI relaxed condition (61.7 ± 28.3% of average baseline) but not for the FDI contract (113.2 ± 32.5% of average baseline). There appeared to be a slight difference in MEP amplitudes between the FDI relaxed and FDI contract conditions for the late response period, although this did not reach statistical significance (FDI relaxed: 85.3 ± 33.2% of average baseline and FDI contract: 123.3 ± 48.5% of average baseline; paired $l_{(7)} = -2.25, P = 0.06$) (Fig. 4B).

**Experiment 5—De-depression by iTBS150**

Average baseline MEP amplitudes for the single and paired cTBS conditions for Experiment 5 were 0.88 ± 0.27 and 0.86 ± 0.30 mV, respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,5} = 0.07, P = 0.80$), nor was there a difference between baseline recording blocks ($F_{2,14} = 1.79, P = 0.20$).

Analysis of the postintervention MEP recording blocks that followed iTBS150 for the single cTBS, iTBS150 condition of Experiment 5 revealed a significant difference compared with the FDI relaxed condition of Experiment 2 (i.e., single cTBS without subsequent voluntary contraction or iTBS150) ($F_{1,18} = 7.31, P = 0.02$), with a trend for an interaction between CONDITION and BLOCK ($F_{6,108} = 2.11, P = 0.06$). Although postintervention MEP amplitudes were depressed compared with baseline for the single cTBS, FDI relaxed condition of Experiment 2, there was no change in MEP amplitudes from baseline values during the postintervention recording period for the single cTBS, iTBS150 condition ($F_{9,81} = 1.79, P = 0.08$) (Fig. 5A). Comparisons of pooled MEP data revealed a significant difference between conditions for the early response period following iTBS150 application (independent $l_{(13)} = -3.59, P = 0.003$) (Fig. 5B).

The postintervention MEP recording blocks that followed iTBS150 for the paired cTBS, iTBS150 condition of Experiment 5 did not differ from those for the paired cTBS, FDI relaxed condition of Experiment 3 ($F_{9,81} = 0.001, P = 0.98$), nor was there an interaction between CONDITION and BLOCK ($F_{6,108} = 0.65, P = 0.69$). As with the FDI relaxed condition of Experiment 3, there was a significant depression of postintervention MEP amplitudes for the paired cTBS, iTBS150 condition ($F_{9,81} = 4.56, P < 0.001$), with depression of MEPs recorded at all postintervention time points compared with baseline ($P ≤ 0.02$ for all; FDRP correction) (Fig. 5C). No differences were observed between conditions when MEP data were pooled into early and late response periods following iTBS150 application (independent $l_{(13)} = 0.18$ and −0.35, respectively, $P > 0.05$ for both) (Fig. 5D).

**Discussion**

The present study confirms that behavioral engagement of M1 by voluntary contraction abolishes LTD-like MEP depression induced by a single cTBS protocol. Here, we show for the first time that the spaced application of repeated cTBS protocols induces MEP depression that is resistant to disruption by voluntary contraction. In addition, findings demonstrate that this MEP depression is also resistant to reversal when an

![Figure 4](https://example.com/figure4.png)

**Figure 4.** The effect of the time interval separating single cTBS and the voluntary contraction on MEP de-depression (Experiment 4). The gray arrow indicates delivery of sham cTBS, while the black arrow indicates delivery of real cTBS. **(A)** As with Experiment 2, MEP amplitudes were depressed following single cTBS for the FDI relaxed condition (filled circles). Despite using a 15-min (instead of 5 min) interval between cTBS and the contraction, there was still MEP reversal back to baseline following the contraction for the FDI contract condition (open circles). **(B)** Analysis of pooled MEP data showed a significant de-depression of MEP amplitudes pooled into the early response period following the contraction. *$P ≤ 0.05$, when FDI relaxed and FDI contract conditions are compared. Data are shown as group means ± standard errors of the mean.
experimental de-depression protocol is used instead of a voluntary contraction.

