Title: Probing changes in corticospinal excitability following theta burst stimulation of the human primary motor cortex

Authors: Mitchell R. Goldsworthy[1,*], Ann-Maree Vallence[1,*], Nicolette A. Hodyl[1], John G. Semmler[2], Julia B. Pitcher[1], Michael C. Ridding[1]

* These authors contributed equally

[1]The Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia.

[2]Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, SA, Australia.

Corresponding author: Dr Mitchell Goldsworthy, DX 650-517 Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide, SA 5005, Australia. Tel: +61 8 8313 1323; Email: mitchell.goldsworthy@adelaide.edu.au

Acknowledgements: MRG is supported by an Alzheimer's Australia Dementia Research Foundation (AADRF) Postdoctoral Fellowship. NAH is supported by a National Health and Medical Research Council (NHMRC) Australian Biomedical (Training) Fellowship (1016379).

Highlights

- Plasticity responses to cTBS and iTBS were assessed using MEP input/output curves.
- LTD-like response to cTBS was greatest when probed using high stimulus intensities.
- LTP-like response to iTBS was greatest when probed using low stimulus intensities.

Abstract

Objective: To determine whether the intensity of transcranial magnetic stimulation (TMS) used to probe changes in corticospinal excitability influences the measured plasticity response to theta burst stimulation (TBS) of the human primary motor cortex.

Methods: Motor evoked potential (MEP) input/output (I/O) curves were recorded before and following continuous TBS (cTBS) (Experiment 1; $n = 18$) and intermittent TBS (iTBS) (Experiment 2; $n = 18$).

Results: The magnitude and consistency of MEP depression induced by cTBS was greatest when probed using stimulus intensities at or above 150% of resting motor threshold (RMT). In contrast, facilitation of MEPs following iTBS was strongest and most consistent at 110% of RMT.

Conclusions: The plasticity response to both cTBS and iTBS is influenced by the stimulus intensity used to probe changes in corticospinal excitability.

Significance: The results highlight the importance of the test stimulus intensity used to assess TBS-induced changes in corticospinal excitability when interpreting neuroplasticity data, and suggest that a number of test intensities may be required to reliably probe the plasticity response.

Keywords: transcranial magnetic stimulation; cTBS; iTBS; motor evoked potential; input/output curve; plasticity

1. Introduction

A number of non-invasive brain stimulation (NIBS) techniques have been developed that provide significant opportunities to gain novel insights into human brain function. In particular, techniques such as transcranial magnetic stimulation (TMS) can be used not only to test the excitability of cortical networks, but also modulate excitability in a bidirectional and reversible manner when applied in trains of repetitive stimuli (i.e., repetitive TMS; rTMS) (Vallence and Ridding, 2014). The changes in excitability induced by rTMS are likely due to processes similar to the long-term potentiation (LTP) and long-term depression (LTD) described in animal models (Huang et al., 2007; Teo et al., 2007), which are key neural mechanisms involved in learning and memory (Cooke and Bliss, 2006). As a result, rTMS is useful for probing human cortical plasticity and may be of potential therapeutic benefit in a range of different neurological and psychiatric disorders (Ridding and Rothwell, 2007).

Conventional rTMS approaches involve a constant rate of stimulation, with low frequencies (≤1 Hz) reducing cortical excitability (Chen et al., 1997) and high frequencies (≥5 Hz) increasing cortical excitability (Berardelli et al., 1998). More recently, however, patterned protocols such as theta burst stimulation (TBS) have been developed which require less stimulation time and lower stimulation intensities than conventional rTMS protocols. Consisting of repeated bursts of high-frequency subthreshold magnetic stimuli, TBS can either depress (when applied as continuous TBS; i.e., cTBS) or increase (when applied as intermittent TBS; i.e., iTBS) cortical excitability (Huang et al., 2005). Although the initial report of TBS demonstrated long-lasting and robust changes, emerging evidence suggests these effects can vary considerably between individuals (for example, Hamada et al., 2013).

When applied to the human primary motor cortex (M1), the plasticity induced by TBS is usually quantified by recording a change in the electromyographic (EMG) response to single-pulse TMS (i.e., the motor evoked potential; MEP) from peripheral muscles. Most studies measure MEPs from a single TMS intensity to probe the plasticity response to TBS, typically using an intensity sufficient to evoke MEPs at baseline with peak-to-peak amplitudes of ~1 mV (SI_{1mV}) (Gentner et al., 2008; Hamada et al., 2013; Huang et al., 2007). However, given that MEP amplitudes are highly variable between subjects, using this arbitrary value potentially results in test MEPs being used that fall on different parts of the input/output (I/O) curve (Burke and Pierrot-Deseilligny, 2010). Therefore, this approach may potentially add to the inter-subject variability of the TBS response. To date, the potential importance of the stimulus intensity used to probe changes in MEPs following TBS has not been investigated systematically.

Generating I/O curves by applying TMS at a range of stimulus intensities can provide a sensitive measure of corticospinal excitability (Devanne et al., 1997; Ridding and Rothwell, 1997; Vallence et al., 2012). Here, we constructed I/O curves before and following cTBS and iTBS (assessed separately), to determine the range of test stimulus intensities that provide the most sensitive and reliable measure of TBS-induced plasticity.

2. Methods

2.1 Subjects

A total of 27 right-handed subjects (16 females) aged from 18 to 32 years (mean ± SEM: 22.1 ± 0.7 years) participated in this study, which consisted of two experiments:

Experiment 1 examined the response to cTBS (18 subjects, including 11 females; 22.7 ± 1.0 years), and Experiment 2 examined the response to iTBS (18 subjects, including 10 females; 22.1 ± 1.0 years). Nine subjects participated in both experiments. This study was performed in accordance with the Declaration of Helsinki and approved by the University of Adelaide Human Research Ethics Committee. All subjects gave informed written consent prior to testing and were screened for any contraindications to TMS (Rossi et al., 2009).

