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The effects of D-Cycloserine on corticospinal excitability after repeated spaced intermittent theta-burst transcranial magnetic stimulation: A randomized controlled trial in healthy individuals

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Repeated spaced TMS protocols, also termed accelerated TMS protocols, are of increasing therapeutic interest. The long-term potentiation (LTP)-like effects of repeated spaced intermittent theta-burst transcranial magnetic stimulation (iTBS) are presumed to be N-Methyl-D-Aspartate receptor (NMDA-R) dependent; however, this has not been tested. We tested whether the LTP-like effects of repeated spaced iTBS are influenced by low-dose D-Cycloserine (100 mg), an NMDA-R partial-agonist. We conducted a randomized, double-blind, placebo-controlled crossover trial in 20 healthy adults from August 2021-Feb 2022. Participants received repeated spaced iTBS, consisting of two iTBS sessions 60 minutes apart, to the primary motor cortex. The peak-to-peak amplitude of the motor evoked potentials (MEP) at 120% resting motor threshold (RMT) was measured after each iTBS. The TMS stimulus-response (TMS-SR; 100–150% RMT) was measured at baseline, +30 min, and +60 min after each iTBS. We found evidence for a significant Drug*iTBS effect in MEP amplitude, revealing that D-Cycloserine enhanced MEP amplitudes relative to the placebo. When examining TMS-SR, pairing iTBS with D-Cycloserine increased the TMS-SR slope relative to placebo after both iTBS tetani, and this was due to an increase in the upper bound of the TMS-SR. This indicates that LTP-like and metaplastic effects of repeated-spaced iTBS involve NMDA-R, as revealed by two measures of corticospinal excitability, and that low-dose D-Cycloserine facilitates the physiological effects of repeated spaced iTBS. However, extension of these findings to clinical populations and therapeutic protocols targeting non-motor regions of cortex requires empirical validation.

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INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive technique that can be used to study and manipulate brain plasticity. The TMS parameter space has been explored to determine the optimal protocols for enhancing plasticity, and this has revealed non-linear effects. For example, changing the frequency, the number of pulses in a session, or the stimulus intensity results in qualitatively different neural adaptations in the brain [1].

TMS is also a therapeutic modality, and in clinical applications, an element of the parameter space under investigation is 'accelerating' well-established once-daily repetitive TMS protocols by delivering repeated spaced interventions within each day of treatment [2]. This is motivated by the practical limitations of daily treatment in clinical populations, and informed by spaced learning theory [3], ex vivo brain slice experiments [4–7], and human TMS experiments in motor cortex suggesting that spaced tetani can lead to compounding LTP through synaptic priming after an initial tetanus or other metaplastic processes [8, 9]. However, it has become increasingly clear that timing is critical to these processes, and that short-interval 'acceleration' does not result in greater synaptic adaptation [10]. Instead, intervals of 40-60 minutes between tetani are required to produce additional increases in potentiation with a second tetanus, consistent with the time required for kinase signaling cascades, transcription factor activation, and replacement of vesicular transporters [3]. Initial observations in the clinical space leveraging these insights indicated that 'accelerated' TMS protocols could achieve clinically significant improvements, leading to extensive investigation of this novel element of the TMS parameter space, ranging from 2-10 daily treatments [11–13]. The mechanistic basis and physiological effects of manipulations within this parameter space, however, are not well characterized.

Intermittent theta-burst stimulation (iTBS) is the dominant protocol within 'accelerated' TMS investigations. iTBS delivers patterned trains of pulses based on endogenous neuronal firing and has been shown to increase cortical excitability following stimulation [14]. However, there is considerable inter-individual variability in the responses to iTBS, with some subjects displaying reduced corticospinal excitability following iTBS [15, 16]. This highlights an important difference between ex vivo hippocampal slice physiology and cortical TMS, where the physiological effects are more variable [17]. Nevertheless, cumulative increases in LTP-like synaptic plasticity can be obtained via the delivery of an increased number of iTBS pulses with spaced delivery of stimulation blocks [18]. Compared to single session iTBS, repeated spaced iTBS increases the magnitude of the excitatory

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effects of iTBS and increases the number of participants that exhibit corticospinal facilitation to iTBS [19, 20].

