The Efficacy of Non-Invasive Brain Stimulation Protocols for Inducing Neuroplasticity in the Primary Motor Cortex

Kym McKenzie Wansbrough

Murdoch University

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary educational institution.

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Kym McKenzie Wansbrough

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Abstract

Neuroplasticity refers to the brain's ability to change with experience. Continuous theta burst stimulation (cTBS) is a non-invasive brain stimulation technique capable of temporarily inducing neuroplasticity in the primary motor cortex (M1), as indicated by changes in the excitability of the stimulated brain region. However, cTBS-induced neuroplasticity shows large inter-individual variability, which limits its potential in research and clinical settings. The present study investigated whether down-regulating motor cortical inhibition, with cTBS applied using a lower than conventional intensity ($cTBS_{low}$), is capable of making the brain more amenable to the neuroplasticity-inducing effects of cTBS applied using the conventional intensity. Thirty-two, right-handed, healthy adults participated in two experimental sessions: 1) cTBS primed by $cTBS_{low}$; 2) cTBS primed by sham stimulation. Due to unforeseen technical issues, there were two groups: group 1 received $cTBS_{low}$ with conventional bursts; group 2 received $cTBS_{low}$ with reduced pulses per burst. Motor cortical excitability and inhibition were measured from an intrinsic hand muscle at baseline, between the two cTBS applications, and following cTBS. In group 1, $cTBS_{low}$ reduced inhibition in M1, however, there was no systematic change in motor cortical excitability following cTBS primed by $cTBS_{low}$ or primed by sham. This lack of effect may be due to unreliable neuroplasticity induction in M1 following cTBS alone. In group 2, long-lasting and less variable changes in motor cortical excitability were found following an unconventional cTBS pattern. These findings confirm the variability of cTBS-induced neuroplasticity and highlight the importance of developing novel protocols to induce less variable neuroplasticity responses.

The Efficacy of Non-Invasive Brain Stimulation Protocols for Inducing Neuroplasticity in the Primary Motor Cortex

The human adult brain is capable of changes with experience (Buonomano & Merzenich, 1998). This phenomenon is referred to as neuroplasticity. Neuroplastic changes can occur in either the brain structure (structural neuroplasticity) or in the strength of existing networks (functional neuroplasticity; Buonomano & Merzenich, 1998). Functional neuroplasticity is particularly important for learning and memory (Buonomano & Merzenich, 1998). Evidence of functional neuroplasticity was first shown in the hippocampus (Kelso, Ganong, & Brown, 1986), however, more recent evidence suggests that the human adult cortex is capable of functional neuroplastic change (Buonomano & Merzenich, 1998).

Specifically, neuroplasticity has been demonstrated in the region of the brain responsible for the execution of voluntary movement (known as the primary motor cortex; Sanes and Donoghue, 2000). There is evidence that an increase in the excitability of the primary motor cortex (M1) is important for motor learning (Sanes and Donoghue, 2000). Consequently, there is much interest in developing techniques that can modulate the excitability of M1, so as to help people relearn movements after brain injury (e.g. stroke; Cramer et al., 2011). Non-invasive brain stimulation (NIBS) techniques are capable of inducing short-lasting neuroplasticity and are commonly used for both research and clinical purposes (Nitsche, Müller-Dahlhaus, Paulus, & Ziemann, 2012).

Transcranial magnetic stimulation (TMS) is a commonly used type of non-invasive brain stimulation technique (Hallett, 2007). It is safe, painless, and has been used for approximately 30 years (Hallett, 2007). TMS can be used in two ways: 1)

single-pulse TMS can measure excitability of M1 (Hallett, 2007); 2) repetitive TMS (rTMS) can be used to temporarily induce short-lasting neuroplastic changes in M1 (Fitzgerald, Fountain, & Daskalakis, 2006).

Single-pulse Transcranial Magnetic stimulation

In TMS, an electric pulse is sent through to a hand-held coil, placed against the scalp, to induce a magnetic field (Hallett, 2007). The magnetic field passes through the skull, with little attenuation, and induces current flow in the underlying tissue (Hallett, 2007). When single-pulse TMS is applied to an area of M1, at a sufficient intensity, it causes the neuronal elements to produce action potentials. These action potentials go on to produce activity in the targeted muscle, referred to as a motor evoked potential (MEP; Hallett, 2007). The amplitude of the MEP, measured peak-to-peak, quantifies the excitability of the pathway from the point of stimulation to the target muscle (that is, it provides a measure of corticospinal excitability; Hallett, 2007).

Repetitive Transcranial Magnetic Stimulation

The repeated application of TMS pulses is referred to as rTMS (Fitzgerald et al., 2006). rTMS is capable of inducing a change in excitability of the stimulated area which outlasts the period of stimulation (Fitzgerald et al., 2006). The effects of rTMS can be quantified using MEP amplitude, where a change in MEP amplitude post-rTMS indicates neuroplasticity induction (Fitzgerald et al., 2006).

The direction of an rTMS-induced change in MEP amplitude depends on a number of stimulation parameters: these include stimulator intensity and the pattern of the rTMS train (Cardenas-Morales, Nowak, Kammer, Wolf, & Schonfeldt-Lecuona, 2010). The first developed rTMS protocols consisted of stimulation at a constant frequency (these protocols will be referred to as conventional rTMS; Fitzgerald et al.,

2006). In conventional rTMS, there are two patterns of stimulation (see panels A and B of Figure 1 for a comparison of the two patterns; Fitzgerald et al., 2006). A train of low-frequency stimulation (≤ 1Hz) induces MEP amplitude suppression that can last for up to 15 minutes following stimulation cessation (Fitzgerald et al., 2006). The mechanisms underlying this decrease in MEP amplitude have been likened to the weakening of connections in neural networks within the stimulated brain region (Ziemann, 2004). This phenomenon is referred to as long-term depression (LTD; Ziemann, 2004). The other conventional rTMS pattern involves a train of high-frequency stimulation (≥ 5Hz), which can induce an increase in MEP amplitude for up to 30 minutes (Fitzgerald et al., 2006). This increase in MEP amplitude is mediated by effects likened to the strengthening of synaptic connections underlying the stimulated neural pathway (Ziemann, 2004). This phenomenon is referred to as long-term potentiation (LTP; Ziemann, 2004). Despite these rTMS protocols being used widely in research and clinical settings, neuroplastic responses to these rTMS patterns vary highly between individuals (Maeda, Keenan, Tormos, Topka, & Pascual-Leone, 2000). Consequently, the potential of rTMS in both research and therapeutic settings remains limited until protocols that induce reliable, and long-lasting, neuroplastic responses are developed (Maeda et al., 2000). Therefore, there is a need to better understand how these protocols work and to develop them to more effectively induce neuroplastic responses (Maeda et al., 2000).

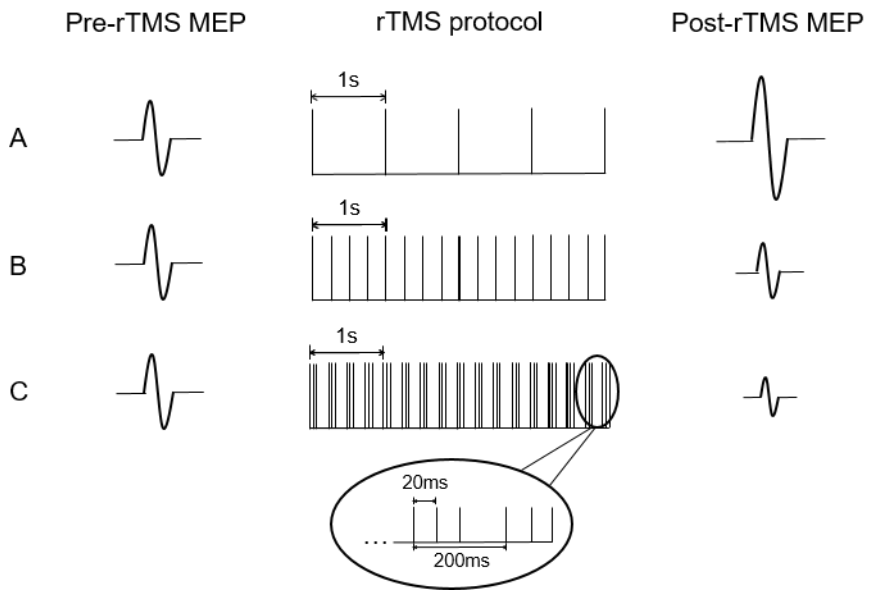

Figure 1. Expected change in motor evoked potentials (MEPs) following different rTMS stimulation patterns. (A) Conventional low-frequency rTMS (1Hz); (B) conventional high-frequency rTMS (5Hz); (C) conventional cTBS.

Continuous Theta Burst Stimulation

A more recent development in rTMS is the patterned rTMS approach (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). The most commonly used patterned rTMS protocol is continuous theta burst stimulation (cTBS; for a review see Chung, Hill, Rogasch, Hoy, & Fitzgerald, 2016). Conventionally, cTBS consists of bursts of three pulses of stimulation at 20ms (50Hz), repeated every 200ms (5Hz), for 40s (600 pulses total; see panel C and insert of Figure 1; Huang et al., 2005). MEP amplitude is reduced following cTBS compared to sham stimulation (where sham stimulation provides auditory stimuli comparable to real cTBS without inducing current flow; Bonato, Miniussi, & Rossini, 2006; Huang et al., 2005). The decrease in MEP amplitude suggests a decrease in corticospinal excitability, which is mediated via LTD-like effects (Cooke & Bliss, 2006; Huang et al., 2007). The mechanism mediating the effect of cTBS is referred to as LTD-like because, while it is very similar to LTD processes, there is insufficient evidence to conclude that this is the case (Cooke & Bliss, 2006). Compared to conventional rTMS, cTBS is the preferred protocol for two

reasons: 1) cTBS induces longer-lasting neuroplastic responses; 2) cTBS uses lower intensity stimulation over a shorter duration (DiLazzaro et al., 2011).

Up until approximately 2011, 18 studies supported the LTD-like effects of cTBS by replicating a significant depression in MEP amplitude post-cTBS (Chung et al., 2016). However, from approximately 2011 onwards, there have been a greater number of reports where cTBS did not induce significant change (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2013; Vernet et al., 2014). For example, when Hamada et al. (2013) investigated the neuroplasticity inducing effects of cTBS in 52 individuals, they found that at the group level there was no significant change in MEP amplitude. Their results revealed large inter-individual variability of responses to cTBS, with 42% of participants showing the expected decrease in MEP amplitude, and 58% showing an unexpected increase in MEP amplitude (Hamada et al., 2013). It is imperative that the inter-individual variability in responses to cTBS be addressed, for, as previously stated, the inability of non-invasive brain stimulation protocols to reliably induce long-lasting neuroplastic responses limits their potential in both research and clinical settings (Maeda et al., 2000).

Previous research has used two approaches in an attempt to reduce cTBS variability: 1) modify cTBS parameters (e.g. Goldsworthy, Pitcher, & Ridding, 2012a); 2) harness factors known to influence neuroplasticity (for a review see Ridding & Ziemann, 2010). These will be discussed in turn. First, variability can be reduced by modifying cTBS parameters. For example, Goldsworthy et al. (2012a) compared the efficacy of two cTBS patterns. The first cTBS pattern was the Huang et al. (2005) paradigm (outlined above), and the second cTBS pattern used stimulation parameters developed by Nyffeler et al. (2006). The Nyffeler et al. (2006) pattern involved bursts of three pulses of stimulation at 33.3ms (30Hz), repeated every 167ms (6Hz), for 33.3s

(see Figure 2 for a comparison of the Huang and Nyffeler cTBS paradigms). Goldsworthy and colleagues' (2012a) results indicated that the Nyffeler et al. (2006) paradigm-induced a more consistent change in MEP amplitude. This finding emphasised the importance of further investigating different patterns to optimise neuroplasticity-induction (Goldsworthy et al., 2012a).