The MEP depression induced by single cTBS in this study was comparable with that observed previously (Huang et al. 2005), and is likely due to LTD-like changes at excitatory synaptic connections within M1 (Di Lazzaro et al. 2005; Huang et al. 2007). A voluntary contraction applied following cTBS abolished this MEP depression, and the extent to which MEPs were reversed was greater in subjects that contracted at higher intensities relative to their maximal effort. The reversal of LTD-like effects by behavioral engagement of M1 is largely consistent with findings from Huang et al. (2008), which showed that a sub-MVC applied immediately following cTBS reversed MEP depression. Although in that study the cTBS-induced depression of MEP amplitudes was converted to a facilitatory effect following voluntary contraction (as opposed to the present study, which showed MEP amplitudes return to baseline levels), this may have been due to differences in the duration of cTBS application (20 s, compared with 40 s, in this study) and/or the duration of the contraction (1 min, compared with 2 min, in this study). Recently, Thirugnanasambandam et al. (2011) found that a mild voluntary contraction applied following cTBS reversed both MEP facilitation and depression induced by tDCS (transcranial direct current stimulation), a non-invasive brain stimulation protocol which, like TBS, can be used to produce LTP and LTD-like plasticity within the human M1 (Nitsche et al. 2003). Similar reversals of LTP and LTD (referred to as depotentiation and de-depression, respectively) have been shown in animal models when normal physiological activity within the stimulated network follows an induction protocol (Xu et al. 1998; Zhou et al. 2003). Therefore, the reversal of cTBS-induced MEP depression by behavioral engagement of the hand motor regions in the present study may reflect a de-depression-like event within the human M1.

In contrast to the present findings, Saglam et al. (2008) showed that a single 40-s train of cTBS depressed MEP amplitudes despite subjects performing a series of sustained sub-MVCs of the targeted hand muscle immediately and at several time points following cTBS application. The reasons for these different findings are unclear, although several key methodological differences make it difficult to compare with the present work. For instance, Saglam et al. required subjects to contract their hand muscles prior to, as well as following, cTBS application, and it is unclear to what extent this prior activity within M1 may have influenced the results (Gentner et al. 2008; Goldsworthy et al. 2012a). Additionally, Saglam et al. do not show the subjects’ responses to cTBS without post-cTBS voluntary contractions, and thus the extent to which the LTD-like response to cTBS was affected by subsequent voluntary contraction was not determined.

An alternate explanation for the reversal of MEP depression following the voluntary contraction in Experiment 2 may have been due to a facilitatory effect of the voluntary contraction itself. While the voluntary contraction applied in the absence of cTBS in Experiment 1 had no lasting effects on MEP amplitudes, it is possible that the reduced excitability of M1 following cTBS application in Experiment 2 may have initiated homeostatic regulatory mechanisms, thus lowering the threshold for induction of LTP-like effects in accordance with the Bienenstock–Cooper–Munro (BCM) theory (Bienenstock et al. 1982).

Figure 5. The influence of iTBS150 (gray column) on MEP responses (expressed as a percentage of the average baseline) to single cTBS (A and B) and paired cTBS (C and D) (Experiment 5). The gray arrow indicates delivery of sham cTBS, while the black arrows indicate delivery of real cTBS. (A) Compared with the MEP depression observed for the FDI relaxed condition of Experiment 2 (i.e., no iTBS; filled circles), there was no change in MEP amplitudes from baseline levels following single cTBS for the iTBS150 condition (open circles). (B) Analysis of pooled MEP data showed that this was due to reduced MEP depression during the early response period following iTBS150 application. (C) As with the FDI relaxed condition of Experiment 3 (i.e., no iTBS; filled circles), there was a pronounced depression of MEP amplitudes from baseline levels following paired cTBS for the iTBS150 condition (open circles). (D) Post-iTBS150 MEPs pooled into early and late response periods did not differ between the conditions. *P ≤ 0.05, when the iTBS150 condition is compared with the FDI relaxed condition of Experiment 2 (i.e., no iTBS). Data are shown as group means ± standard errors of the mean.
Such homeostatic regulation of neuroplastic change has been shown to occur within the human M1 (Siebner et al. 2004; Ziemann et al. 2004; Stefan et al. 2006; Müller et al. 2007) and may have promoted facilitation of MEPs following voluntary contraction. To investigate this further, correlation analyses were performed to determine whether the level of MEP depression following cTBS and prior to the contraction in Experiment 2 was related to the extent to which MEPs were reversed following the contraction. Although the relationship did not reach statistical significance, there was a tendency for a smaller reversal of MEPs in subjects who responded to cTBS with greater MEP depression. Thus, we consider it unlikely that the reversal of MEPs following a contraction in Experiment 2 was due to homeostatic processes initiated by a cTBS-induced depression of M1 excitability.