The effects of single iTBS interventions on corticospinal excitability are dependent on the ionotropic glutamate receptor N-Methyl-D-Aspartate (NMDA-R) [21, 22], and pairing iTBS with a low-dose of the partial NMDA-R agonist D-Cycloserine has been shown to enhance therapeutic outcomes in major depressive disorder [23]. It is assumed that the effects of repeated spaced iTBS on corticospinal excitability also involve NMDA-R-dependent processes [19, 20]. However, there has been no direct test of the mechanisms of these effects and demonstration of NMDA-R involvement may have important treatment implications.

The aim of the present study was to characterize repeated spaced iTBS and its effects on corticospinal excitability and to examine the involvement of NMDA-R using a low dose of the NMDA-R partial agonist D-Cycloserine. Our hypotheses were twofold: (1) metaplastic effects would not be occluded by pairing repeated spaced iTBS with the partial NMDA receptor agonist, D-Cycloserine, and (2) we hypothesized that repeated spaced iTBS paired with D-Cycloserine would result in increased LTP-like plastic effects, evidenced by larger increases in motor evoked potential (MEP) amplitude and slope of the TMS stimulus-response curve (TMS-SR). Accordingly, we present data normalized to immediately prior to each iTBS as well as the baseline normalized time course for both the MEP amplitude and the TMS-SR.

METHOD

Participants

The results of Tse et al. [19] show a standardized effect size (within-participant Cohen's *d*) of 0.8 [24] for the effect of repeated spaced iTBS on MEP amplitude. Power analysis (alpha = 0.05, beta = 0.9, $d = 0.8$) revealed that 19 participants are required to detect these effects. We recruited 20 healthy adults (mean age = 33.7 years, 12 males, 17 right-handed) via community and online advertisement. The CONSORT flow diagram is illustrated in Supplementary Figure 1. Inclusion criteria were individuals of any sex or gender aged 18–65 who did not have chronic medical conditions. Exclusion criteria included a) allergy to D-Cycloserine, (b) currently pregnant, lactating, or intending to become pregnant, (c) a substance use disorder within the last 3 months, (d) current psychiatric conditions, (e) intracranial implant or metallic object, (f) benzodiazepine use, and (g) any chronic medical condition. All participants gave written informed consent, and the protocol was approved by the University of Calgary Conjoint Health Research Ethics Board. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines.

Experimental procedures

This experiment was a pre-registered, randomized, double-blind, placebo-controlled crossover trial (NCT05081986). Data was collected between August 18th, 2021, and February 23rd, 2022 at the University of Calgary. A randomization key was generated using atmospheric noise by an independent statistician, allocation was concealed with sequentially numbered blister packs, and both experimenters and participants were blind to the sequence of interventions. Individuals were randomly assigned to one of two experimental arms of the crossover study: (a) Placebo followed by D-Cycloserine and (b) D-Cycloserine followed by placebo. In the D-Cycloserine arm, participants received one oral dose of 100 mg D-Cycloserine one hour before the first iTBS. This dose of D-Cycloserine (100 mg) has consistently been associated with enhancement of synaptic plasticity [25] and has been used to successfully modulate the effects of non-invasive brain stimulation for up to 60 minutes [26–28]. In the counterbalanced placebo arm, they received an identical microcrystalline cellulose capsule (100 mg). Participants attended the laboratory on two occasions to complete both arms of the crossover design. Time of arrival was standardized across the crossover arms, and these were separated by at least seven days.

TMS procedure

All TMS pulses were delivered using a neuro-navigated MagPro stimulator (X100 system, MagVenture, Denmark) with a biphasic Cool-B70 figure-eight coil. The participant's head and the TMS coil were registered in three-dimensional space using a model MNI brain in neuronavigation software (Visor 2, ANT Neuro, Germany). The coil was positioned so that the initial phase of the biphasic pulse delivered an electric field with an orientation posterior to anterior in the brain, relative to the central sulcus. The peak-to-peak amplitude of MEPs were recorded from two surface electromyographic (EMG) electrodes placed on the right first dorsal interosseous muscle (FDI), with the ground electrode placed on the right styloid process. EMG signals were amplified (×1,000) and filtered (20–2000 Hz) using a CED 1401 signal analog/digital converter (Cambridge Electronic Design, UK) and digitized at 5000 Hz using Signal 6.0 software (Cambridge Electronic Design).