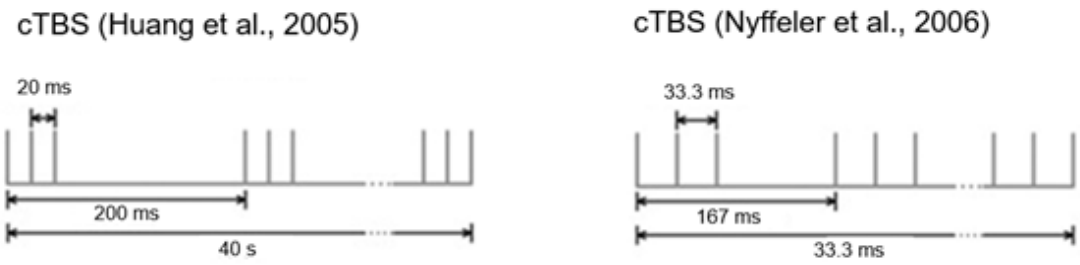

Figure 2. Stimulation patterns of two cTBS protocols. Adapted from "A Comparison of Two Different Continuous Theta Burst Stimulation Paradigms Applied to the Human Primary Motor Cortex," by Goldsworthy et al., 2012a, *Clinical Neurophysiology, 123,* p. 2257.

Second, there are a number of inter-individual factors that influence neuroplasticity induction which can be harnessed to reduce cTBS response variability (see Ridding & Ziemann, 2010). For example, attention and exercise are neuroplasticity-influencing factors that have recently been reviewed (Ridding & Ziemann, 2010). Of identified factors, there is strong evidence to suggest that an easily modifiable factor, inhibition, plays a major role in neuroplasticity induction (Ziemann, Corwell, & Cohen, 1998).

Inhibition and Neuroplasticity Induction

There are inhibitory networks all throughout the brain, including M1 (Kalat, 2013). Inhibition is an active process which, in M1, is capable of suppressing the excitability of cortical motor neurons (Kalat, 2013). A number of animal studies have investigated the role of inhibition in neuroplasticity induction (Hess, Aizenman, & Donoghue, 1996). These studies show that reducing inhibition (pharmacologically)

enhances neuroplasticity in the motor cortex by unmasking pre-existing excitatory processes (Jacobs & Donoghue, 1991). The role of inhibition in neuroplasticity induction has also been examined in the M1 of conscious humans (e.g. Ziemann et al., 1998). In Ziemann and colleagues' (1998) study, rTMS was primed by a protocol that reduces inhibition in M1 (through the temporary removal of sensory input from the hand to the brain). Results showed that rTMS led to a significant increase in MEP amplitude when applied following the priming condition, but not when applied alone (Ziemann et al., 1998). In light of this evidence, it is plausible that reducing inhibition prior to the application of rTMS would reduce response variability.

Paired-pulse TMS can be used to measure the inhibitory processes acting within M1 (i.e. intracortical inhibition; Kujrai et al., 1993). As previously explained, when single-pulse TMS is applied to an area of M1, an MEP is elicited, which provides a measure of corticospinal excitability (Hallett, 2007). In contrast, paired-pulse TMS involves two stimuli: a conditioning stimulus which precedes a test stimulus at an interval of 3ms (Kujrai et al., 1993). While the conditioning stimulus is not of a sufficient intensity to elicit an MEP, it activates intracortical inhibitory circuits (Kujrai et al., 1993). Activation of these circuits, by the conditioning stimulus, results in an inhibitory effect on the MEP elicited by the test stimulus (Kujrai et al., 1993). By comparing the MEP elicited by single-pulse TMS to the MEP elicited by paired-pulse TMS it is possible to obtain a measure of intracortical inhibition, referred to as short-interval intracortical inhibition (SICI; Kujrai et al., 1993).

Recent work has investigated whether a modified cTBS protocol can be used to reduce intracortical inhibition (McAllister, Rothwell, and Ridding, 2009). McAllister et al., (2009) found that cTBS, set at a lower than conventional intensity, down-regulates inhibition in the stimulated region of M1. In light of the research above, this inhibition-

reducing, low intensity, cTBS protocol ($cTBS_{low}$) might make the brain more amenable to plasticity induction. If so, $cTBS_{low}$ may be able to prime cTBS of a conventional intensity to induce a more reliable plastic response. Consequently, priming with $cTBS_{low}$ might reduce the impact of inter-individual variability as a limitation of cTBS.

The priming effect of $cTBS_{low}$, on conventional cTBS, has been investigated by Murakami and colleagues (2012). Their results showed that the change in MEP after cTBS primed by $cTBS_{low}$ was not different from the change in MEP after cTBS alone (Murakami et al., 2012). This finding suggests that priming with $cTBS_{low}$ does not affect cTBS-induced neuroplasticity (Murakami et al., 2012). However, Murakami et al. (2012), investigated the priming effect of $cTBS_{low}$ on the Huang et al. (2005) protocol, which is not the most consistent neuroplasticity-inducing cTBS protocol (Goldsworthy et al., 2012a). As previously mentioned, the change in MEP amplitude following the Nyffeler et al. (2006) cTBS pattern is greater and more consistent compared to the Huang et al. (2005) pattern (Goldsworthy et al., 2012a). Given that slight changes in stimulation, characteristics can influence the neuroplastic response, it is important to investigate the priming effect of $cTBS_{low}$ on the most effective single-train protocol, based on the available literature (Goldsworthy et al., 2012a). Therefore, instead of priming the Huang et al. (2005) cTBS protocol, the present study investigated the priming effects of $cTBS_{low}$ on the Nyffeler et al. (2006) protocol ($cTBS_{Nyffeler}$).

Aim and Hypotheses

The aim of the current study was to determine whether $cTBS_{low}$ could reduce inhibition, and prime M1, so that $cTBS_{Nyffeler}$ could induce a more consistent neuroplastic response compared to $cTBS_{Nyffeler}$ primed by sham stimulation ($cTBS_{sham}$). To investigate this, responses to single- and paired-pulse TMS were recorded from an

intrinsic hand muscle. As mentioned previously, both single- and paired-pulse TMS allows the investigation of changes in corticospinal excitability (indicative of neuroplasticity induction) and SICI (indicative of intracortical inhibition modulation), respectively. Thus MEPs and SICI were recorded at baseline, following the first train of cTBS (i.e. $cTBS_{low}$ or $cTBS_{sham}$), and intermittently over 45 minutes post-$cTBS_{Nyffeler}$. To adequately compare the two proposed protocols, the current study implemented a sham-controlled, within-subjects design. The within-subjects design ensured that inter-variability in responses to the different neuroplasticity-inducing protocols would not confound the results. Additionally, the application of $cTBS_{sham}$ in the control condition, in place of $cTBS_{low}$, reduced the possibility that the effects of $cTBS_{low}$ were due to factors other than the stimulation itself.

The research aim (stated above) was addressed by three key hypotheses. As previously mentioned, McAllister et al. (2009) found that $cTBS_{low}$ down-regulated SICI. In light of this result, it was first hypothesised that SICI would decrease following $cTBS_{low}$, but would not change following $cTBS_{sham}$ (that is, SICI would not significantly change after no stimulation). As per evidence from animal and human studies, a reduction in intracortical inhibition will make the brain more amenable to neuroplasticity induction (Ziemann et al., 1998). Therefore, the second hypothesis was that there would be a greater decrease in MEP amplitude following $cTBS_{Nyffeler}$ primed by $cTBS_{low}$ (i.e. primed $cTBS_{Nyffeler}$), compared to $cTBS_{Nyffeler}$ primed by $cTBS_{sham}$ (i.e. $cTBS_{Nyffeler}$ alone). Furthermore, if a reduction in SICI makes the brain more amenable to neuroplasticity induction (as per the rationale of the second hypothesis), then a greater reduction in SICI would be associated with a greater neuroplastic response to $cTBS_{Nyffeler}$ (i.e. a greater decrease in MEP amplitude). Thus, the third hypothesis was

that there would be a positive linear relationship between a decrease in SICI following $cTBS_{low}$ and a decrease in MEP amplitude following primed $cTBS_{Nyffeler}$.

Method

Participants

Thirty-eight participants completed data collection (23 females; 18-33 years of age; M = 22.79, SD = 3.85). Six participants (4 females; 21-30 years of age; M = 24.83, SD = 3.92) attended a single pilot session, and the remaining 32 participants (19 females; 18-33 years of age; M = 22.41, SD = 3.77) participated in the experimental phase, which comprised of two sessions. The sample size of the experimental phase (n = 32) allowed for the results to be comparable to most previous research regarding the effects of cTBS on M1 in young adults ($N \leq 20$; Chung et al., 2016), and comparable to a number of studies assessing the inter-individual variability of TBS responses ($N \leq 30$; e.g. Hinder et al., 2014; Vallence et al., 2015). Participants were recruited from the student population via an electronic portal as part of their course credit, and from the general public by word-of-mouth. All 38 participants were deemed eligible for TMS following a screening of their relevant medical history, as per the international guidelines for the safe use of TMS (see Appendix A; Rossi, Hallett, Rossini, & Pascual-Leone, 2011). Additionally, recruited participants were restricted to right-handed individuals, as indicated by the Edinburgh Handedness Inventory (see Appendix B; Oldfield, 1971). Handedness was restricted due to evidence demonstrating differences in hemispheric asymmetries in left- and right-handed people (Triggs, Subramanium, Rossi, 1999), and evidence of greater TMS-induced neuroplasticity in the dominant hemisphere (Ridding & Flavel, 2006).

Prior to their involvement, participants read an information letter regarding the methods used in the experiment, were given the opportunity to ask questions, and gave written informed consent (see Appendices C and D). Data was collected from all participants at a psychophysiology laboratory at Murdoch University. This study had ethical approval from the Murdoch University Human Research Ethics Committee (see Appendix E).

Design

The experimental phase was a participant-blinded, within-subjects design. The independent variable was protocol, of which there were two conditions: the protocol applied in the $cTBS_{low}$ condition was $cTBS_{low}$ primed $cTBS_{Nyffeler}$, and the protocol delivered in the $cTBS_{sham}$ condition was $cTBS_{sham}$ primed $cTBS_{Nyffeler}$. Order of protocol was counter-balanced across participants. MEP amplitude and SICI were the two dependent variables.

Materials and Procedures

Recordings. Electromyographic (EMG) signals were recorded from the right first-dorsal interosseous (FDI) using Ag/AgCl cup electrodes placed in a belly-tendon configuration (see Figure 3). The FDI was targeted due to its large representation in M1, which allows corticospinal excitability to be reliably measured (Malcolm et al., 2006; Reilly & Mercier, 2007). Furthermore, the FDI is an intrinsic hand muscle for motor learning and fine motor control, therefore targeting this muscle was functionally valuable (Lang & Schieber, 2003). The raw electrode signal from the FDI was amplified 1000 times (CED 1902 amplifier) and band pass filtered (20-1000Hz). The signal was then digitized at a sampling value of 5kHz (CED Power1401), and EMG data were stored on a computer for offline analysis (Signal version 6.02).

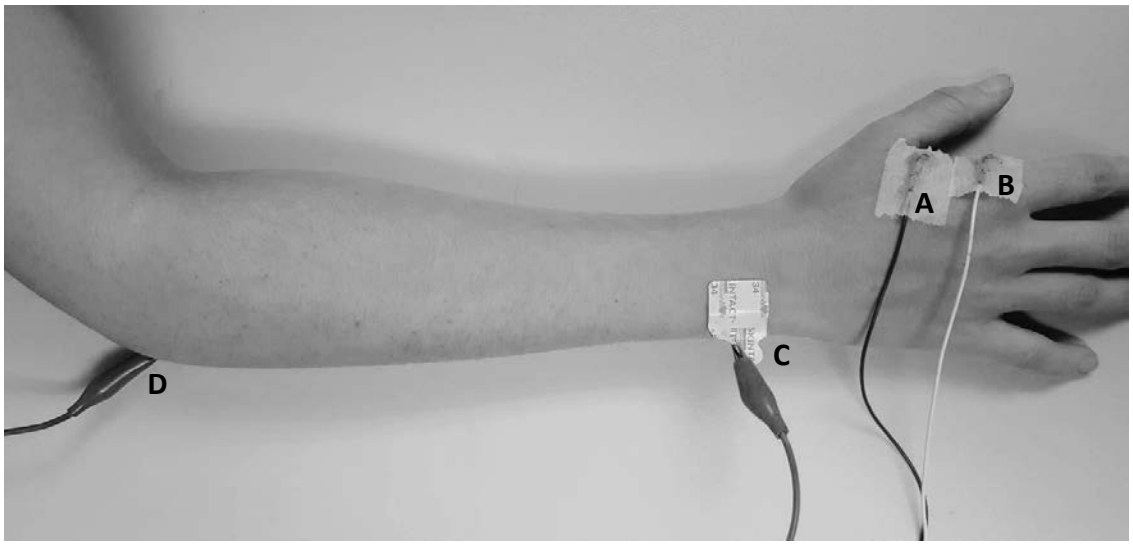

Figure 3. Belly-tendon electrode configuration for FDI. Electrode A recorded muscle activity from the belly of the FDI. Electrode B was placed on a metacarpophalangeal joint to account for measurements of non-muscular activity in electrode A. The recorded EMG activity was the difference between electrodes A and B, which provided muscle activity with a good signal-to-noise ratio. Electrodes C and D were grounding electrodes for electrical activity.