Although the depression of MEP amplitudes following a single cTBS application in this study is consistent with the initial findings by Huang et al. (2005), a number of more recent studies have shown considerable intersubject variability in MEP responses to cTBS (McAllister et al. 2011; Goldsworthy et al. 2012a, 2012b). In particular, Hamada et al. (2013) investigated a relatively large sample of 52 subjects and showed no overall effect of cTBS, with fewer than 50% of individuals responding with the expected MEP depression. The reason for the lack of consistency in the response to cTBS application across studies is unclear. However, there is one important methodological difference between the present study and that of Hamada et al. that might help explain at least some of this difference. Whereas the preactivation history of M1 was controlled in the present study by requiring that the subject’s targeted hand was at rest prior to cTBS application, subjects in the study by Hamada et al. performed extensive contractions of the targeted hand muscle prior to cTBS. Although there is evidence that a prior contraction of the targeted hand muscle can affect the induced response to cTBS applied to M1 (Gentner et al. 2008; Goldsworthy et al. 2012a), the contribution of this preactivation to the intersubject variability of responses has not been explored. Therefore, it may be of interest for future studies to investigate the impact of prior voluntary contraction on the response variability to cTBS.

While MEP depression induced by a single cTBS protocol was reversed by a voluntary contraction, the MEP depression induced by paired cTBS remained stable. One possible factor which may have contributed to this finding was the difference in the time interval separating cTBS application and the voluntary contraction for the single and paired cTBS conditions. For single cTBS, subjects performed the contraction at 5 min following stimulation. However, for the paired cTBS condition, the time interval between the first cTBS protocol and the voluntary contraction was 15 min. Experiments in both hippocampal slice preparations and freely moving animals have shown a time-dependency of reversal effects, with less reversal observed when disruptive stimuli were applied after a certain time period (usually tens of minutes) following plasticity induction (Fuji et al. 1991; Xu et al. 1998; Huang et al. 1999; Chen et al. 2001). Therefore, subjects for Experiment 4 received single cTBS with the order of the sham and real cTBS protocols reversed such that the interval between the real cTBS and the contraction was 15 min. Despite the interval being the same as that used for the paired cTBS condition of Experiment 3, the voluntary contraction was still able to reverse single cTBS-induced MEP depression. Therefore, the stability of MEP depression observed in Experiment 3 was likely due to the repeated application of cTBS and not the timing of the contraction. Although the greater number of pulses for the paired cTBS condition may have contributed to the enhanced stability of induced MEP depression, we consider this unlikely since doubling the length of a cTBS train has been shown in a previous study to facilitate, rather than depress, MEP amplitudes (Gamboa et al. 2010).