TMS was delivered to the left primary motor cortex to identify the area that, when stimulated, elicited the largest peak-to-peak FDI MEP amplitude. This area was marked on the neuronavigation software, and all subsequent stimulations were performed at this site. The resting motor threshold (RMT) was determined as the lowest stimulator output, which elicited peak-to-peak MEP amplitudes ≥0.05 mV in 5/10 trials using an established algorithm [29].

iTBS. iTBS was delivered twice, separated by 60 minutes in each arm of the crossover design. iTBS was delivered to the FDI hotspot at 80% RMT. Six-hundred pulses were delivered in each stimulation, which consisted of 20 trains of bursts, each composed of 3-pulses at 50 Hz, repeated at 5 Hz with 8-second inter-train intervals.

Measures

Two measures of corticospinal excitability were recorded: the average peak-to-peak amplitude during stimulation at an intensity equal to 120% of the RMT and the TMS-SR curve at six different stimulus intensities (100–150% RMT).

Bins of 20 MEPs were collected (interstimulus interval 4 s, 0.25 Hz) 15 minutes prior to the first iTBS, and then immediately, 5, 10, and 15 minutes after. MEPs were then sampled immediately prior to the second iTBS (60 minutes after the first iTBS) and then again immediately, 5, 10, and 15 minutes after. The mean MEP amplitude was recorded for each bin. Frequency tables were used to confirm there was no systematic alteration of MEP amplitude across time in each bin. MEPs following the first iTBS were normalized to the mean of the MEPs recorded 15 minutes prior to the first iTBS. MEPs following the second iTBS were normalized to the mean of the MEPS recorded immediately before the second iTBS (60 minutes post the first iTBS). To control for the possible confounding effects of an increase in the MEP amplitude at 60 minutes post the first iTBS, a second model was run where the MEPs following the second iTBS were normalized to the mean of the MEPs recorded 15 minutes prior to the first iTBS.

The TMS-SR was measured by delivering bins of 10 single-pulse TMS (interstimulus interval 4 s, 0.25 Hz) at six different stimulus intensities presented in a pseudo-randomized order. TMS-SR measurement was performed five times: at baseline and then 30 and 60 minutes after each iTBS, and measurements after the first and second iTBS were normalized to the TMS-SR measurement performed at baseline.

Data and statistical analysis

EMG data were exported from Signal and analyzed offline using a custom R programming language script. Individual MEPs were isolated for data cleaning. A total of 1188 MEPs were removed. Pre-stimulus muscle activation, identified via blinded visual inspection of pre-stimulation EMG, was the removal reason for 243 MEPs (1.24%). Outlier (>+/− 2 SD) identification analysis resulted in the removal of 888 (4.5%) MEPs. Experimental issues resulted in the removal of 57 (0.29%) MEPs. In no individual bin of MEPs were more than 20% of trials removed.

We intended to quantify the TMS-SR by fitting a sigmoidal curve to the collated bins of MEPs (10 at each % of RMT). However, in many instances, models failed to converge, or the model fit (estimated using the R^2 values) was poor (<0.7). We believe this was a consequence of using a biphasic coil to measure the TMS-SR, as it has been reported that SR curves are much steeper using this coil, primarily driven by response to high stimulator outputs [30]. We, therefore, used two alternative measures of the TMS-SR. We used a method recommended in the IFCN guidelines for TMS use [31, 32], where the MEP amplitude at 140% of RMT is expressed relative to 120% RMT. For this analysis, we used the MEP values recorded during the SR measurement. We also calculated the effect of DRUG (D-Cycloserine, Placebo) on the normalized (relative to

Table 1. Participant Demographics.

ID	Age	Sex at birth	Gender Identity	Ethnicity	Handedness
001	38	M	M	European Origins	Right
002	26	F	F	European Origins	Right
003	34	M	M	Asian Origins	Right
004	34	M	M	Asian Origins	Left
005	32	F	F	European Origins	Right
006	28	F	F	European Origins	Right
007	43	M	M	European Origins	Right
008	44	M	M	European Origins	Right
009	46	F	F	Latin, Central and South American Origins	Right
010	25	M	M	European Origins	Right
011	24	M	M	European Origins	Right
012	38	M	M	European Origins	Right
013	44	M	M	African Origins	Right
014	23	F	F	European Origins	Left
015	20	M	M	European Origins	Right
016	57	M	M	European Origins	Right
017	27	F	F	European Origins	Right
018	29	F	F	European Origins	Right
019	28	M	M	European Origins	Left
020	27	M	M	European Origins	Right

baseline) MEP amplitude at each STIMULATION (i.e., 100%, 110%, 120%, 130%, 140%, and 150% RMT).