Transcranial magnetic stimulation. Single- and paired-pulse stimulation was delivered with a Magstim BiStim 200^2 (Magstim, Whitland, Dyfed, UK) via a 90mm figure-of-eight coil. Additionally, cTBS was delivered using a Magstim Rapid (Magstim, Whitland, Dyfed, UK) via a 70mm air-cooled figure-of-eight coil. The coil was placed tangentially to the scalp with the handle pointed backward, 45° away from the midline (that is, at the optimal coil orientation for inducing current flow in M1; Janssen, Oostendorp, & Stegeman, 2015).

The optimal site on the scalp for evoking MEPs for contralateral FDI of the right hand was located using suprathreshold single-pulse TMS. The optimal site evokes the largest and most reliable MEPs and is referred to as the hotspot (Saisanen et al., 2008). Once the hotspot was determined, the scalp was marked for all following placements of the coil. The hotspot was determined and marked separately using the Magstim BiStim 200^2 (i.e. BiStim) and Magstim Rapid (i.e. Rapid), due to differences in coil size.

Single-pulse TMS was used to determine three intensities: resting motor threshold (RMT), active motor threshold (AMT), and the stimulator intensity required to evoke MEP amplitudes between approximately 0.5 and 1mV (known as SI_{1mV}). RMT was defined as the minimum stimulator intensity to evoke MEPs of at least 50μV in amplitude, in at least five out of ten consecutive trials, while the right FDI was relaxed (Hamada et al., 2013; Mori et al., 2013). RMT was measured twice; once using the BiStim (i.e. RMT_{BiStim}) and once with the Rapid (i.e. RMT_{Rapid}). Second, AMT was determined. AMT was defined as the minimum stimulator intensity required to evoke MEPs of at least 200μV, in at least five out of ten consecutive trials, whilst the participant maintained a voluntary FDI contraction of 10% of their maximum voluntary contraction (Hamada et al., 2013; Mori et al., 2013). To aid participants in maintaining this contraction, real-time feedback was given via target EMG activity presented on screen. AMT was measured using the Rapid at the end of the second experimental session. Third, SI_{1mV} was obtained by adjusting the stimulator intensity to evoke MEP amplitudes between approximately 0.5 and 1mV (Silbert, Patterson, Pevcic, Windnagel, & Thickbroom, 2013). SI_{1mV} was measured with the BiStim.

Pilot data collection. Previous studies have either set the stimulator intensity for cTBS application relative to RMT or AMT (Chung et al., 2016). However, setting cTBS intensities relative to AMT can be problematic, for sustaining a contraction prior to cTBS can reduce the neuroplastic response (Huang, Rothwell, Edwards, & Chen, 2008). To eliminate the potential effect of contraction on cTBS response in the current study cTBS could not be set relative to AMT. However, previous research using $cTBS_{low}$ set cTBS intensity relative to AMT (McAllister et al., 2009). Therefore, before running the experiment, pilot data was collected so that AMT could be reliably estimated from each individual's RMT.

During pilot data collection, single-pulse TMS was delivered with the Rapid to determine each participant's FDI hotspot, RMT, and AMT (as described above). To generate a formula for estimating AMT, the actual AMT was expressed as a ratio of AMT to RMT for each participant. The group level AMT was 82% of RMT (data presented in Table 1). Therefore, to estimate AMT, the current study used the following formula: estimated AMT = $RMT_{Rapid} \times .82$.

Table 1

Pilot Participants' Resting and Active Motor Thresholds

Participant	RMT (% of MSO)	AMT (% of MSO)	(AMT/RMT) × 100 (%)
1	56	46	82
2	57	45	79
3	57	47	82
4	50	32	64
5	53	48	91
6	65	60	92
		Mean	82

Note. RMT = resting motor threshold. AMT = active motor threshold. % of MSO = percentage of maximum stimulator output.

Experimental protocols.

MEP amplitude and SICI. Single-pulse TMS was applied at SI_{1mV} to measure MEP amplitude. SI_{1mV} was used because 1mV is considered a moderately sized response, which has the capacity to increase or decrease (Silbert et al., 2013). To measure SICI, paired-pulse TMS was applied. The intensity of the first pulse (i.e. the conditioning stimulus) was set at 70% RMT (Vucic, Cheah, Krishnan, Burke, & Kiernan, 2009), the intensity of the second pulse (i.e. the test stimulus) was set at SI_{1mV}

(Silbert et al., 2013), and the inter-stimulus interval was 3ms (McAllister et al., 2009). For a reliable measure of MEP amplitude and SICI, baseline measurements comprised of three blocks of 20 single- and 20 paired-pulse stimuli (Goldsworthy, Hordacre, & Ridding, 2016). Single and paired-pulse trials were psuedorandomised within each block, and the inter-trial interval was five seconds (± 20% to reduce anticipation). In-between each block, the mean and standard deviation of the 20 single-pulse trials was calculated to ensure that there was a consistent and stable measure of corticospinal excitability at baseline.

cTBS$_{low}$. Short bursts of three pulses were delivered at 20ms (50Hz), repeated every 200ms (5Hz), for 40s (600 pulses total; Huang et al., 2005). The intensity of was set to 70% of estimated AMT (calculated for each individual by multiplying RMT_{Rapid} by 0.82; to evaluate how closely this study estimated AMT, each participant's actual AMT was determined at the end of the second session; McAllister et al., 2009).

cTBS$_{Nyffeler}$. Short bursts of three pulses were delivered at 33ms (30Hz), every 167ms (6Hz), for 33.3s (600 pulses total; see panel B of Figure 5; Nyffeler et al., 2006). The intensity of $cTBS_{Nyffeler}$ was set to 80% of RMT (Nyffeler et al., 2006).

cTBS$_{sham}$. In the control session, a sham coil was used to mimic the auditory sensation of $cTBS_{low}$, without inducing current flow in the brain (Bonato et al., 2006).

Experiment procedure. Each participant attended two sessions; an experimental session and a control session. Sessions were separated by at least 2 days to allow for any cTBS-induced changes to washout (Goldsworthy, Pitcher, & Ridding, 2012b; Todd, Flavel, & Ridding, 2009). An rTMS-induced change in MEP is greater in the afternoon, therefore individual participants were tested at same time of day so that individual differences in MEP amplitude between sessions could not be attributed to the time of

testing (Sale, Ridding, & Nordstrom, 2007). See Figure 4 for a schematic of the experimental timeline. RMT_{BiStim}, $SI_{1mV,}$ and RMT_{Rapid} were measured prior to baseline measurements, to tailor the intensity of the conditioning stimulus, test stimulus, and cTBS protocols to each individual, respectively. Both MEP amplitude and SICI were measured at baseline, after the priming paradigm (either $cTBS_{low}$ or $cTBS_{sham}$), and at six time-points following $cTBS_{Nyffeler}$ (0, 5, 10, 20, 30, and 45 min post). The inter-train interval was 10 minutes, in both conditions. There were two reasons for choosing this inter-interval: 1) the greatest reduction in SICI occurs between 5 and 20 minutes post-$cTBS_{low}$ (McAllister et al., 2009); 2) response to cTBS is greater following two is of cTBS spaced at 10 minutes (relative to a single-application of cTBS; Goldsworthy et al., 2012b).

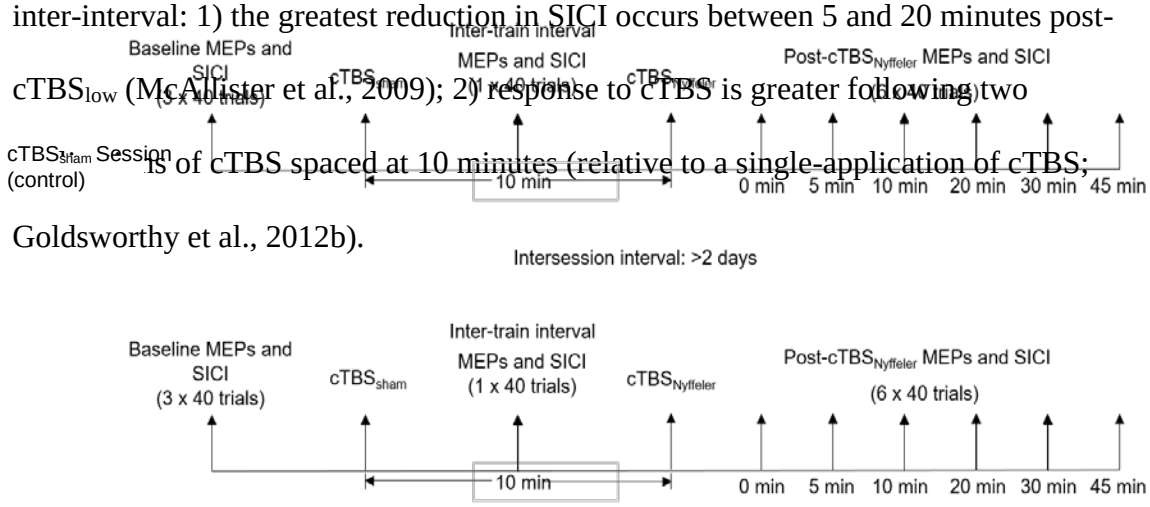

Figure 4. Schematic of the experimental timeline.

Unforeseen Technical Issue Identified During Data Collection

Distinct auditory stimuli are associated with bursts of cTBS pulses. Part-way through data collection (after 43 sessions) a distortion in auditory stimuli was noticed by the experimenter. The session was immediately stopped, and a systematic investigation was conducted to determine which stimulator intensities skipped pulses, to identify

where in a cTBS train pulses were skipped, and to determine whether the issue was persistent.

The output of cTBS pulses was recorded for each cTBS intensity that had been used to stimulate participants. Outputs were recorded for both $cTBS_{low}$ and $cTBS_{Nyffeler}$ stimulator intensities. Each intensity was tested three times (in a randomised order). For each recorded stimulator intensity, all 200 bursts of stimulation were viewed to determine if a pulse had skipped, and if so, at what point in the train this had occurred. This systematic investigation showed that, for the $cTBS_{low/sham}$, and $cTBS_{Nyffeler}$ paradigms, there was a systematic skipping of pulses (see Figure 5 for a schematic of the conventional and unconventional cTBS patterns). For $cTBS_{low}$, the middle pulse was skipped for intensities ≥ 30% of maximum stimulator output (MSO; as shown in panel C of Figure 5). For $cTBS_{Nyffeler}$, the middle pulse was skipped for intensities ≥ 53% of MSO (as shown in panel D of Figure 5). For both $cTBS_{low}$ and $cTBS_{Nyffeler}$, there was one stimulator intensity (52% MSO) for which pulses skipped intermittently. It was for this intensity only that the auditory stimuli of bursts were distorted, and it was during an experimental session of this intensity that the experimenter identified the technical issue. It was determined that this issue was not the result of a human error in configuring the triggers, but the failure of the Rapid in delivering pulses.