2.2 Stimulation and recording

Surface EMG was recorded from the relaxed right first dorsal interosseous (FDI) using two Ag/AgCl electrodes arranged in a belly-tendon configuration. EMG activity was amplified with a gain of 1000, band-pass filtered between 20 and 1000 Hz (Cambridge Electrical Design 1902 amplifier, Cambridge, UK), and digitised at a sampling rate of 5 kHz (Cambridge Electrical Design 1401, Cambridge, UK).

Single-pulse TMS was applied with monophasic waveform using a figure-of-eight coil (90 mm external wing diameter) connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, UK). The coil was positioned over the left M1 tangential to the scalp, with the handle pointing posterolaterally at a 45° angle to the sagittal plane (i.e., posterior–anterior current flow across M1). Stimuli were applied systematically to different scalp locations using a suprathreshold stimulus intensity to identify the optimal site for consistently evoking MEPs in the relaxed FDI. Once located, this site was marked on the scalp using a felt marker, and resting motor threshold (RMT) was determined. RMT was defined as the minimum stimulus intensity (expressed as percentage of maximum stimulator output; MSO) required to elicit an MEP in the

relaxed FDI with peak-to-peak amplitude >50 μV in at least 5 out of 10 consecutive trials.

2.3 Theta burst stimulation

TBS was applied with biphasic waveform (posterior–anterior/anterior–posterior current flow) using an air-cooled figure-of-eight coil connected to a Magstim Super Rapid magnetic stimulator (Magstim, Whitland, UK). The pattern consisted of short bursts of three stimuli at 50 Hz, repeated at a frequency of 5 Hz. For cTBS (Experiment 1), this pattern of stimuli was applied as a continuous 40-s train, whereas for iTBS (Experiment 2), bursts of stimuli were applied for 2 s at 10-s intervals for a total duration of 190 s (Huang et al., 2005). Stimulation intensity was set to 70% of RMT (Gentner et al., 2008; Goldsworthy et al., 2014a; Goldsworthy et al., 2012a), which was assessed just prior to TBS application using the same coil and biphasic pulse waveform.

2.4 Input/output curves

I/O curves were constructed using monophasic single TMS pulses applied at 10 different stimulus intensities between 90 and 180% RMT (inclusive), with increments of 10% RMT. Stimulus intensities were determined at baseline for each experiment and remained constant for all I/O curve measurements. For each I/O curve, eight stimuli were delivered at each intensity in a pseudo-randomised order, using an interstimulus interval of 5 s (± 10% variance). The time taken to obtain each curve was ~7 min. Curves were measured at five time periods during each experiment: twice at baseline (B1 and B2), and during the periods 0–7, 15–22, and 30–37 min post-TBS (P1, P2, and P3, respectively) (Fig. 1). EMG activity was monitored at all times post-TBS in both experiments to ensure complete relaxation of the right FDI

and minimise the influence of voluntary contraction on the TBS response (Goldsworthy et al., 2014b; Huang et al., 2008).

2.5 Data analysis

Statistical analyses were performed with IBM SPSS Statistics 20 (IBM SPSS, Armonk, NY, USA). Identical analyses were performed in parallel for cTBS (Experiment 1) and iTBS (Experiment 2) data.

Peak-to-peak MEP amplitudes were calculated for each trial; those contaminated with background EMG activity during the 200 ms prior to TMS were excluded from analysis. Mean MEP amplitudes were calculated for each stimulus intensity at each time period. To test for differences between the two I/O curves obtained at baseline, two-way repeated measures ANOVA (RM-ANOVA) with within-subject factors TIME (2 levels: B1 and B2) and INTENSITY (10 levels: 90, 100, 110, 120, 130, 140, 150, 160, 170, 180% RMT) were performed on raw MEP amplitudes. Since there were no significant differences between baseline curves in either Experiment (see Results), the two baseline I/O curves were averaged. The maximum mean MEP amplitude (MEP_{max}) of the average baseline I/O curve was determined for each subject, and was defined as the largest recorded mean MEP amplitude at any stimulus intensity after averaging between baseline curves. All data were normalised to this average baseline MEP_{max} value for the main analysis.

As with the baseline curves, post-TBS I/O curves did not differ between P1, P2, and P3 in either Experiment (see Results), and therefore were pooled across time periods. For the main analysis examining the plasticity response to each of the TBS protocols, two-way RM-ANOVA were performed with TIME (2 levels: average baseline and average post-TBS) and INTENSITY (10 levels) as within-subject factors. Conditional

on a significant interaction between main effects, *post hoc* comparisons between baseline and post-TBS MEP amplitudes were conducted for each stimulus intensity using Fisher's PLSD test.

Further analyses were performed to examine the variability of TBS-induced effects probed using four different test stimulus intensities: (1) 110% RMT (the lowest suprathreshold intensity tested), (2) SI_{1mV} (defined as the intensity between 110–180% RMT, inclusive, at which average baseline MEP amplitudes were closest to 1 mV), (3) SI_{50} (defined as the intensity at which average baseline MEP amplitudes were closest to 50% of the average baseline MEP_{max}), and (4) 180% RMT (the highest stimulus intensity tested). Paired *t* tests were performed to compare raw MEP amplitudes at baseline (average of B1 and B2) with those recorded post-TBS (average of P1, P2, and P3) at each intensity.