Statistical analysis was performed using the R programming language. Details of the R packages used can be found in the supplementary materials. Baseline data for MEP amplitude and TMS-SR were compared between DRUG using paired students' *T*-tests. The difference in MEP amplitude prior to the first iTBS (baseline) compared to immediately prior to the second iTBS (60 minutes) was compared using two-tailed paired students' *T*-tests. The effect of iTBS was examined in normalized data using linear mixed models with maximum likelihood estimation, where DRUG (Placebo, D-Cycloserine), STIMULATION (first iTBS, second iTBS), and TIME (MEP acquisitions at 5, 10, 15-minute time points following each iTBS) were used as fixed predictors. Random intercepts were included across participants. Because three participants were identified as left-handed, models for the main dependent variables (MEP amplitude and the 140%/120% RMT stimulus-response relationship) were rerun with these participants removed. All statistically significant predictors remained significant with these participants removed, and thus results from the model with the full sample are reported. The distribution of the residuals for each model was assessed using a visual inspection of a Q-Q plot. If residuals violated the assumption of normality, two checks for robustness were performed. The first was to identify if any participants' data was >5 SD from the mean of the sample, and these participants were removed and the analysis rerun. If the residuals were still not normally distributed, a robust linear mixed model [33] was used to check the model. Where main effects were identified as being significant predictors (p value <0.05), a-priori pairwise comparisons using the Kenward-Roger method to adjust degrees of freedom were performed to identify the direction and magnitude of the effect. Where necessary, p values were adjusted by controlling the false discovery rate [34].

RESULTS

One individual randomized to the Placebo-D-Cycloserine sequence did not receive the allocated intervention and was withdrawn due to poor MEP data quality. Twenty healthy participants completed the study between August 18th, 2021 and February 23rd, 2022. No adverse events occurred during the study.

Full participant demographic data are shown in Table 1. There were no differences in the RMT between arms ($t_{(19)} = 0.5$, $p = 0.289$, Placebo arm median RMT = 43% maximum stimulator output, D-Cycloserine = 41% maximum stimulator output).

Blinding effectiveness was assessed by asking participants to identify which arm they believed they had been assigned after the first session. Twelve participants (60%) correctly guessed the order of interventions.

The effect of repeated spaced iTBS on MEP amplitude

MEP amplitude at baseline (prior to the first iTBS) was not different between the arms of the crossover design ($t_{(19)} = 0.4$, $p = 0.684$). There was no difference between the MEP amplitude at baseline and at 60 minutes in both D-Cycloserine arm (DCS) and placebo arm (PLA) ($p > 0.05$). A linear mixed model revealed an interaction between DRUG and STIMULATION ($p = 0.035$). The increase in normalized MEP amplitude (averaged across TIME) was facilitated by D-Cycloserine after both iTBS administrations, and the degree of facilitation did not decrease between each iTBS administration (1st ITBS: 1.27 [1.03, 1.51], 2nd iTBS: 1.24 [1.00, 1.48], $t_{(315)} = 0.2$, $p_{(fdr)} = 0.8080$). In the placebo arm, the normalized MEP amplitude did not significantly increase (on average) after the first iTBS but did after the second iTBS (1st iTBS: 1.01 [0.78, 1.26], 2nd iTBS: 1.34 [1.01, 1.58], $t_{(315)} = 2.6$, $p_{(fdr)} = 0.009$, Fig. 1A). The model examining baseline normalized MEP amplitudes revealed a similar pattern of results (DRUG $t = 2.1$, $p = 0.035$; Fig. 1B). Individual data, both normalized to baseline and raw, are shown in Figure S2.