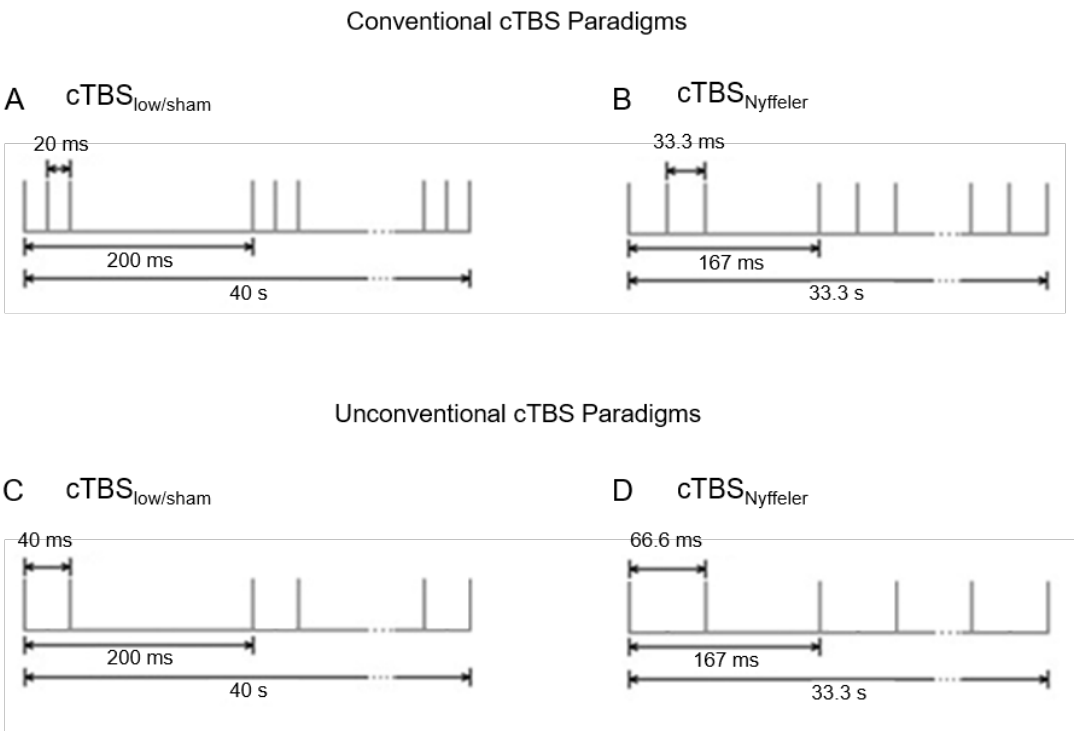

Figure 5. Schematic of the pulse configurations in each of the cTBS paradigms. Panel's A and B illustrate the conventional patterns of stimulation, with a three-pulse burst. Panel's C and D illustrate the unconventional patterns of stimulation, with only two pulses per burst. Adapted from "A Comparison of Two Different Continuous Theta Burst Stimulation Paradigms Applied to the Human Primary Motor Cortex," by Goldsworthy et al., 2012a, *Clinical Neurophysiology, 123,* p. 2257.

For 21 (of 25) participants whose data had been collected up until this point, patterns of either one or both of the cTBS trains were not delivered as planned. Given that pulses were systematically and consistently skipped, individual participants were divided into three groups based on the pattern of stimulation they had received for both paradigms: the planned group, planned Nyffeler group, or unplanned group. Firstly, the planned group (n = 14) received both paradigms at their planned pattern of stimulation (i.e. three pulses per burst). Secondly, the planned Nyffeler group (n = 12) received the $cTBS_{Nyffeler}$ paradigm at its planned pattern but received $cTBS_{low}$ at an unplanned

pattern (i.e. two pulses at 25Hz; 400 pulses total; see Figure 5C). Third, the unplanned group (n = 6) received both paradigms at an unplanned pattern (i.e. two pulses per burst in each paradigm; 400 pulses total per paradigm; see panels C and D of Figure 5). It is important to note the small sample size for the unplanned group (n = 6), relative to the intended sample of N = 30. This small sample does not allow for these data to be interpreted, therefore these results have not been presented in the main text of the current study.

Data Exclusions and Analyses

Participant exclusion during data collection. Forty-nine healthy adults were originally recruited for data collection. Three participants completed the first session but could not attend the second session due to unforeseen circumstances, and eight were excluded from completing the first session. There were two reasons for excluding participants from completing the first session, these will be discussed in turn. First, three participants were excluded from further testing because the stimulator intensity required to evoke MEPs exceeded 75% of BiStim MSO (MSOs greater than 75% can overheat the coil). Second, five participants were excluded because more than 30% of trials had EMG activity of > 0.02mV in the 265ms leading up to the MEP, which would have contaminated the recorded MEPs. If less than 30% of trials were contaminated, participants continued and contaminated trials were excluded from data analysis.

Data screening. Prior to data analysis, baseline MEP amplitudes and SICI ratios were screened for outliers. Outliers were identified as values ±2 SD above the mean. For baseline MEP amplitude, one individual in the planned group was identified as an outlier. This individual was excluded from all MEP analyses. Additionally, two outliers

were identified in baseline SICI ratios: one in the planned group; the other in the planned Nyffeler group. These two participants were excluded from all SICI analyses.

Data analysis. For each trial, MEP amplitude was measured peak-to-peak between 12ms and 42ms post-test stimulus. SICI was quantified by creating a ratio of the average paired-pulse MEP amplitudes in a block to the average single-pulse MEP amplitudes in a block. For all analyses post-cTBS (that is, immediately post-$cTBS_{low}$, immediately post-$cTBS_{sham}$, or post-$cTBS_{Nyffeler}$) raw MEP amplitude and SICI ratios were normalised to baseline. That is, for each post-cTBS block, MEP amplitude and SICI was expressed as a ratio of average baseline (where average baseline was the mean of the three baseline blocks). Due to the different patterns of cTBS the groups received, these data were analysed separately. Given that technical issues during data collection resulted in three separate and likely underpowered groups, statistical analyses in the current study were considered exploratory. Consequently, multiple comparisons were not corrected for, therefore the results should be interpreted with caution as they are at a high risk of type I error. Statistical significance was accepted at a p value of $< .05$. Due to the technical difficulties and likely underpowered samples in the current study, results approaching significance at a p value of $\leq .06$ have also been highlighted.

Across groups. Independent-samples t-tests were performed to test for group differences in baseline neurophysiological characteristics (including age, RMT_{Rapid}, SI_{1mV}, average raw baseline MEP amplitude, and average baseline SICI ratios). Separate t-tests were performed for each characteristic.

Within groups. Within each group, baseline neurophysiological characteristics (as above) were compared between the two conditions with paired-samples t-tests (where the $cTBS_{low}$ condition refers to $cTBS_{low}$ primed $cTBS_{Nyffeler}$, and the $cTBS_{sham}$ condition

refers to $cTBS_{sham}$ primed $cTBS_{Nyffeler}$). To investigate whether MEP or SICI changed from baseline following the first train of cTBS (i.e. post-$cTBS_{low}$ or post-$cTBS_{sham}$), normalised data at the mid-point were compared to baseline (i.e. 1.00) with single-sample t-tests. To determine whether MEP and SICI changed post-$cTBS_{Nyffeler}$ primed by $cTBS_{low}$ or $cTBS_{sham}$, two-way repeated measures (RM) ANOVAs were performed on the normalised MEP amplitude and normalised SICI of the two conditions. Separate analyses were performed for MEP and SICI. The within-subjects factors were PROTOCOL (2 levels: cTBS_low and cTBS_sham) and TIME (6 levels: 0, 5, 10, 20, 30, and 45 min). To determine whether MEP and SICI post-$cTBS_{Nyffeler}$ significantly differed from baseline, single-sample t-tests were conducted on normalised MEPs and normalised SICI at each time-point following $cTBS_{Nyffeler}$, for both conditions.

Associations. To determine whether a change in SICI immediately following $cTBS_{low}$ was associated with the average change in MEP post-$cTBS_{Nyffeler}$ in the planned group, Pearson's r correlations were performed on normalised SICI at the mid-point and the mean change in normalised MEP amplitude post-$cTB_{Nyffeler}$. The average change in MEP amplitude was determined by calculating mean MEP amplitude across all time points following the second train of stimulation. Correlates were performed separately for $cTBS_{low}$ and $cTBS_{sham}$ conditions. To determine whether the change in MEP at the mid-point was associated with a change in MEP post-Nyffeler in the planned Nyffeler group, Pearson's r correlations were also performed on MEP amplitude at the normalised mid-point, and the average change in normalised MEP amplitude post-$cTB_{Nyffeler}$. The average change in MEP amplitude was determined in the same manner as in the planned group (above). Separate correlations for $cTBS_{low}$ and $cTBS_{sham}$ conditions.

AMT. To gauge whether estimating AMT might have influenced responses to $cTBS_{low}$, a paired-samples t-test was used to compare $cTBS_{low}$ stimulator intensities set using estimated AMT and $cTBS_{low}$ stimulator intensities that would have been set with actual AMT.

Results

Assumptions

Across all data analyses, assumptions of normality, normality of difference scores, homogeneity of variance, linearity, and homoscedasticity were tested. Normality and normality of difference scores were assessed with the Shapiro-Wilk statistic and, where relevant, through visual inspection of histograms. Homogeneity of variance was assessed for with Levene's statistic for independent-samples t-tests and F_{max} for two-way RM ANOVAs (Tabachnick & Fidell, 2007). While a number of measures violated a number of these assumptions, most parametric tests are relatively robust to moderate violations (Tabachnick & Fidell, 2007). Therefore, all measures that violated any of the above assumptions were analysed and interpreted with non-corrected parametric tests. However, corrections were made in analyses where the assumption of sphericity was violated. For all two-way repeated measures ANOVAs the assumption of sphericity was assessed with Mauchly's test. Where violated, degrees of freedom were adjusted with the Huynh-Feldt Epsilon (Tabachnick & Fidell, 2007).

Demographic and Baseline Neurophysiological Characteristics

Table 2 outlines the demographic characteristics of the planned and planned Nyffeler groups. An independent-samples t-test revealed that the planned group was significantly younger than the planned Nyffeler group ($t(24) = -3.24$, $p = .003$, $d = .76$).

Table 2

Demographic Characteristics for Participants in the Planned and Planned Nyffeler Groups

Group	N	% Female	Age Range (M, SD)	% tested in afternoon
Planned	14	78.57	18 - 24 (20.07, 1.64)	71.43
Planned Nyffeler	12	41.67	18 - 29 (23.25, 3.22)	58.33

Table 3 summarises the average stimulator intensities used in each group. An independent-samples t-test revealed that the planned group had a significantly lower RMT_{Rapid} than the planned Nyffeler group ($t(50) = -2.46$, $p = .02$, $d = 0.67$). Separate independent-samples t-tests showed that the planned group had a significantly larger mean baseline MEP than the planned Nyffeler group ($t(46) = 2.38$, $p = .02$, $d = .52$) while mean baseline SICI ratios were not significantly different ($t(46) = -1.28$, $p = .21$ $d = 0.13$).

Table 3

Mean (Standard Deviation) Stimulator Intensities (expressed as a percentage of maximum stimulator output) For the Planned and Planned Nyffeler Groups

Group	RMT_{BiStim}	RMT_{Rapid}	SI_{1mV}	Estimated AMT	True AMT
Planned	45.86 (6.04)	53.57 (7.22)	53.61(7.84)	44.43 (5.37)	45.64 (8.04)
Planned Nyffeler	49.54 (4.46)	57.58 (3.66)	60.21 (7.35)	46.50 (3.12)	46.00 (3.67)

Note. RMT_{BiStim} = resting motor threshold obtained with the Magstim BiStim 200^2. RMT_{Rapid} = resting motor threshold obtained with the Magstim Rapid. SI_{1mV} = the stimulus intensity required to produce motor evoked potentials of approximately 0.5-1mV in amplitude. AMT = active motor threshold. Estimated AMT = RMT × 0.82.