The responses to cTBS and iTBS were compared in the subset of nine subjects that participated in both experiments. For this analysis, average post-TBS mean MEP amplitudes for both the cTBS and iTBS protocols were expressed as a ratio of the average baseline mean MEP amplitude for each suprathreshold test stimulus intensity (i.e., between 110–180% RMT, inclusive). Comparisons between the two TBS protocols were performed using two-way RM-ANOVA with PROTOCOL (2 levels: cTBS and iTBS) and INTENSITY (8 levels: 110, 120, 130, 140, 150, 160, 170, 180% RMT) as within-subject factors, and *post hoc* analyses were conducted using Fisher's PLSD test when a significant interaction was identified.

Finally, to examine possible ceiling and floor effects (that is, biases in the direction and magnitude of the induced response, whereby maximal MEPs are more likely to be depressed following stimulation and liminal MEPs are more likely to be facilitated),

correlation analyses were performed to determine the relationship between the plasticity response probed at high (150–180% RMT) and low (100–120% RMT) stimulus intensities, and the likelihood that baseline MEP amplitudes elicited at those intensities were near the ceiling or floor of the testable range, respectively. Ceiling and floor assessments were based on the slope of the linear regression line fitted to average baseline mean MEP amplitudes (normalised to average baseline MEP_{max}) between 150–180% RMT for the ceiling, and between 100–120% RMT for the floor. In both instances, slope values close to 0 indicated that baseline MEPs elicited using these intensities were nearer the ceiling or floor of the testable range. The Pearson correlation coefficient was used for all correlation analyses, except where assumptions of normality were violated (in which case, Spearman's rho was used).

All analyses were two-tailed, and all data represent group mean ± SEM. Data were checked for normality using the Kolmogorov–Smirnov test. Mauchly's Test of Sphericity was performed and, where necessary, degrees of freedom were adjusted using Huynh-Feldt corrections. Statistical significance was accepted for $P < 0.05$.

3. Results

3.1 Baseline measures

Mean RMT at baseline was 39.2 ± 1.0% MSO and 42.3 ± 1.3% MSO for Experiments 1 and 2, respectively. No significant differences were observed between raw I/O curves recorded at B1 and B2 in Experiment 1 (TIME: $F_{1,17} = 0.49$, $P = 0.49$; TIME × INTENSITY: $F_{5,92} = 0.64$, $P = 0.68$) or Experiment 2 (TIME: $F_{1,17} = 2.91$, $P = 0.11$; TIME × INTENSITY: $F_{6,105} = 1.53$, $P = 0.17$). Raw mean MEP amplitudes recorded

at baseline (average of B1 and B2 trials) for each stimulus intensity are shown in Table 1.

3.2 Experiment 1 – cTBS

There was no significant difference between post-cTBS I/O curves recorded at P1, P2, and P3 in Experiment 1 (TIME: $F_{2,34} = 0.43$, $P = 0.65$; TIME × INTENSITY: $F_{12,205} = 0.62$, $P = 0.83$). Two-way RM-ANOVA on normalised data pooled into average baseline and average post-cTBS revealed significant main effects of TIME ($F_{1,17} = 5.33$, $P = 0.03$) and INTENSITY ($F_{5,82} = 320.6$, $P < 0.0001$), as well as a significant interaction between these two factors ($F_{4,73} = 4.01$, $P = 0.004$). This was due to a reduction in post-cTBS MEP amplitudes, compared with baseline, at stimulus intensities 120, 150, 160, 170, and 180% RMT (for all, paired $t_{17} \geq 2.16$, $P \leq 0.04$) (Fig. 2A).

SI_{1mV} varied between 110 and 180% RMT (median: 125% RMT), and produced baseline MEPs with mean amplitude 0.94 ± 0.06 mV. The range for SI_{50} was between 120 and 140% RMT (median: 130% RMT), evoking MEPs at baseline with mean amplitude 1.46 ± 0.26 mV. While raw MEP amplitudes recorded post-cTBS did not differ from those recorded at baseline for the 110% RMT (Fig. 2B), SI_{1mV} (Fig. 2C), and SI_{50} (Fig. 2D) stimulus intensities, a significant MEP depression was observed at 180% RMT (paired $t_{17} = 3.27$, $P = 0.004$) (Fig. 2E). The expected decrease in MEP amplitudes occurred in 33% of subjects at 110% RMT (Fig. 2F), 72% at SI_{1mV} (Fig. 2G), and 61% at SI_{50} (Fig 2H), compared with 83% of subjects at 180% RMT (Fig. 2I).

3.3 Experiment 2 – iTBS

Post-iTBS I/O curves were not significantly different between P1, P2, and P3 in Experiment 2 (TIME: $F_{2,26} = 0.86$, $P = 0.41$; TIME × INTENSITY: $F_{11,188} = 0.95$, $P = 0.49$). Two-way RM-ANOVA on normalised data pooled into average baseline and average post-iTBS revealed a significant main effect of INTENSITY ($F_{4,66} = 289.4$, $P < 0.0001$) but not TIME ($F_{1,17} = 0.053$, $P = 0.82$). However, a significant TIME × INTENSITY interaction was observed ($F_{5,76} = 2.98$, $P = 0.02$), and this was due to the increased amplitude of post-iTBS MEP amplitudes, compared with baseline, at the 110% RMT stimulus intensity (paired $t_{17} = -2.67$, $P = 0.02$) (Fig. 3A).