The effect of repeated spaced iTBS on the TMS stimulus-response relationship

The 140%/120% RMT MEP amplitude ratio was used as the primary measure of the TMS-SR. TMS-SR was not different at baseline between placebo or D-Cycloserine arms ($t_{(19)} = 0.7$, $p = 0.488$). We did not observe evidence of a priming effect on the TMS-SR after a first iTBS when normalized to the 140%/120% immediately preceding each iTBS (Fig. 2A). There was a main effect of DRUG on baseline normalized 140%/120% ratio, where the ratio was higher in the D-Cycloserine arm compared to the placebo arm (D-Cycloserine; 1.6 [1.2, 1.9], placebo; 0.99 [0.6, 1.4], $t_{(126)} = 3.1$, $p_{(fdr)} = 0.003$, Fig. 2B). There was no effect of

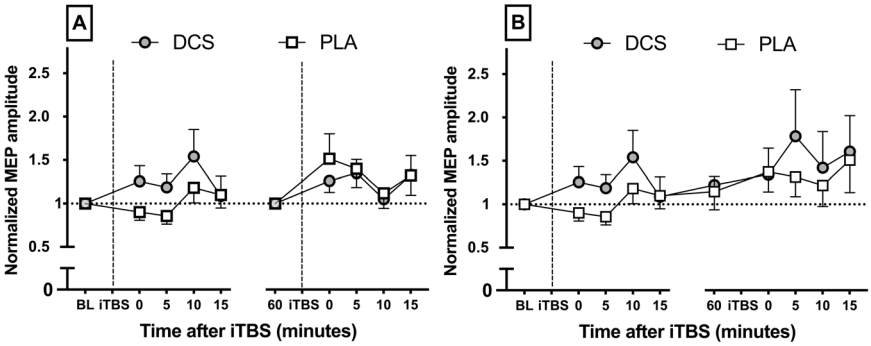

Fig. 1 Peak-to-peak MEP amplitude after repeated spaced iTBS. Mean ± SE of the MEP amplitude normalized to baseline (MEPs after first iTBS) and the MEP amplitude 60 minutes (MEPS after second iTBS) (**A**) and normalized baseline for all time points (**B**). The first and second iTBS are represented by vertical lines. Panel **A**: There was a significant interaction ($p = 0.035$) between DRUG and BLOCK when MEP amplitude was normalized to baseline and 60 minutes after the first iTBS, where the normalized MEP amplitude, averaged across TIME increased following the second iTBS in the placebo arm only. Panel **B**: There was a main effect of DRUG ($p = 0.035$) when MEP amplitude was normalized to baseline values only, where normalized MEP amplitude was higher in the D-Cycloserine arm. BL; Baseline (pre-first iTBS) measurement time point, iTBS1; the first iTBS, iTBS2; the second iTBS, DCS; D-Cycloserine arm, PLA; placebo arm.

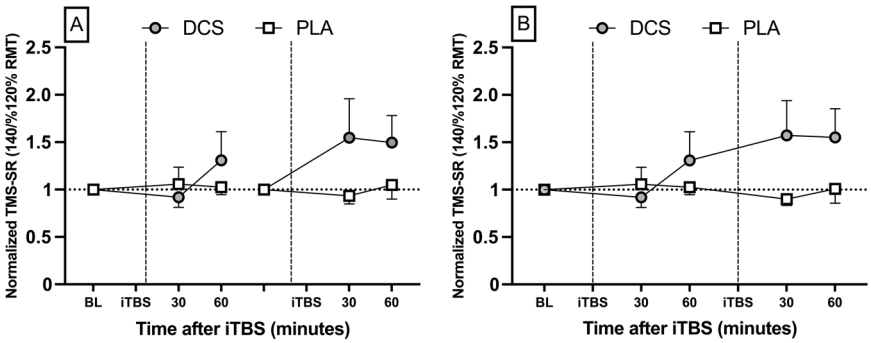

Fig. 2 Stimulus response curves after repeated spaced iTBS. Mean + SE of the TMS-SR normalized to baseline and to 60 minutes (for TMS-SR) following second iTBS, **A** and normalized to baseline (**B**). In both panels, there was a main effect of DRUG (**A**: $p = 0.035$, **B**: $p = 0.003$) on the normalized TMS-SR, where the TMS-SR was higher in the D-Cycloserine arm. BL; Baseline (pre-first iTBS) measurement time point, iTBS1; the first iTBS, iTBS2; the second iTBS, DCS; D-cycloserine arm, PLA; placebo arm.

STIMULATION or TIME, nor any interaction. The group average SR curves and individual SR curves are shown in the supplementary material in Supplementary Figures 3 and 4.

The change in MEP amplitude relative to baseline recorded at each TMS intensity collected as part of the TMS-SR are shown in Fig. 3. There was no effect of DRUG on the baseline MEP amplitude at any of the stimulation intensities collected during the TMS-SR. There was no effect of DRUG or STIMULATION on the normalized MEP amplitude at 100%, 110%, 120%, and 130% RMT. However, at 140% RMT and 150% RMT, there was a significant main effect of DRUG (both $p_{(fdr)} < 0.030$), where the normalized MEP amplitudes were higher in the DCS arm compared to the placebo (Fig. 3).