Planned Group

Baseline neurophysiological characteristics. Table 4 summarises the baseline neurophysiological characteristics of each condition in the planned group (where the $cTBS_{low}$ condition refers to $cTBS_{low}$ primed $cTBS_{Nyffeler}$, and the $cTBS_{sham}$ condition refers to $cTBS_{sham}$ primed $cTBS_{Nyffeler}$). Paired-samples t-tests were performed on several baseline neurophysiological characteristics to determine whether there was a systematic difference between the $cTBS_{low}$ and $cTBS_{sham}$ conditions. The two conditions did not significantly differ in RMT_{Rapid}, SI_{1mV}, mean baseline MEP amplitude, or mean baseline SICI ratio ($t(12) \leq 2.19$, $p \geq .05$, $d \leq 0.32$).

Table 4

Baseline Neurophysiological Characteristics of the $cTBS_{low}$ and $cTBS_{sham}$ Conditions in the Planned Group

	$cTBS_{low}$		$cTBS_{sham}$	
	M	*SD*	*M*	*SD*
RMT_{Rapid} (% of MSO)	53.64	7.71	53.50	6.98
SI_{1mV} (% of MSO)	54.36	8.40	52.86	7.48
Baseline MEP amplitude (mV)	1.11	0.18	1.06	0.13
Baseline SICI ratio (mV)	0.30	0.28	0.37	0.28

Note. % of MSO = percentage of maximum stimulator output. RMT_{Rapid} = resting motor threshold obtained with the Magstim Rapid. SI_{1mV} = the stimulus intensity required to produce motor evoked potentials of approximately 0.5-1mV in amplitude. MEP = motor evoked potential. SICI = short-interval intracortical inhibition.

Post-$cTBS_{low}$. Figure 6 shows the change in MEP amplitude in the planned group, and panel A shows the time-course of this change. The orange frame in panel A of Figure 6 highlights the change in MEP amplitude immediately following $cTBS_{low}$ and $cTBS_{sham}$. Single-sample t-tests were performed on normalised MEP amplitude at the mid-point (i.e. the point between the first and second train of cTBS) to determine whether MEP was modulated by the first train of cTBS (i.e. $cTBS_{low}$ or $cTBS_{sham}$). Following the first train of cTBS, MEP amplitude did not significantly change from baseline (i.e. 1.00) in either the $cTBS_{low}$ ($t(12) = -1.77$, $p = .10$, $d = 0.48$) or $cTBS_{sham}$ ($t(12) = -1.13$, $p = .28$, $d = 0.32$) conditions. Figure 7 shows the time course of change in SICI. The orange frame in Figure 7 highlights the change in SICI immediately following $cTBS_{low}$ and $cTBS_{sham}$. Single-sample t-tests were performed on normalised SICI at the mid-point to determine whether SICI was modulated by the first train of cTBS. Relative to baseline, SICI was significantly reduced post-$cTBS_{low}$ ($t(12) = 2.61$, $p = .02$, $d = 0.73$), but not post-$cTBS_{sham}$ ($t(12) = 1.63$, $p = .13$, $d = 0.46$).

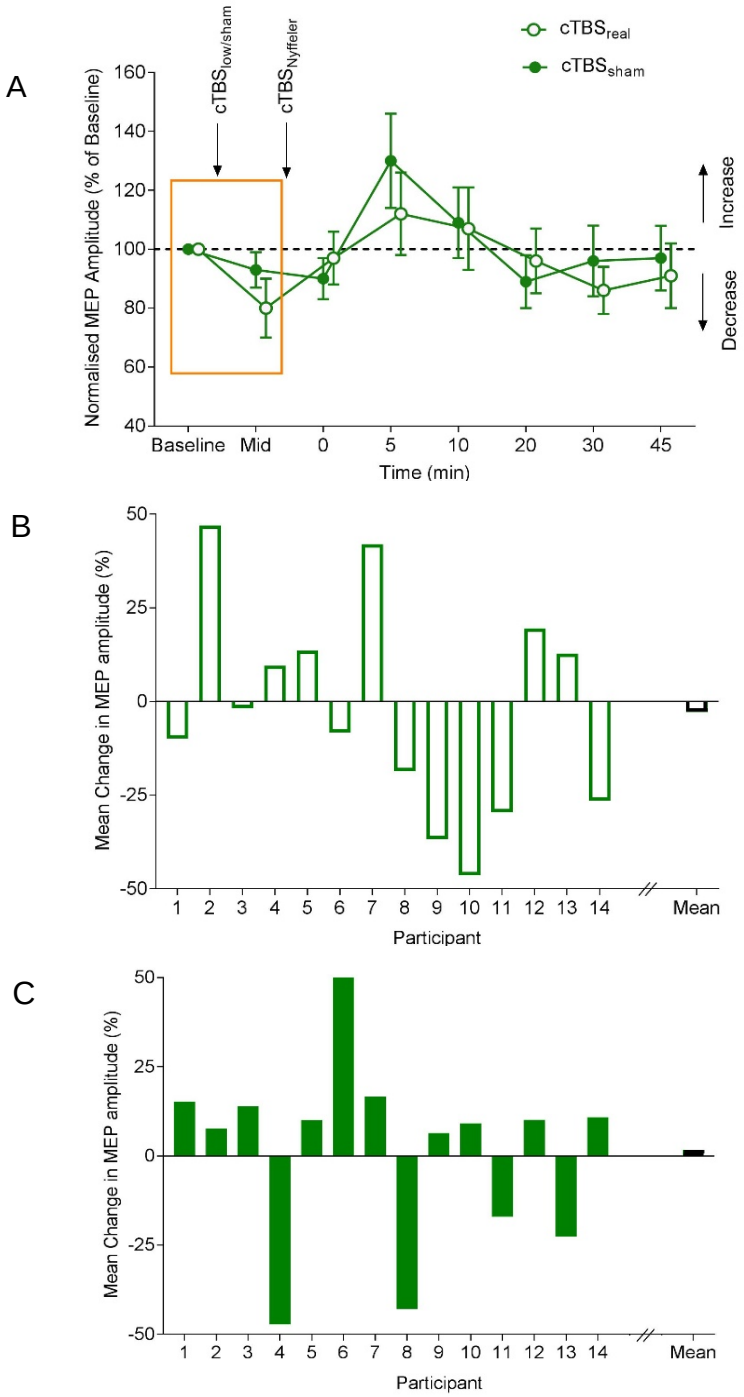

Figure 6. MEP amplitude expressed as a percentage of baseline post-cTBS. (A) The time course of change in both $cTBS_{low}$ (open symbol) and $cTBS_{sham}$ (filled symbol) conditions. Time-points are horizontally offset so that error bars are visible. Error bars represent standard error of the mean. The orange frame highlights the change in MEP following the first train of cTBS. (B and C) Comparison of inter-individual variability in responses, to both $cTBS_{low}$ and $cTBS_{sham}$ protocols, respectively. Response profiles are expressed as the mean percentage change in MEP amplitude (bars below 0 reflect expected decrease in MEP amplitude, bars above 0 reflect increase in MEP amplitude post-$cTBS_{Nyffeler}$). The mean percentage change in MEP amplitude was determined by calculating the average change in MEP amplitude post-$cTBS_{Nyffeler}$ from baseline for each individual.

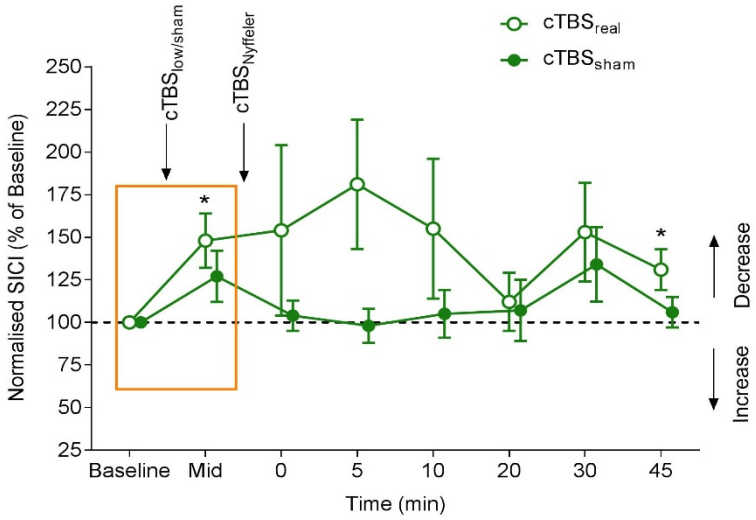

Figure 7. SICI expressed as a percentage of baseline post-cTBS for the two conditions. Depicts the time course of SICI change in both $cTBS_{low}$ (open symbols) and $cTBS_{sham}$ (filled symbols) conditions. The orange frame highlights the change in SICI following the first train of cTBS. Black * = reflects that this time-point in the $cTBS_{low}$ condition is significantly different from baseline ($p < .05$). Time-points are horizontally offset so that error bars are visible. Error bars represent standard error of the mean.

Post-cTBS$_{Nyffeler}$. All data points outside of the orange frame in panel A of Figure 6 show the time-course of change in MEP following $cTBS_{Nyffeler}$ primed by either $cTBS_{low}$ or $cTBS_{sham}$. A two-way RM ANOVA was performed to determine whether the change in MEP amplitude post-$cTBS_{Nyffeler}$ in the $cTBS_{low}$ condition was significantly different than the change in MEP amplitude post$cTBS_{sham}$ $cTBS_{Nyffeler}$ in the $cTBS_{sham}$ condition. There were no significant main effects or interactions ($F \leq 2.64$, $p \geq .08$, $\eta_p^2 \leq 0.18$). Single-sample t-tests were performed on normalised MEP amplitude, at all post-$cTBS_{Nyffeler}$ time-points, to determine whether MEP was modulated post-$cTBS_{Nyffeler}$ in either condition. MEP amplitude post-$cTBS_{Nyffeler}$ did not significantly differ from baseline (i.e. 1.00) at any of the time-points in either the $cTBS_{low}$ ($t(12) \leq 0.50$, $p \geq .13$) or $cTBS_{sham}$ condition ($t(12) \leq 2.01$, $p \geq .07$). Panel B of Figure 6 shows the mean change in MEP amplitude post-$cTBS_{Nyffeler}$ for the $cTBS_{low}$ condition, and panel C of Figure 6 shows the mean change in MEP amplitude post-$cTBS_{Nyffeler}$ for the $cTBS_{sham}$ condition. In the $cTBS_{low}$ condition, 62% of participants responded with

overall MEP depression, while 23% of participants in the $cTBS_{sham}$ condition responded with an overall MEP depression.

All data points outside of the orange frame in Figure 7 show the time-course of change in SICI following $cTBS_{Nyffeler}$ primed by either $cTBS_{low}$ or $cTBS_{sham}$. A two-way RM ANOVA was performed to determine whether the change in SICI post-$cTBS_{Nyffeler}$ in the $cTBS_{low}$ condition was significantly different from the change in SICI in the $cTBS_{sham}$ condition. The main effect of PROTOCOL approached statistical significance (F (1, 12) = 4.93, p = .05, η_p^2 = 0.29). There were no other main effects or interactions approaching or reaching statistical significance ($F \leq 0.84$, $p \geq .55$, $\eta_p^2 \leq 0.07$). Single-sample t-tests were performed on normalised SICI at all post-$cTBS_{Nyffeler}$ time-points to determine whether SICI was modulated post-$cTBS_{Nyffeler,}$ in either condition. There was a significant decrease in SICI, relative to baseline (i.e. 1.00), at 45 minutes post-$cTBS_{Nyffeler}$ in the $cTBS_{low}$ condition (t(12) = 2.37, p =.04, d = 0.67). Additionally, there was a numerical decrease in SICI at 5 and 30 minutes post-$cTBS_{Nyffeler}$, in the $cTBS_{low}$ condition, that approached statistical significance (5 minutes: t(12) = 2.17, p = .05, d = 0.60; 30 minutes: t(12) = 2.08, p = .06, d = 0.57). There was no change in SICI for the remaining time-points (i.e. 0, 10, and 20 minutes) post-$cTBS_{Nyffeler}$ in the $cTBS_{low}$ condition (t(12) ≤ 1.37, $p \geq .20$). Furthermore, single-sample t-tests showed no significant change in SICI post-$cTBS_{Nyffeler}$ in the $cTBS_{sham}$ condition (t(12) ≤ 1.37, $p \geq$ .20).