SI_{1mV} was between 110 and 180% RMT in Experiment 2 (median: 120% RMT), resulting in mean MEP amplitudes of 0.94 ± 0.13 mV at baseline. SI_{50} was between 120 and 140% RMT (median: 130% RMT), evoking baseline MEPs with mean amplitude 1.86 ± 0.26 mV. Raw post-iTBS MEP amplitudes were significantly facilitated compared with baseline for the 110% RMT (paired $t_{17} = -3.43$, $P = 0.003$) (Fig. 3B) and SI_{1mV} (paired $t_{17} = -2.73$, $P = 0.01$) (Fig. 3C) stimulus intensities; however, no change was observed at SI_{50} (Fig. 3D) or 180% RMT (Fig. 3E). In contrast to Experiment 1, the largest proportion of subjects responding in the expected direction to iTBS was observed at 110% RMT, with 83% showing an increase in MEP amplitudes (Fig. 3F). The proportion of subjects responding in the expected direction decreased at higher intensities, with the expected increase in MEP amplitudes occurring in 72%, 56%, and 44% of subjects for SI_{1mV} (Fig. 3G), SI_{50} (Fig. 3H), and 180% RMT (Fig. 3I), respectively.

3.4 Comparison between TBS protocols

In the subset of nine subjects who participated in both experiments, average baseline I/O curves for the cTBS and iTBS protocols did not differ (PROTOCOL: $F_{1,8} = 0.27$, $P = 0.62$; PROTOCOL × INTENSITY: $F_{2,19} = 0.19$, $P = 0.86$). However, comparison of average post-TBS mean MEP amplitudes expressed as a ratio of average baseline for all suprathreshold intensities showed a difference between cTBS and iTBS, with significant main effects of PROTOCOL ($F_{1,8} = 5.87$, $P = 0.04$) and INTENSITY ($F_{2,18} = 6.10$, $P = 0.008$), as well as a PROTOCOL × INTENSITY interaction ($F_{3,27} = 3.65$, $P = 0.02$). This was due to differences between protocols at the 110 and 120% RMT stimulus intensities (for both, paired $t_8 \geq 2.49$, $P \leq 0.04$), with a greater increase in MEP amplitudes at these stimulus intensities following iTBS compared with cTBS (Fig. 4).

3.5 Potential ceiling and floor influences on TBS-induced plasticity

While the slope between 150–180% RMT showed no association with the plasticity response to cTBS probed using these high stimulus intensities ($r = 0.11$, $P = 0.68$) (Fig. 5A), a significant correlation was observed for iTBS, with steeper slope associated with greater MEP facilitation ($r = 0.48$, $P = 0.04$) (Fig. 5B). Conversely, while there was evidence for a floor effect for cTBS, with a trend observed between the plasticity response probed at 100–120% RMT and the slope of average baseline mean MEP amplitudes elicited at these intensities ($r = -0.46$, $P = 0.06$) (Fig. 5C), no association was observed for iTBS ($r_s = -0.20$, $P = 0.43$) (Fig. 5D).

4. Discussion

The present study shows that the measured plasticity response (indexed as a change in MEP amplitude) to both cTBS and iTBS is influenced by the stimulus intensity used to probe corticospinal excitability. While a consistent cTBS-induced MEP depression was observed at higher stimulus intensities, lower intensities were optimal for detecting iTBS-induced MEP potentiation.

To date, few studies have investigated the influence of plasticity-inducing NIBS techniques on corticospinal excitability using test intensities that cover the full range of MEP responses. Muellbacher et al. (2000) recorded I/O curves before and at two time points following 1 Hz rTMS and, although an initial depression of MEP amplitudes was observed for all stimulus intensities, only MEPs evoked using high stimulus intensities (≥140% RMT) were still depressed 30 min following stimulation. Similarly, Gangitano et al. (2002) showed that the depressive effects of 1 Hz rTMS and the facilitatory effects of 20 Hz rTMS were most significant for MEPs evoked using higher stimulus intensities (≥160% RMT for 1 Hz rTMS, and ≥130% RMT for 20 Hz rTMS). The changes in corticospinal excitability evoked by cTBS in the present study were largely consistent with these previous findings: although a significant depression of MEP amplitudes was observed at the 120% RMT stimulus intensity following cTBS, the largest effect occurred at stimulus intensities at or above 150% RMT.

There are at least two possible explanations for this finding. First, the more robust inhibitory effect at higher stimulus intensities might be related to differences in the neuronal pool (and thus the nature of the descending volley) activated by TMS at these intensities. Hamada et al. (2013) showed that inter-individual differences in the

intracortical network of neurons preferentially activated by single-pulse TMS contributed to ~50% of the variability in subjects' responses to both cTBS and iTBS, with those subjects in whom late indirect waves (I-waves) were preferentially recruited displaying a greater plasticity response (characterised by a change in MEP amplitudes in the expected direction) to both protocols compared with those subjects with preferential early I-wave recruitment. TMS applied at low intensities with a posterior–anterior current direction (such as that used here) usually initially evokes a single early I-wave (termed I1); as stimulus intensity increases, late I-waves are recruited in addition to I1 (Di Lazzaro and Rothwell, 2014). Therefore, it is possible that the larger pool of M1 synaptic connections activated at high stimulus intensities, resulting in the recruitment of both early and late I-waves in the majority of subjects, may have increased the likelihood of testing those networks preferentially affected by TBS.

It is worth noting that, in addition to early and late I-waves, an even earlier wave resulting from direct excitation of corticospinal tract axons (termed D-wave) can sometimes be observed when TMS is applied with a posterior–anterior current direction at high stimulus intensities (Di Lazzaro and Rothwell, 2014). While reduced amplitude of the D-wave, but not I-waves, could potentially explain the greater response to cTBS in Experiment 1 when probed using higher intensities, we consider this unlikely as the D-wave has previously been shown to be unaffected by cTBS (Di Lazzaro et al., 2005).