The increased stability of paired cTBS-induced MEP depression in the present study extends our previous finding that paired cTBS prolongs the duration of MEP depression when applied to the human M1 (Goldsworthy et al. 2012a). Similarly, repeated cTBS has been found to produce robust after-effects when applied in a single session to the human frontal eye field (Nyffeler et al. 2006) and parietal cortical regions (Nyffeler et al. 2009; Cazzoli et al. 2012). In contrast to these results, several studies have shown homeostatic interactions between paired cTBS protocols when applied to the human M1 (Gamboa et al. 2011; Murakami et al. 2012; Mastroeni et al. 2013). However, these studies have required subjects to sustain a voluntary contraction of the targeted hand muscle prior to paired cTBS application to set the stimulation intensity, and this may have influenced the way in which the 2 cTBS protocols interacted to produce changes in MEP amplitudes (Goldsworthy et al. 2012a). Additionally, we employed a slightly different interval between cTBS protocols compared with these previous studies. Studies in both animals (Zhou et al. 2005) and humans (Gamboa et al. 2011) have shown that the length of time separating successive stimulation protocols is important in determining the neural response to repeated stimulation. For example, Zhou et al. (2003) showed that the application of repeated stimulation trains to the developing Xenopus visual system was only effective at stabilizing LTP when using intertrain intervals within a short range (i.e., ±2.5 min) of the optimal interval, which in their study was 5 min. Similarly, Gamboa et al. (2011) showed that the repeated application of cTBS trains to the hand area of the human M1 induced greater MEP depression when long intervals (i.e., 20 min) separated cTBS trains compared with short intervals (i.e., 2 and 5 min). Therefore, although not within the scope of this study, it may be of interest for future studies to further examine the importance of the interval between cTBS protocols applied in a single session to the human M1.

The prolonged duration of cTBS-induced effects with repeated applications bears resemblance to data in animal studies showing long-lasting synaptic plasticity following repeated stimulation protocols (Bliss and Gardner-Medwin 1973; Abraham et al. 1993; Huang and Kandel 1994; Trepel and Racine 1998; Abraham et al. 2002). Furthermore, Zhou et al. (2003) showed that the repeated application of simulation protocols to the developing Xenopus visual system in a spaced manner produced lasting LTP at retinotectal synapses that was resistant to depotentiation by spontaneous activation of the postsynaptic tectal neuron. A similar resistance to depotentiation has been achieved in the rodent hippocampus using repeated stimulation protocols (Woo and Nguyen 2003) and was likely due to consolidation of LTP through increases in de novo protein synthesis and gene transcription (Krug et al. 1984; Huang and Kandel 1994; Nguyen et al. 1994; Woo and Nguyen 2003). Thus, a similar consolidation of LTD-like effects following repeated cTBS applications may underlie the
resistance to de-depression by voluntary contraction observed in the present study.

A feature of consolidated synaptic plasticity in animal models is that it is stable not only in the presence of behaviorally relevant physiological activity, but also when the network is stimulated artificially shortly after plasticity induction (Woo and Nguyen 2003). The stimulation protocols used to reverse plasticity are typically weaker than those used for plasticity induction and do not produce lasting effects when applied on their own (Zhou and Poo 2004). In this study, we tested whether paired cTBS-induced MEP depression was also resistant to reversal by externally generated activity within M1. The iTBS150 paradigm was specifically chosen as it has no effect on MEP amplitude when given alone (Huang et al. 2010), compared with longer trains (e.g., iTBS with 300 pulses) which increase MEP amplitudes (Huang et al. 2005; Murakami et al. 2012). A novel finding of the present study was that while single cTBS-induced MEP depression was reversed by iTBS150, the MEP depression induced by paired cTBS remained stable. We suggest that this provides an additional line of evidence linking the M1 excitability changes induced by TBS protocols with the LTP and LTD observed in animal models.

The instability of rTMS-induced neuroplasticity in the face of normal physiological activity impacts greatly on the therapeutic potential of rTMS protocols. The results of the present study have shown that the repeated application of cTBS may be an effective approach for consolidating MEP depression, making it resistant to reversal by behavioral engagement of the motor regions. We also show that this MEP depression is stable in the presence of a stimulation protocol designed to reverse cTBS-induced neuroplasticity. These findings may have significant implications for the clinical application of rTMS.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes
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References