Individual responses to repeated spaced iTBS

There is significant inter-individual variability in response to iTBS [35]. It has previously been suggested that stimulation effects are mediated by whether individuals can be considered to be 'responders' to plasticity-inducing stimulation protocols [35]. To address the possibility that the present results were also mediated by whether individuals could be considered 'responders' to iTBS, post-hoc we identified how many participants demonstrated facilitated MEP amplitudes after iTBS and how D-Cycloserine influenced the effects of stimulation in these individuals. For each person, we calculated the grand mean of the normalized MEP across the four TIME points (0, 5, 10, 15 minutes) following each iTBS in the placebo arm. Participants in whom the grand mean was >1, i.e., there was an increase in the MEP amplitude compared to baseline, were identified as responders. After the first iTBS, there were 12/20 participants identified as responders, which increased to 15 participants following the second iTBS (Fig. 4).

Responders to the first iTBS were classified as responders for the subsequent analyses. The linear mixed models for the normalized MEP and 140/120% ratio were rerun in participants who were classified as responders despite the drastic drop in statistical power. With this limitation, there was no effect of DRUG, STIMULATION, or TIME on the normalized MEP amplitude in those classified as responders (D-Cycloserine 1st iTBS: 1.6 [1.2, 1.9], 2nd iTBS: 1.3 [1.0, 1.6], Placebo 1st iTBS: 1.3 [1.0, 1.6], PLA 2nd iTBS: 1.5 [1.2, 1.8]). For the TMS-SR, the main effect of DRUG remained, where the normalized TMS-SR was higher in the D-Cycloserine arm (1.3 [1.0, 1.57]) compared to the placebo arm (0.9 [0.6, 1.2], $t_{(126)} = 2.4$ $p = 0.0209$).

DISCUSSION

The aim of the present study was to characterize the effects of repeated spaced iTBS on corticospinal excitability and the role of NMDA-R by using two measures of excitability, namely MEP amplitude and the TMS-SR. In accordance with our hypotheses, adjunctive D-Cycloserine did not occlude metaplastic processes and resulted in a significant increase in MEP amplitude and TMS-SR that was superior to adjunctive placebo, and furthermore this facilitation was of comparable magnitude after both the first and second iTBS tetani. This indicates that NMDA receptor agonism enhances some measures of LTP-like phenomena without