Associations. Pearson's r correlations were performed to determine whether the change in SICI immediately following the first train of cTBS (i.e. $cTBS_{low}$ or $cTBS_{sham}$) was associated with the average change in MEP post-$cTBS_{Nyffeler}$ in either condition. The average change in MEP amplitude post-cTBS and the change in SICI following the

first train of cTBS were not significantly related in either the $cTBS_{low}$ ($r(12) = .15$, $p = .64$) or $cTBS_{sham}$ ($r(12) = .03$, $p = .93$) conditions.

Planned Nyffeler Group

Baseline neurophysiological characteristics. Table 5 summarises the baseline neurophysiological characteristics of each condition in the planned Nyffeler group. Paired-samples t-tests were performed on several baseline neurophysiological characteristics to determine whether there was a systematic difference between the $cTBS_{low}$ and $cTBS_{sham}$ conditions. The two conditions did not significantly differ in SI_{1mV}, raw mean baseline MEP amplitude, or mean baseline SICI ratio ($t \leq 0.88$, $p \geq .15$, $d \leq 0.46$). RMT was significantly higher in the $cTBS_{sham}$ condition compared to the $cTBS_{low}$ condition ($t(11) = -2.38$, $p = .04$, $d = 0.46$).

Table 5

Baseline Neurophysiological Characteristics of the Real and Sham Conditions in the Planned Nyffeler Group

	$cTBS_{low}$		$cTBS_{sham}$	
	M	*SD*	*M*	*SD*
RMT_{Rapid} (% of MSO)	56.75	3.84	58.42	3.42
SI_{1mV} (% of MSO)	59.75	8.11	60.67	6.83
Baseline MEP amplitude (mV)	1.00	0.21	0.94	0.17
Baseline SICI ratio (mV)	0.42	0.37	0.49	0.43

Note. % of MSO = percentage of maximum stimulator output. RMT_{Rapid} = resting motor threshold obtained with the Magstim Rapid. SI_{1mV} = the stimulus intensity required to produce motor evoked potentials of approximately 0.5-1mV in amplitude. MEP = motor evoked potential. SICI = short-interval intracortical inhibition.

Post-$cTBS_{low}$. Figure 8 shows the change in MEP amplitude in the planned Nyffeler group, and panel A shows the time-course of this change. The orange frame in panel A of Figure 8 highlights the change in MEP amplitude immediately following $cTBS_{low}$ and $cTBS_{sham}$. Single-sample t-tests were performed on normalised MEP amplitude at the mid-point to determine whether MEP was modulated by the first train of cTBS (i.e. $cTBS_{low}$ or $cTBS_{sham}$). There was a significant decrease in MEP amplitude

from baseline (i.e. 1.00), post-$cTBS_{low}$ ($t(11) = -2.66$, $p = .02$, $d = 0.79$), but no change post-$cTBS_{sham}$ ($t(11) = 0.55$, $p = .59$, $d = 0.15$). The orange frame in panel A of Figure 9 highlights the change in SICI immediately following $cTBS_{low}$ and $cTBS_{sham}$. Single-sample t-tests were performed on normalised SICI at the mid-point to determine whether SICI was modulated by the first train of cTBS (i.e. $cTBS_{low}$ or $cTBS_{sham}$). SICI was not significantly different from baseline (i.e. 1.00) post-$cTBS_{low}$ ($t(10) = 0.64$, $p = .54$, $d = 0.20$) or post-$cTBS_{sham}$ ($t(10) = -1.83$, $p = .10$, $d = 0.56$).

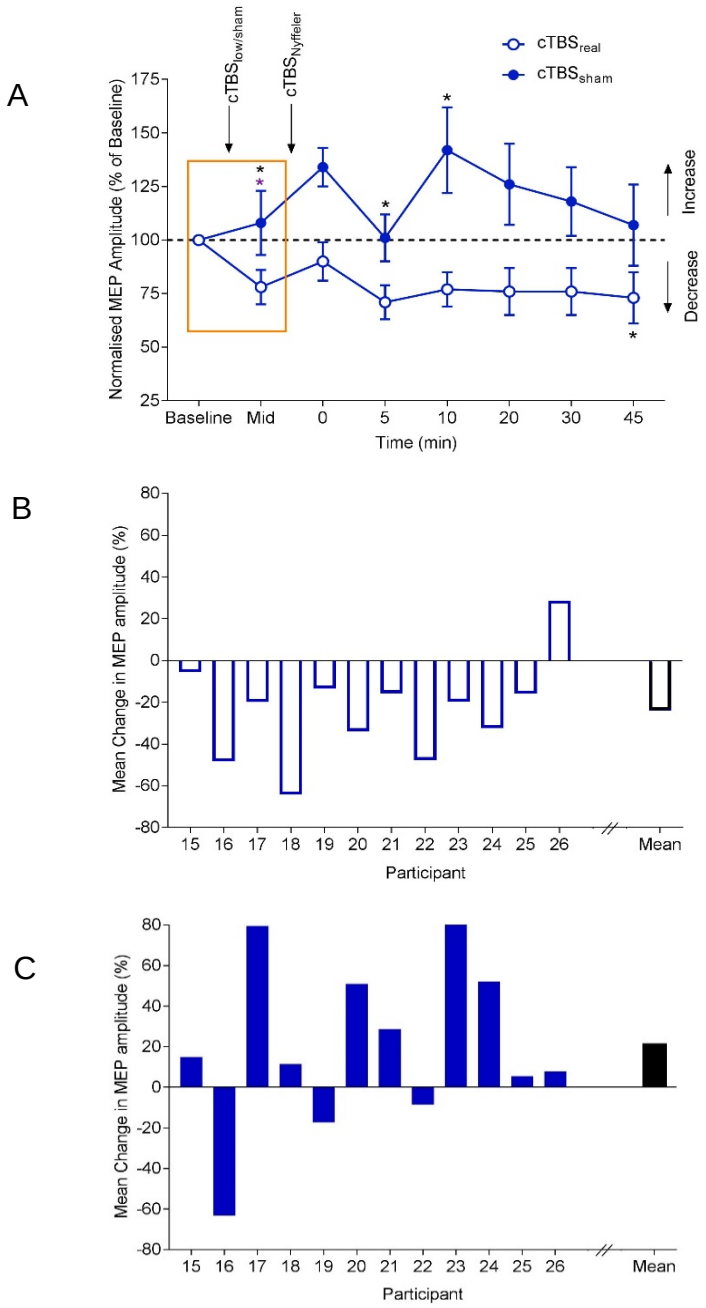

Figure 8. MEP amplitude expressed as a percentage of baseline post-cTBS. (A) The time course of change in both $cTBS_{low}$ (open symbol) and $cTBS_{sham}$ (filled symbol) conditions. Time-points are horizontally offset so that error bars are visible. Error bars represent standard error of the mean. The orange frame highlights the change in MEP following the first train of cTBS. (B and C) Comparison of inter-individual variability in responses, to both $cTBS_{low}$ and $cTBS_{sham}$ protocols, respectively. Response profiles are expressed as the mean percentage change in MEP amplitude (bars below 0 reflect expected decrease in MEP amplitude, bars above 0 reflect increase in MEP amplitude post-$cTBS_{Nyffeler}$). The mean percentage change in MEP amplitude was determined by calculating the average change in MEP amplitude post-$cTBS_{Nyffeler}$ from baseline for each individual. Black * = reflects that this time-point in the $cTBS_{low}$ condition is significantly different from baseline ($p < .05$). Purple * reflects that this time-point in the $cTBS_{sham}$ condition is significantly different from baseline ($p < .05$).

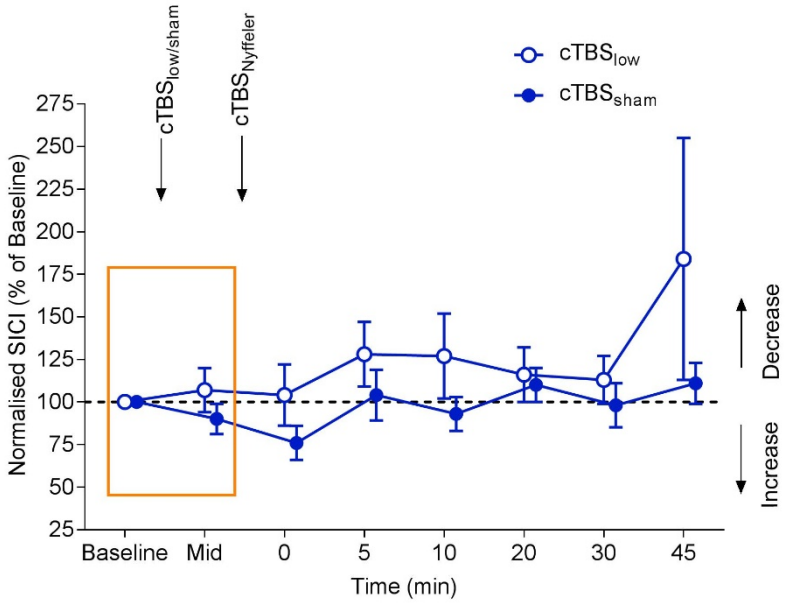

Figure 9. SICI expressed as a percentage of baseline post-cTBS for the two conditions. Depicts the time course of SICI change in both cTBS$_{low}$ (open symbols) and cTBS$_{sham}$ (filled symbols) conditions. The orange frame highlights the change in SICI following the first train of cTBS. Time-points are horizontally offset so that error bars are visible. Error bars represent standard error of the mean.

Post-cTBS$_{Nyffeler}$. All data points outside the orange frame in panel A of Figure 8 show the time-course of change in MEP following cTBS$_{Nyffeler}$ primed by either cTBS$_{low}$ or cTBS$_{sham}$. A two-way RM ANOVA was performed to determine whether the change in MEP amplitude post-cTBS$_{Nyffeler}$ in the cTBS$_{low}$ condition was significantly different than the change in MEP amplitude post-cTBS$_{Nyffeler}$ in the cTBS$_{sham}$ condition. There was a significant main effect of PROTOCOL (F (1, 11) = 11.09, p = .01, η_p^2 = .50), and a main effect of TIME that approached statistical significance (F (5, 55) = 2.24, p = .06, η_p^2 = .17). There was no significant interaction between TIME and PROTOCOL (F (5, 55) = 0.88, p = .50, η_p^2 = 0.07). Single-sample t-tests were performed on normalised MEP amplitude at all post-cTBS$_{Nyffeler}$ time-points to determine whether MEP was modulated post-cTBS$_{Nyffeler}$ in either the cTBS$_{low}$ or cTBS$_{sham}$ condition. In the cTBS$_{low}$ condition, there was a significant decrease in MEP amplitude from baseline (i.e. 1.00) at 5 (t(11) = -3.67, p = .004, d = 1.07), 10 (t(11) = -2.91, p = .01, d = 0.85), and 45 (t(11) = -2.31, p = .04, d = 0.66) minutes post-cTBS$_{Nyffeler}$, and a numerical decrease in MEP that

approached statistical significance at 20 ($t(11) = -2.08$, $p = .06$, $d = .62$) and 30 ($t(11) = -2.13$, $p = .06$, $d = 0.63$) minutes post-$cTBS_{Nyffeler}$. In the $cTBS_{sham}$ condition, single-samples t-tests showed a significant increase in MEP amplitude from baseline immediately post-$cTBS_{Nyffeler}$ ($t(11) = 3.83$, $p = .003$, $d = 1.10$), and a numerical increase in MEP amplitude that approached statistical significance at 10 minutes post-$cTBS_{Nyffeler}$ ($t(11) = 2.10$, $p = .06$, $d = 0.60$). Single-sample t-tests further revealed that MEP amplitude did not significantly differ from baseline at the remaining time-points (i.e. 5, 20, 30 and 45 minutes) post-$cTBS_{Nyffeler}$ in the $cTBS_{sham}$ condition ($t(11) \leq 1.38$, $p \geq .20$). Panel B of Figure 8 shows the mean change in MEP amplitude post-$cTBS_{Nyffeler}$ for the $cTBS_{low}$ condition, and panel C of Figure 8 shows the mean change in MEP amplitude post-$cTBS_{Nyffeler}$ for the $cTBS_{sham}$ condition. Numerically, responses in the $cTBS_{sham}$ condition were more variable than responses in the $cTBS_{low}$ condition. In the $cTBS_{low}$ condition, 91% of participants responded with overall MEP depression, while 27% of participants in the $cTBS_{sham}$ condition responded with an overall MEP depression.