If the greater response to cTBS at higher stimulus intensities was due to the activation of a larger (and more consistent) pool of intracortical neuronal networks, then it might be expected that the response to iTBS would also be optimal at higher stimulus

intensities. This was examined in Experiment 2. However, in contrast to the results of Experiment 1, only MEPs evoked using low stimulus intensities were significantly potentiated following iTBS. Studies which recorded descending volleys from the epidural space in chronic pain patients showed that cTBS reduced the amplitude of I1 (Di Lazzaro et al., 2005), whereas iTBS increased the amplitude of late I-waves (Di Lazzaro et al., 2008). It is possible that iTBS-induced increases in the excitability of late I-wave circuitry resulted in greater late I-wave recruitment at lower stimulus intensities, thereby facilitating MEP amplitudes. While this would be consistent with the findings of Di Lazzaro et al., it might be expected that smaller MEPs generated by more discrete I1 recruitment following cTBS would demonstrate a greater degree of inhibition at lower stimulus intensities. However, this was not the case. Given this, it appears that the relationship between changes in MEP amplitudes induced by TBS protocols and the components of the descending volley responsible for this change are highly complex.

Another possible explanation for the results of the present study is that ceiling and floor effects influenced the intensity range over which optimal responses to cTBS and iTBS were observed. This explanation would suggest that the strongest response to cTBS would be seen at near-maximal MEP amplitudes where facilitation of the response is unlikely, biasing the response towards inhibition. In contrast, the strongest response to iTBS would be seen with small MEP amplitudes where reductions in amplitude are limited, biasing the response towards facilitation. The possible influence of ceiling and floor effects on our results is difficult to exclude based on the current data. Although the lack of difference between TBS protocols at higher stimulus intensities may be indicative of a ceiling effect for the cTBS response (see

Fig. 4), it should be remembered that this comparison was performed on only a small subset of subjects.

An important consideration is that, for both Experiments 1 and 2, not all subjects' baseline MEP amplitudes had reached a plateau at the upper end of the I/O curve. We reasoned that if a ceiling effect was responsible for the different responses observed for cTBS and iTBS when probed using high stimulus intensities, then those subjects whose MEPs had reached an upper plateau (that is, the ceiling of the testable range) at these intensities should show a greater inhibitory response to cTBS in Experiment 1, and a lesser facilitatory response to iTBS in Experiment 2. While this was the case for iTBS, suggesting the lack of iTBS-induced facilitation of MEPs probed using stimulus intensities above 150% RMT may be at least partially explained by a ceiling effect, the MEP depression induced by cTBS was not influenced by whether subjects' MEPs had reached a plateau. Conversely, whereas the inhibitory response to cTBS probed using low stimulus intensities between 100–120% RMT tended to be reduced in those subjects whose baseline MEPs were nearer the floor of the testable range, similar floor effects were not seen for iTBS-induced MEP facilitation at the lower end of the I/O curve. This provides evidence against the notion that ceiling and floor influences are solely responsible for the strong plasticity responses to cTBS and iTBS probed using high and low stimulus intensities, respectively. While we do not exclude the possibility that differences in the makeup of the descending volley evoked at these intensities may have contributed to our findings, it is clear that additional experimentation directly recording descending volleys is required to further examine this issue.

In recent years, there has been a considerable body of research investigating the determinants of NIBS-induced plasticity. A number of factors have been identified that influence the direction and magnitude of induced effects, including the age of study participants, their genetics, and their history of physical activity (Ridding and Ziemann, 2010). Although initial studies suggested TBS protocols were capable of inducing robust LTP and LTD-like plasticity in the human M1 (Huang et al., 2007; Huang et al., 2005), the response to stimulation can vary considerably between individuals (Hamada et al., 2013) and between studies (Di Lazzaro et al., 2011; Gentner et al., 2008; Goldsworthy et al., 2014a, b; Goldsworthy et al., 2012a, b; Hasan et al., 2012; McAllister et al., 2011; Todd et al., 2009; Vallence et al., 2013; Zafar et al., 2008). Most studies use a standard MEP amplitude of ~1 mV to probe the plasticity response to TBS. For both cTBS and iTBS, 72% of subjects responded with changes in MEP amplitudes in the expected direction when probed using SI_{1mV} in the present study, resulting in a significant group response for iTBS, but not cTBS. It should be noted that SI_{1mV} corresponded to a wide range of stimulus intensities, varying between near threshold in some subjects and near maximal in others. Considering this, it is somewhat surprising that for both protocols, the plasticity responses at this intensity were relatively consistent between subjects. Nevertheless, the potential for MEP amplitudes probed at SI_{1mV} to fall on different parts of the I/O curve in different subjects may have contributed to some of the variability in subjects' responses to TBS in previous studies.

The rationale for using SI_{1mV} is that, in most subjects, this provides a baseline measure of excitability that is roughly midway between the smallest and largest response, thus providing an equal opportunity for change in either direction. Corresponding to a stimulus intensity that produces an MEP amplitude that is ~50%

of the largest response, SI_{50} represents a more effective approach for consistently targeting the dynamic linear portion of the stimulus-response curve (Devanne et al., 1997), and should therefore be more sensitive to excitability changes following TBS. However, in the present study, neither cTBS nor iTBS induced a significant group-level response when probed at this intensity, with large variability observed between individual subject response profiles in both experiments. Although, by definition, the SI_{50} point of the I/O curve is consistent, the nature of the descending volley (i.e., D- and I-wave contributions) at this intensity may differ significantly between subjects. This highlights the difficulties with selecting a single appropriate test stimulus intensity. On this basis, we suggest that, where possible, the MEP response to a number of test intensities should be recorded to reliably probe plastic changes in corticospinal excitability.