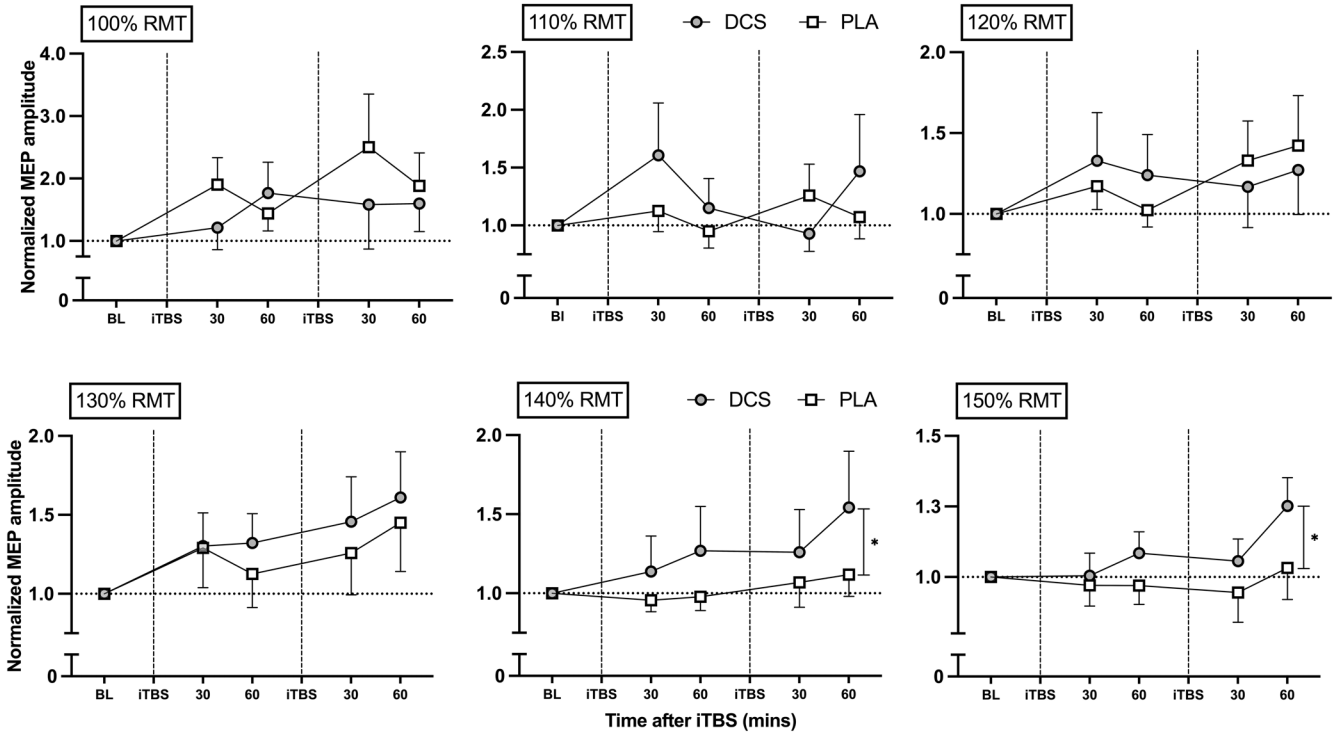

Fig. 3 Normalized peak-to-peak amplitude at each stimulation intensity within the stimulus response curve after repeated spaced iTBS. Mean±SE normalized MEP amplitude collected as part of the TMS-SR at 100%, 110%, 120%, 130%, 140%, and 150% RMT. *represents a main effect of DRUG (both $p < 0.030$), normalized MEP amplitude was higher in the DCS arm at 140% and 150% RMT. DCS; D-Cycloserine arm, PLA; Placebo arm.

disrupting metaplastic and/or priming processes underlying accelerated iTBS. In comparison, iTBS with placebo had limited effects on MEP amplitudes after the first iTBS, followed by significant facilitation after the second iTBS, informing its clinical utility in Major Depressive Disorder. Using TMS-SR as another metric of corticospinal excitability, we found evidence that adjunctive D-Cycloserine paired with repeated spaced iTBS enhanced the 140%/120% ratio through an expansion of neuronal assemblies at higher intensities of the TMS-SR, which may be consistent with synaptic priming of neurons peripheral to the FDI representation after the first iTBS. Our data support the involvement of NMDA-R in the repeated spaced iTBS LTP-like and metaplastic effects in motor cortex in healthy individuals.

The mechanisms of LTP-like neuroplastic effects after repeated spaced iTBS have been presumed to be regulated by NMDA-R activity [36], however, this has not been explicitly tested. As expected and reported by others [19, 20], repeated spaced iTBS increased motor cortex excitability as measured by MEP amplitude to a fixed intensity of stimulation. Our data indicate that partial agonism of the NMDA-R receptor can further enhance this effect. D-Cycloserine preferentially binds to the glycine site on the NR2C subunit of the NMDA-R, where it acts as a partial agonist [37, 38], and therefore low-doses serve to agonize and facilitate NMDA-R signaling. Although previous studies examining MEP amplitude combining D-Cycloserine with neurostimulation have been inconsistent in the direction of effect, they have shown that D-Cycloserine consistently impacted MEP amplitude and TMS-SR [27, 28, 39], highlighting the importance of the NMDA-R in iTBS induced synaptic plasticity and metaplasticity.