All data points outside of the orange frame in Figure 9 show the time-course of change in SICI following $cTBS_{Nyffeler}$ primed by either $cTBS_{low}$ or $cTBS_{sham}$. A two-way RM ANOVA was performed to determine whether the change in SICI post-$cTBS_{Nyffeler}$ in the $cTBS_{low}$ condition was significantly different from the change in SICI post-$cTBS_{Nyffeler}$ in the $cTBS_{sham}$ condition. There were no significant main effects or interactions ($F \leq 3.38$, $p \geq .10$, $\eta_p^2 \leq 0.25$). Single-sample t-tests were performed on normalised SICI at all post-$cTBS_{Nyffeler}$ time-points to determine whether SICI was modulated post-$cTBS_{Nyffeler}$ in either condition. There was a numerical increase in SICI from baseline (i.e. 1.00) immediately post-$cTBS_{Nyffeler}$ in the $cTBS_{sham}$ condition that approached statistical significance ($t(10) = -2.10$, $p = .06$, $d = 0.64$). SICI did not

significantly differ from baseline at the remaining time-points post-$cTBS_{Nyffeler}$ (i.e. 5, 10, 20, 30, and 45 minutes) in the $cTBS_{sham}$ condition ($t(10) \leq 1.04$, $p \geq .32$), or at any time-point in the $cTBS_{low}$ condition ($t(10) \leq 1.70$, $p \geq .12$).

Associations. Pearson's r correlations were performed to determine whether a change in MEP amplitude immediately following the first train of cTBS was associated with the average change in MEP post-$cTBS_{Nyffeler}$ in either condition. In the $cTBS_{low}$ condition, a change in MEP amplitude post-$cTBS_{low}$ was significantly related to the average change in MEP post-$cTBS_{Nyffeler}$ ($r(11) = .83$, $p = .001$). Furthermore, in the $cTBS_{sham}$ condition, the relationship between MEP amplitude post-$cTBS_{low}$ and the average change in MEP post-$cTBS_{Nyffeler}$ approached statistical significance ($r(11) = .56$, $p = .06$). That is, individuals who showed a large decrease in MEP amplitude post-$cTBS_{low}$ or post-$cTBS_{sham}$ showed a greater depression in MEP post-$cTBS_{Nyffeler}$.

Estimated AMT

As described in Methods, the intensity for $cTBS_{low}$ was set at 70% of the estimated AMT. At the completion of the second testing session, true AMT was obtained for each individual. Figure 10 shows the stimulator intensity of $cTBS_{low}$ set with estimated AMT (left) and the stimulator intensity of $cTBS_{low}$ set with true AMT (right) for each participant (N = 32). Paired-samples t-tests were performed on all 38 participants to determine whether the stimulator intensity of $cTBS_{low}$ set with estimated AMT was significantly different from true AMT. Across all groups (including the unplanned group), estimated AMT was not significantly different from actual AMT ($t(32) = 0.29$, $p = .77$, $d = 0.03$). On average, AMT was 82% of RMT for participants in the experimental phase, which was the same as the ratio obtained from pilot data collection.

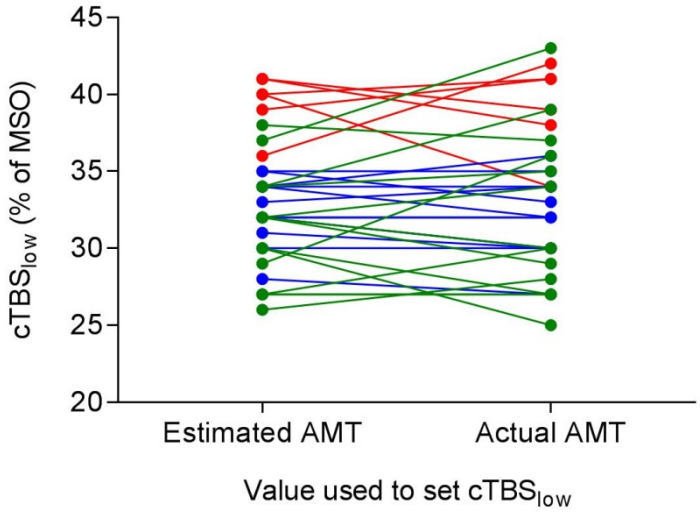

Figure 10. cTBS$_{low}$ stimulator intensities set with estimated AMT (left) and actual AMT (right). % of MSO = percentage of maximum stimulator output. Green = planned group, blue = planned Nyffeler group, red = unplanned group.

Discussion

The aim of the current study was to determine whether cTBS$_{low}$ could reduce inhibition, and lead to more consistent neuroplasticity induction following cTBS$_{Nyffeler}$. The original design of this study incorporated one sample of participants (with a planned N of 30), however, due to unforeseen technical issues, the study comprised of three groups: planned (n = 14), in which both cTBS$_{low}$ and cTBS$_{Nyffeler}$ were applied with the conventional three-pulse bursts; planned Nyffeler (n = 12), in which cTBS$_{low}$ was applied with unconventional two-pulse bursts and cTBS$_{Nyffeler}$ was applied with conventional three-pulse bursts; unplanned (n = 6), in which cTBS$_{low}$ and cTBS$_{Nyffeler}$ were applied with unconventional two-pulse bursts. Given that there was such a small sample size, data analyses and interpretations of the unplanned group were not presented in the main text of the current study. Furthermore, due to the different applications of cTBS and statistically significant differences in baseline neurophysiological characteristics (i.e. RMT and age), no direct comparisons have been made between groups.

The results of the planned group were used to address the original hypotheses. In agreement with the first hypothesis, SICI significantly decreased immediately following $cTBS_{low}$ but not $cTBS_{sham}$. Secondly, it was hypothesised that there would be a greater decrease in MEP amplitude following $cTBS_{Nyffeler}$ primed by $cTBS_{low}$ (i.e. primed $cTBS_{Nyffeler}$), compared to $cTBS_{Nyffeler}$ primed by $cTBS_{sham}$ (i.e. $cTBS_{Nyffeler}$ alone). This hypothesis was not supported. Lastly, in contrast to the third hypothesis, a reduction in SICI following $cTBS_{low}$ was not positively related to a depression in MEP amplitude following $cTBS_{Nyffeler}$. Interestingly, while MEP amplitude did not significantly change in the planned group, the primed $cTBS_{Nyffeler}$ protocol in the planned Nyffeler group induced a long-lasting, and less-variable, MEP depression.

Planned Group

Current results show a decrease in SICI immediately following $cTBS_{low}$, as expected and consistent with McAllister et al. (2009). SICI provides a measure of intracortical inhibition that is mediated by GABAergic inhibition (Illic et al., 2002), the main inhibitory neurotransmitter in the central nervous system (Kalat, 2013). Pharmacological evidence suggests that SICI is mediated by a type of GABA receptor, $GABA_A$ (Di Lazzaro et al., 2006). Therefore, the reduction in SICI post-$cTBS_{low}$ found in the current study likely reflects a decrease in GABAAergic inhibition (McAllister et al., 2009). Furthermore, there was no change in MEP amplitude following $cTBS_{low}$, consistent with McAllister et al. (2009). This suggests that $cTBS_{low}$ selectively modulates the excitability of intracortical inhibitory circuits, mediated by $GABA_A$, but not the excitability of intracortical excitatory circuits.

Current results do not show a significant difference in SICI following $cTBS_{Nyffeler}$ primed by $cTBS_{low}$, or following $cTBS_{Nyffeler}$ alone. However, there was a trend of a

greater decrease in SICI following primed $cTBS_{Nyffeler}$ compared to $cTBS_{Nyffeler}$ alone. Following primed $cTBS_{Nyffeler}$, SICI was variable and lacked a significant change. Surprisingly, at 45 minutes post-primed $cTBS_{Nyffeler}$, variability in the SICI circuits was reduced, and there was a significant reduction in SICI relative to baseline. From these data, it is not possible to determine whether the decrease in SICI at 45 minutes is due to $cTBS_{Nyffeler}$, or due to a lasting decrease in SICI following $cTBS_{low}$. McAllister et al. (2009) showed a significant reduction in SICI between 5 and 20 minutes post-$cTBS_{low}$, and a numerical but not statistically significant decrease in SICI at 20 to 30 minutes post-$cTBS_{low}$. Future research should systematically investigate the time-course of SICI change following $cTBS_{low}$.

Despite the reduction in SICI following $cTBS_{low}$, and contrary to the second hypothesis, primed $cTBS_{Nyffeler}$ had no systematic effect on MEP amplitude. Additionally, $cTBS_{Nyffeler}$ alone did not lead to a significant decrease in MEP amplitude. The absence of a change in MEP amplitude following $cTBS_{Nyffeler}$ alone suggests that this protocol does not reliably induce LTD-like effects in M1. This finding contradicts previous research where $cTBS_{Nyffeler}$ produced a more consistent and long-lasting depression in MEP amplitude compared to the Huang et al. (2005) paradigm (Goldsworthy et al., 2012a). It is worth noting that $cTBS_{Nyffeler}$ has not been used a lot in the literature. Therefore, the data from this study are valuable and suggest that, in contrast to Goldsworthy et al. (2012a), the inter-individual variability in response to $cTBS_{Nyffeler}$ might be comparable to the variability of the Huang et al. (2005) cTBS paradigm (Hamada et al., 2013). Thus, if $cTBS_{Nyffeler}$ alone does not reliably induce LTD-like neuroplasticity, then applying a priming protocol that decreases inhibition (like $cTBS_{low}$) will not be effective at enhancing neuroplasticity induction. That is to say, $cTBS_{Nyffeler}$ will not induce LTD-like effects, irrespective of the plasticity state of

M1. This explanation is consistent with the null finding regarding the third hypothesis, where the response to primed $cTBS_{Nyffeler}$ was not associated with a changed in SICI following $cTBS_{low}$.

An alternative explanation for the absence of change in MEP amplitude post-primed $cTBS_{Nyffeler}$ is that the interval between $cTBS_{low}$ and $cTBS_{Nyffeler}$ was sub-optimal. The rationale for employing a 10-minute interval was two-fold: 1) the greatest reduction in SICI occurs between five and twenty minutes post-$cTBS_{low}$ (McAllister et al., 2009); 2) response to cTBS is greater following two applications of cTBS spaced at 10 minutes (Goldsworthy et al., 2012b). This rationale was limited by the lack of research regarding the time-course of change in SICI post-$cTBS_{low}$. However, the sub-optimal inter-trial interval explanation would not account for both protocols failing to induce a significant depression of MEP amplitude, as $cTBS_{Nyffeler}$ alone was expected to induce LTD-like effects. Therefore, it is more likely that $cTBS_{Nyffeler}$ is not a reliable protocol for inducing LTD-like effects in M1.

It is worth noting that, Murakami et al. (2012) examined the priming effect of $cTBS_{low}$ on cTBS and, although the cTBS paradigm was different (i.e. Huang et al., 2005), there was no change in MEP amplitude (Murakami et al., 2012). When the present study and the Murakami et al. (2012) study are taken together, these results suggests that down-regulating intracortical inhibition with $cTBS_{low}$ might not make M1 more amenable to neuroplasticity induction. However, with highly variable test protocols, and a lack of knowledge regarding the time-course of change in SICI post-$cTBS_{low}$, it is not currently possible to conclude whether the null finding is a true effect.