5. Conclusions

The present study shows that the stimulus intensity used to probe changes in corticospinal excitability following TBS is an important factor determining the magnitude and consistency of the induced plasticity response, with high stimulus intensities more sensitive to the LTD-like after-effects to cTBS and low intensities more sensitive to the LTP-like after-effects of iTBS. These findings have important implications for future studies investigating TBS-induced M1 plasticity.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed

References

Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Curra A, Gilio F, et al. Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res. 1998; 122:79-84.

Burke D, Pierrot-Deseilligny E. Caveats when studying motor cortex excitability and the cortical control of movement using transcranial magnetic stimulation. Clin Neurophysiol. 2010; 121:121-3. 10.1016/j.clinph.2009.10.009

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology. 1997; 48:1398-403.

Cooke SF, Bliss TV. Plasticity in the human central nervous system. Brain. 2006; 129:1659-73. 10.1093/brain/awl082

Devanne H, Lavoie BA, Capaday C. Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res. 1997; 114:329-38.

Di Lazzaro V, Dileone M, Pilato F, Capone F, Musumeci G, Ranieri F, et al. Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. J Neurophysiol. 2011; 105:2150-6. 10.1152/jn.00781.2010

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol. 2008; 586:3871-9. 10.1113/jphysiol.2008.152736

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, et al. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. J Physiol. 2005; 565:945-50. 10.1113/jphysiol.2005.087288

Di Lazzaro V, Rothwell JC. Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. J Physiol. 2014; 592:4115-28. 10.1113/jphysiol.2014.274316

Gangitano M, Valero-Cabre A, Tormos JM, Mottaghy FM, Romero JR, Pascual-Leone A. Modulation of input-output curves by low and high frequency repetitive transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol. 2002; 113:1249-57. 10.1016/S1388-2457(02)00109-8

Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J. Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. Cereb Cortex. 2008; 18:2046-53. 10.1093/cercor/bhm239

Goldsworthy MR, Müller-Dahlhaus F, Ridding MC, Ziemann U. Inter-subject Variability of LTD-like Plasticity in Human Motor Cortex: A Matter of Preceding Motor Activation. Brain Stimul. 2014a. 10.1016/j.brs.2014.08.004

Goldsworthy MR, Müller-Dahlhaus F, Ridding MC, Ziemann U. Resistant Against De-depression: LTD-Like Plasticity in the Human Motor Cortex Induced by Spaced cTBS. Cereb Cortex. 2014b. 10.1093/cercor/bht353

Goldsworthy MR, Pitcher JB, Ridding MC. The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci. 2012a; 35:125-34. 10.1111/j.1460-9568.2011.07924.x

Goldsworthy MR, Pitcher JB, Ridding MC. A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. Clin Neurophysiol. 2012b; 123:2256-63. 10.1016/j.clinph.2012.05.001

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex. 2013; 23:1593-605. 10.1093/cercor/bhs147

Hasan A, Hamada M, Nitsche MA, Ruge D, Galea JM, Wobrock T, et al. Direct-current-dependent shift of theta-burst-induced plasticity in the human motor cortex. Exp Brain Res. 2012; 217:15-23. 10.1007/s00221-011-2968-5

Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol. 2007; 118:1028-32. 10.1016/j.clinph.2007.01.021

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron. 2005; 45:201-6. 10.1016/j.neuron.2004.12.033

Huang YZ, Rothwell JC, Edwards MJ, Chen RS. Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cereb Cortex. 2008; 18:563-70. 10.1093/cercor/bhm087

McAllister SM, Rothwell JC, Ridding MC. Cortical oscillatory activity and the induction of plasticity in the human motor cortex. Eur J Neurosci. 2011; 33:1916-24. 10.1111/j.1460-9568.2011.07673.x

Muellbacher W, Ziemann U, Boroojerdi B, Hallett M. Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. Clin Neurophysiol. 2000; 111:1002-7.

Ridding MC, Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. Electroencephalogr Clin Neurophysiol. 1997; 105:340-4.

Ridding MC, Rothwell JC. Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci. 2007; 8:559-67. 10.1038/nrn2169

Ridding MC, Ziemann U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J Physiol. 2010; 588:2291-304. 10.1113/jphysiol.2010.190314

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009; 120:2008-39. 10.1016/j.clinph.2009.08.016

Teo JT, Swayne OB, Rothwell JC. Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol. 2007; 118:1649-51. 10.1016/j.clinph.2007.04.010

Todd G, Flavel SC, Ridding MC. Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. Exp Brain Res. 2009; 195:307-15. 10.1007/s00221-009-1791-8

Vallence AM, Hammond GR, Reilly KT. Increase in flexor but not extensor corticospinal motor outputs following ischemic nerve block. J Neurophysiol. 2012; 107:3417-27. 10.1152/jn.01118.2010

Vallence AM, Kurylowicz L, Ridding MC. A comparison of neuroplastic responses to non-invasive brain stimulation protocols and motor learning in healthy adults. Neurosci Lett. 2013; 549:151-6. 10.1016/j.neulet.2013.05.064

Vallence AM, Ridding MC. Non-invasive induction of plasticity in the human cortex: Uses and limitations. Cortex. 2014; 58C:261-71. 10.1016/j.cortex.2013.12.006

Zafar N, Paulus W, Sommer M. Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. Clin Neurophysiol. 2008; 119:1393-9. 10.1016/j.clinph.2008.02.006

Figure captions

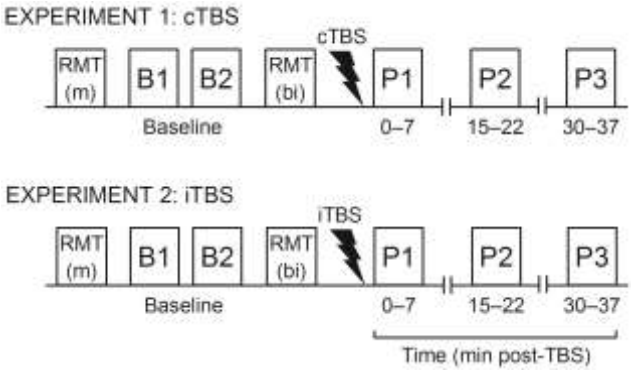

Figure 1. Experimental design. I/O curves were recorded twice at baseline (B1 and B2) and at three time periods post-TBS (P1, P2, and P3) for both experiments. RMT was determined using both monophasic (m) and biphasic (bi) pulse waveforms.