Planned Nyffeler Group

In the planned Nyffeler group, a significant decrease in MEP amplitude was found following two-burst $cTBS_{low}$, but not two-burst $cTBS_{sham}$. This result was further supported by preliminary data analyses of the unplanned group (which received the same two-burst $cTBS_{low}$ priming stimulation; see Appendix F for preliminary data analyses and interpretations of the unplanned group). This result suggests that two-burst $cTBS_{low}$ suppressed corticospinal excitability. Additionally, SICI did not change significantly from baseline in either the two-burst $cTBS_{low}$ or two-burst $cTBS_{sham}$ conditions. This suggests that the suppression of corticospinal excitability induced by two-burst $cTBS_{low}$ was not driven by a change in $GABA_A$-mediated inhibition. However, the two-burst protocol was unplanned, and with no previous research on two-burst cTBS, it is difficult to interpret these results.

A highly speculative interpretation of these data stems from a recent TMS-EEG study (Premoli et al., 2014). Combined TMS-EEG allows measurement of electrical activity from the scalp, in response to TMS (Premoli et al., 2014). TMS-EEG measures waveforms known as TMS-evoked potentials (TEPs), which have both inhibitory and excitatory components (Premoli et al., 2014). Premoli et al. (2014) revealed that there is an inhibitory component at a 45ms post-TMS. The latency of this process is similar to the timing of two-burst $cTBS_{low}$ intra-burst stimuli (i.e. 40ms). The inhibitory component at 45ms is thought to be mediated by the inhibitory circuits of another type of GABA receptor, $GABA_B$ (Premoli et al., 2014). Therefore, a speculative interpretation of the effect of two-burst $cTBS_{low}$ would be that this protocol might have influenced the excitability of inhibitory processes, that were preferentially active at around 40ms, which led to a net reduction in corticospinal excitability. To better understand the mechanisms underlying this change, further research should investigate

the effect of two-burst $cTBS_{low}$ with combined TMS-EEG. To investigate this, TEPs would be measured before and after the application of two-burst $cTBS_{low}$ and two-burst $cTBS_{sham}$ (at several time points). If two-burst $cTBS_{low}$ does excite this particular inhibitory process, which can be measured by the size of the peak at 45ms, it would be expected that the peak of this component would be larger after two-burst $cTBS_{low}$ compared to two-burst $cTBS_{sham}$ (see Figure 11 for an illustration of the expected change in TEP following two-burst $cTBS_{low}$).

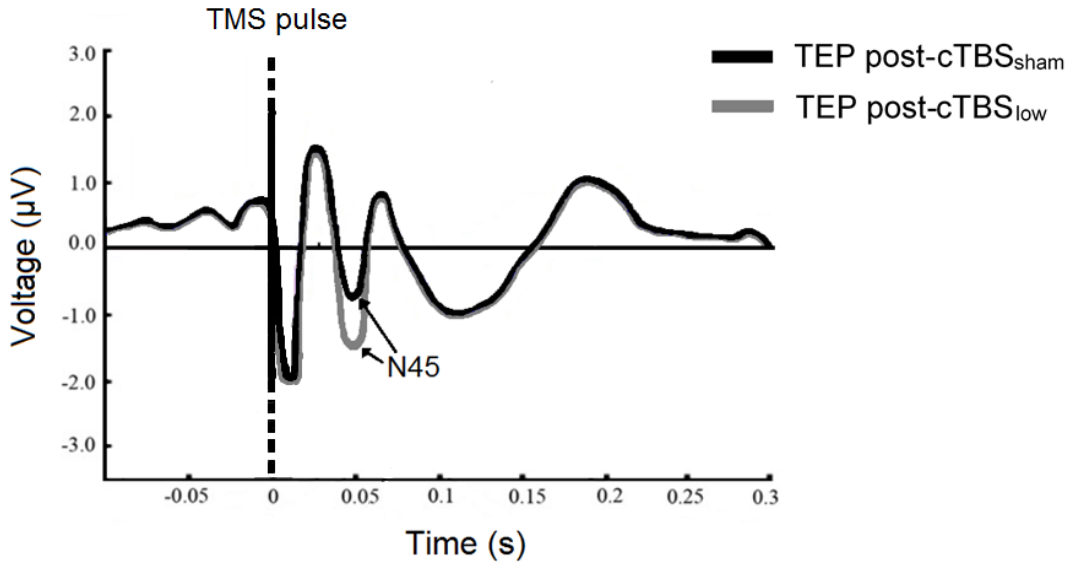

Figure 11. Expected change in TMS-evoked potential following two-burst $cTBS_{low}$. N45 = inhibitory component that occurs 45ms following a suprathreshold TMS pulse. Vertical dashed lines indicated the time of the suprathreshold TMS pulse.

Additionally, in the planned Nyffeler group, $cTBS_{Nyffeler}$ primed by two-pulse $cTBS_{low}$ led to greater, longer-lasting, and less-variable MEP depression than $cTBS_{Nyffeler}$ alone. This result suggests that the two-burst $cTBS_{low}$ primed $cTBS_{Nyffeler}$ protocol suppresses corticospinal excitability. Furthermore, SICI did not significantly change following two-burst $cTBS_{low}$ or two-burst $cTBS_{low}$-primed $cTBS_{Nyffeler}$. This suggests that it is unlikely that depression in corticospinal excitability was driven by a change in GABAAergic inhibition. Furthermore, there was a positive linear relationship

between a change in MEP amplitude following two-burst $cTBS_{low}$ and the average change in MEP amplitude post-primed-$cTBS_{Nyffeler}$. This might suggest that a long-lasting LTD-like effect, driven by two-burst $cBTS_{low}$, might be mediating the decrease in corticospinal excitability following $cTBS_{Nyffeler}$ However, this explanation is speculative and cannot be concluded from these data.

Alternatively, the two-burst $cTBS_{low}$ paradigm might have primed $cTBS_{Nyffeler}$ to induce longer-lasting depression in corticospinal excitability through spaced cTBS-like mechanisms. Similar to the protocol in this study, spaced-cTBS involves the application of two trains of cTBS (following the Huang et al., 2005 paradigm), at an inter-train interval of 10 minutes (Goldsworthy et al., 2012b). Spaced-cTBS has been shown to lead to longer-lasting depression in corticospinal excitability relative to a single train of cTBS (Goldsworthy et al., 2012b). Goldsworthy et al. (2012b) suggested that the increased efficacy of spaced-cTBS was the result of an accumulative effect, whereby applying a greater number of pulses led to longer-lasting depression of corticospinal excitability. While the stimulation parameters of spaced-cTBS used by Goldsworthy et al. (2012b) are slightly different from the parameters of $cTBS_{low}$ primed $cTBS_{Nyffeler}$ used here, it is possible that the depression in corticospinal excitability observed in the current study occurred through similar mechanisms.

While the mechanisms underlying the observed change in corticospinal excitability are unknown, the findings of the current study suggest that the two-burst $cTBS_{low}$ paradigm could potentially contribute to the development of a consistent plasticity-inducing cTBS protocol. It is possible that two-burst $cTBS_{low}$ has a lasting effect on corticospinal excitability, however, this needs to be tested systematically. Therefore, future research, with a more powerful sample, is required to fully characterise the change in MEP amplitude following two-burst $cTBS_{low}$ and determine

whether this paradigm can reliably induce LTD-like plasticity. To investigate this, MEP amplitude would be measured before and after the application of two-burst $cTBS_{low}$ and two-burst $cTBS_{sham}$ (at several time points).

In addition to MEP depression in the $cTBS_{low}$ condition, there were two time-points in the $cTBS_{sham}$ condition where MEP amplitude was significantly facilitated (specifically, at 0 and 10 minutes post-$cTBS_{Nyffeler}$). Given the lack of MEP facilitation post-$cTBS_{Nyffeler}$ alone in the planned group, it is unclear as to whether this is a true facilitation or noise from inter-individual variability. It is worth noting that Hamada et al. (2013) found that 42% of participants showed the expected decrease in MEP amplitude to the cTBS Huang et al. (2005) paradigm, and 58% showed an increase in MEP amplitude. It is speculated that there may be a similar proportion of individuals showing expected and unexpected responses to $cTBS_{Nyffeler}$ alone. Future research with a more powerful sample is required to determine whether $cTBS_{Nyffeler}$ alone induces an increase in MEP.

It is important to note that there was a significant difference in RMT between sessions in this group. Previous research has demonstrated that the RMT of individuals is subject to a small and unsystematic amount of change between sessions (Hermsen et al., 2016). In light of this research, it was unlikely that the small but significant difference in RMT in the current study was systematic. If this was a chance finding, then the 1.67% difference in average RMT_{Rapid} between conditions was unlikely to have affected the results. Nonetheless, the presented results and interpretations should be considered with caution.

Estimated AMT

McAllister et al. (2009) set the stimulator intensity for $cTBS_{low}$ relative to each individual's actual AMT. However, actual AMT requires sustaining a voluntary contraction prior to cTBS, which has been shown to reduce the neuroplastic response of cTBS (Huang et al., 2008). Therefore, to eliminate the potential effect of contraction on the cTBS response in the current study, the intensity of $cTBS_{low}$ was set by estimating AMT from each individual's RMT. When the pilot and experimental data were compared, the ratio of AMT to RMT was exactly the same, which suggests that at the group level this formula was an accurate and reliable method for estimating AMT. Thus, the above results were not likely affected by using a formula to estimating the intensity of $cTBS_{low}$. While the formula was accurate at the group level, AMT would have been under- or over-estimated at the individual level. This might have had an influence on the $cTBS_{low}$ stimulator intensity at the individual level. While the difference between estimated and true AMT could be added as a covariate in analyses, the current study was not sufficiently powered for this analysis. Therefore, this study could not determine the influence of variability in estimated AMT on cTBS-induced neuroplasticity.

Limitations, Clinical Implications, and Future Research

It is important to note that the findings of the current study may have been limited by the influence of intra-individual variability on neuroplasticity-induction. Intra-individual variability refers to factors within the individual that vary on a day-to-day (or more frequent) basis (e.g. stress hormone levels; Sale, Ridding, & Nordstrom, 2008). There is evidence that neuroplasticity-inducing NIBS protocols are influenced, and thus limited by, intra-individual variability (Vallence et al., 2015). This limitation can be

overcome in future research by applying the same protocol to individuals over multiple sessions.

Nonetheless, the current results make a valuable contribution to the understanding of non-invasive brain stimulation-induced (NIBS-induced) neuroplasticity. The results do, however, confirm the current view in the literature that NIBS-induced neuroplasticity is variable. Even so, NIBS is already used widely in clinical settings, therefore it is important to continue better understand it. Specifically, it is clinically important to identify methods for optimising the way neuroplasticity is induced in conscious humans. An approach to optimising neuroplasticity induction is through NIBS priming. Although the efficacy of priming in the current study is unclear, incorporating primers in NIBS protocols is a promising method for reliable M1 neuroplasticity induction (Ridding & Ziemann, 2010). For example, a NIBS technique that delivers low current electrical stimulation (known as tDCS), has been effectively used to prime motor learning (Christova, Rafolt, & Gallasch, 2015). Therefore, with promising effects in inducing functionally relevant neuroplasticity, it is worth continuing to investigate priming as a method of optimising neuroplasticity-induction.

Conclusion

The current study offers three important findings. First, when three-burst $cTBS_{low}$ was applied to M1, intracortical inhibition in the stimulated brain region was down-regulated. This replicated the findings of previous research (McAllister et al., 2009). However, the current results also suggest that this reduction in intracortical inhibition has no effect on neuroplasticity-induction following $cTBS_{Nyffeler}$. Second, unexpected technical issues in data collection led to preliminary evidence for a cTBS-protocol that consistently reduces MEP amplitude (that is, two-burst $cTBS_{low}$). It is recommended

that the effect of two-burst $cTBS_{low}$ continue to be investigated in a larger sample of healthy populations, with more complex methods (e.g. combined TMS-EEG). Third, the unexpected protocol that reduced MEP amplitude (i.e. two-burst $cTBS_{low}$) was also associated with long-lasting and less-variable neuroplasticity-induction following a test-cTBS protocol (i.e. three-burst $cTBS_{Nyffeler}$). Taken together, the current findings offer evidence that non-invasive brain stimulation techniques can induce neuroplastic responses, and therefore offer promise for the therapeutic application of these techniques.

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