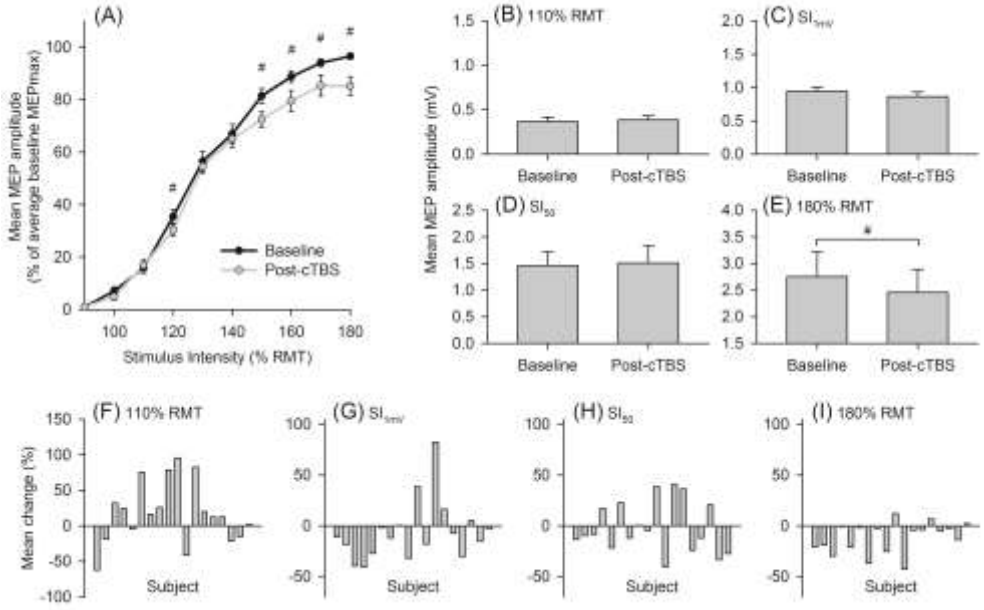

Figure 2. Probing cTBS-induced after-effects on corticospinal excitability using TMS I/O curves (Experiment 1). (A–E) Influence of cTBS on (A) averaged I/O curves (normalised to average baseline MEP_{max}), and on raw MEP amplitudes evoked using

stimulus intensities (B) 110% RMT, (C) SI_{1mV}, (D) SI_{50}, and (E) 180% RMT. # denotes a significant difference between baseline (average of B1 and B2) and post-cTBS (average of P1, P2, and P3) MEP amplitudes ($P < 0.05$). Data represent group means ± SEM. (F–I) Inter-subject response variability to cTBS probed using stimulus intensities (F) 110% RMT, (G) SI_{1mV}, (H) SI_{50}, and (I) 180% RMT. Data represent mean percentage change of post-cTBS MEP amplitudes from baseline, with positive values indicating an increase in MEP amplitudes following cTBS, and negative values indicating a decrease in MEP amplitudes.

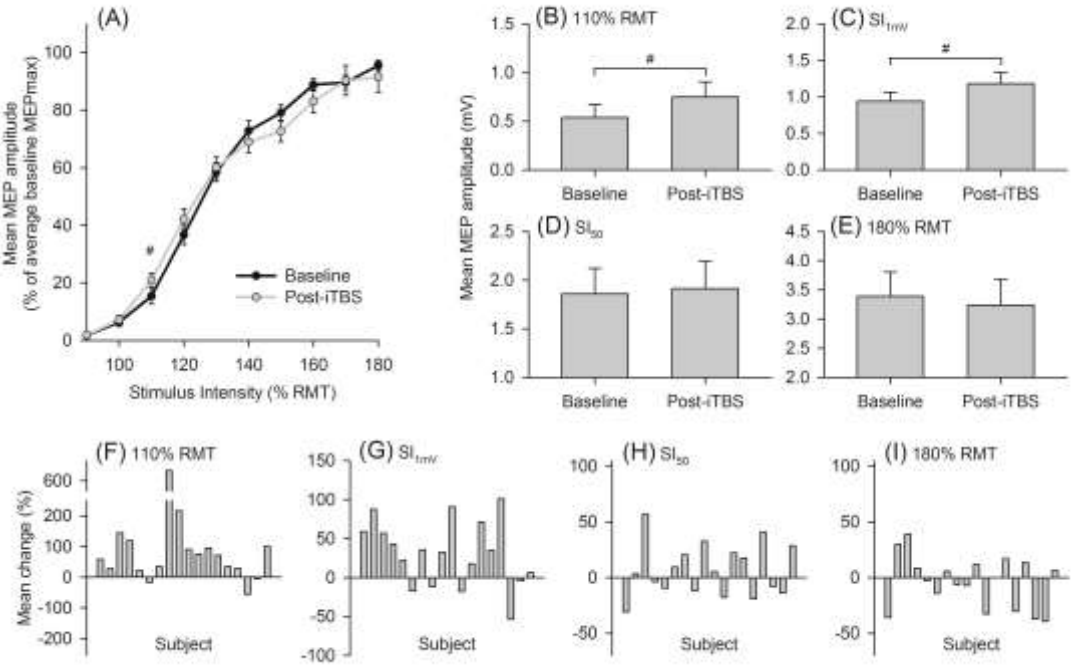

Figure 3. Probing iTBS-induced after-effects on corticospinal excitability using TMS I/O curves (Experiment 2). (A–E) Influence of iTBS on (A) averaged I/O curves (normalised to average baseline MEP_{max}), and on raw MEP amplitudes evoked using stimulus intensities (B) 110% RMT, (C) SI_{1mV}, (D) SI_{50}, and (E) 180% RMT. #

denotes a significant difference between baseline (average of B1 and B2) and post-iTBS (average of P1, P2, and P3) MEP amplitudes ($P < 0.05$). Data represent group means ± SEM. (F–I) Inter-subject response variability to iTBS probed using stimulus intensities (F) 110% RMT, (G) SI_{1mV}, (H) SI_{50}, and (I) 180% RMT. Data represent mean percentage change of post-iTBS MEP amplitudes from baseline, with positive values indicating an increase in MEP amplitudes following iTBS, and negative values indicating a decrease in MEP amplitudes.

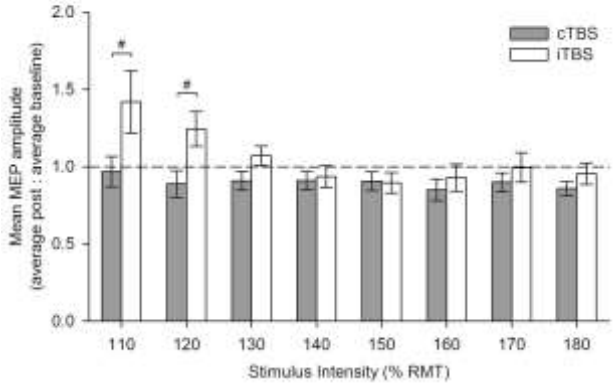

Figure 4. Comparison between the MEP response to cTBS and iTBS at different suprathreshold stimulus intensities. Data are shown as average post-TBS mean MEP amplitudes expressed as a ratio of the average baseline mean MEP amplitude, with values >1.0 indicating an increase in MEP amplitudes following TBS, and values <1.0 indicating a decrease in MEP amplitudes. Data are group means ± SEM from subjects that participated in both experiments ($n = 9$). # denotes a significant difference between TBS protocols ($P < 0.05$).

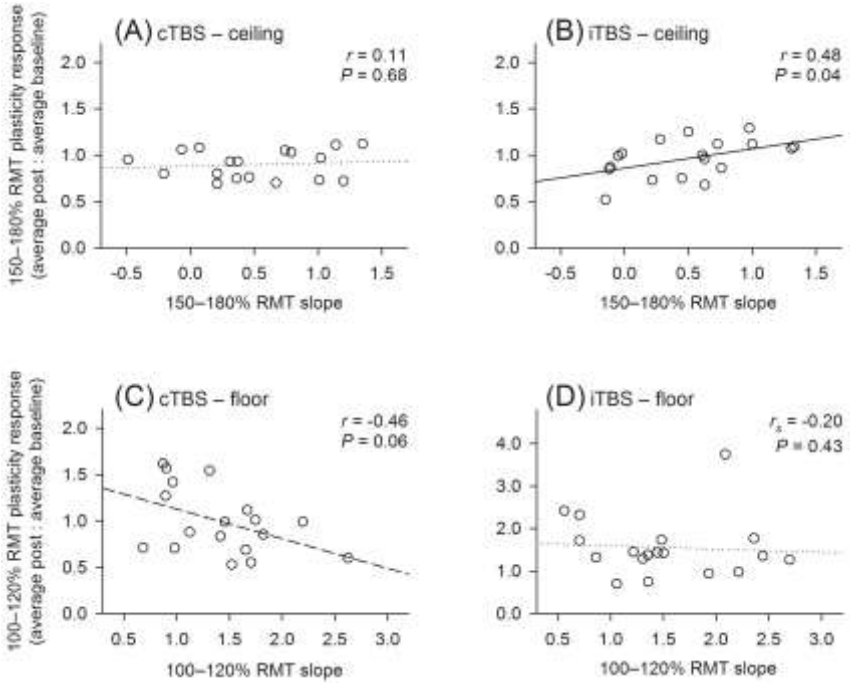

Figure 5. Contribution of ceiling and floor effects to TBS-induced plasticity probed using high (150–180% RMT) and low (100–120% RMT) stimulus intensities. Smaller slope values indicate average baseline MEP amplitudes were nearer either the ceiling (A: cTBS; B: iTBS) or floor (C: cTBS; D: iTBS) of the testable range. Each data point represents results from an individual subject.

Tables

Table 1. Average baseline MEP amplitudes

Stimulus intensity (% RMT)	Mean amplitude (mV) ± SEM	
	Experiment 1 (cTBS)	Experiment 2 (iTBS)
90	0.02 ± 0.00	0.02 ± 0.00
100	0.14 ± 0.03	0.17 ± 0.03
110	0.37 ± 0.05	0.54 ± 0.13
120	0.99 ± 0.20	1.34 ± 0.24
130	1.59 ± 0.29	2.12 ± 0.34
140	1.96 ± 0.38	2.63 ± 0.39
150	2.37 ± 0.42	2.90 ± 0.42
160	2.57 ± 0.45	3.16 ± 0.41
170	2.69 ± 0.46	3.17 ± 0.41
180	2.75 ± 0.46	3.38 ± 0.42