We used the ratio of MEP amplitudes at 140% and 120% RMT recorded during the TMS-SR assessment (see methods) as a measure of the slope of the TMS-SR [31]. D-Cycloserine increased the post-iTBS MEP amplitude at 140% and 150%, but not 120% of RMT, resulting in an increased and stable TMS-SR slope, congruent with the effects of D-Cycloserine on LTP in-vitro [40]. Higher stimulation intensities, and the subsequent spread of the electric field, result in the activation of a much larger population of neurons, possibly including more direct activation of the corticospinal tract neurons [31, 41]. The effect of D-Cycloserine at 140% and 150% RMT, but not 100-120% RMT, thus may represent an NMDA-R activity-related reduction in the firing threshold after iTBS in neurons that would not otherwise be involved in the FDI map, indicating an expansion of the neuronal ensemble. A further consideration is the nature of the synaptic connections between the activated interneurons and pyramidal neurons. iTBS has been suggested to increase MEP amplitude via the activation of late indirect waves (I-waves) [42, 43]. Late I-waves may represent increased recruitment of neurons preferentially activated when the electric field is orientated anterior-posterior across the central sulcus [27], and these neurons typically have a higher threshold for activation [44]. In the present study, we used a biphasic pulse to measure the TMS-SR, the reverse phase of which induces an anterior-posterior orientated electric field. A monophasic pulse likely recruits neurons that are sensitive to a posterior-anterior orientated electric field and which contribute to early I-wave volleys [45]. In contrast, the reverse phase of a biphasic pulse likely increases the recruitment of neuronal populations, which contributes to late I-waves [44]. Speculatively, D-Cycloserine as an adjunct to iTBS may therefore result in a lowered stimulation threshold for these neurons after the tetanus. It should be possible to test this suggestion by examining the effects of combined iTBS and D-Cycloserine on MEP amplitude elicited with a posterior-anterior electric current induced by a monophasic pulse. We do not believe these results indicate that combined facilitative effects of D-Cycloserine and repeated spaced iTBS will only occur at high stimulation intensities. Instead, we conclude that these data indicate that D-Cycloserine lowers the threshold for neurons not typically activated by single-pulse TMS of the motor cortex at greater than 120% RMT. Interestingly, it was only at these higher stimulation intensities that a qualitative effect

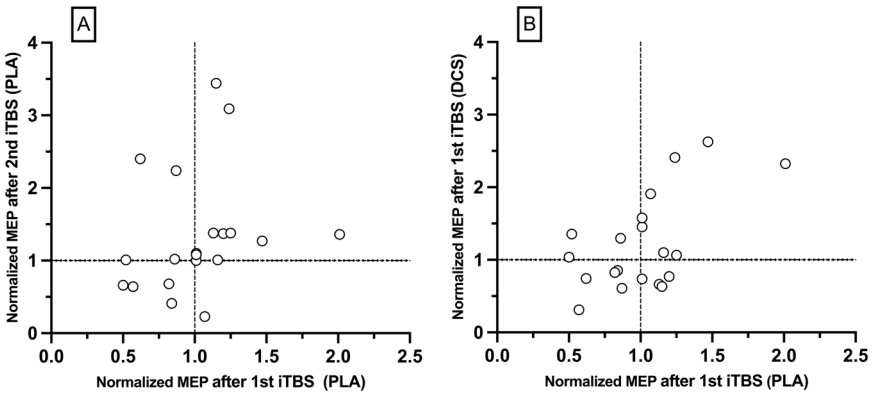

Fig. 4 Individual variability and consistency of the MEP amplitude after iTBS. A individual variability after the first and second iTBS in the placebo arm (PLA). **B** individual variability after the first iTBS in the placebo and D-Cycloserine arm (DCS). Illustrated are means of the normalized MEP at 0, 5, 10, and 15 minutes following iTBS. Participants in whom the normalized MEP was >1 following the first iTBS in PLA were classed as responders.

of repeated spaced iTBS was observed, though the interaction term did not reach significance in the statistical model (Fig. 3). In addition to insufficient statistical power, one explanation for this may be that these effects are not, solely, dependent on NMDA-R activity, or possible regulation of plasticity by homeostatic metaplastic mechanisms [46].

It is important to note that we did not find group-wide facilitation of the MEP after the first iTBS in the placebo arm. This contrasts with Huang et al. [14], who reported that facilitation of the MEP occurred following a single session of iTBS. Many others have also failed to replicate this result [19, 35, 47], such that it is now widely recognized that there is considerable interindividual variability in response to iTBS [15, 35, 48]. Indeed, it is estimated that only ~40–60% of participants' MEP amplitude will increase following iTBS [15, 35], and the present results are in accordance with this estimate. However, it is also important to note although the difference (~0.3 mV) between the MEP amplitude at baseline and at 60 minutes after the first iTBS (immediately prior to the second iTBS) was not statistically different, the study may have lacked the power to detect this effect. Addressing the inter-individual variability in response to stimulation is one of the rationales for using repeated spaced stimulations [2]. We found tentative evidence for stronger effects of repeated spaced iTBS on the MEP amplitude, and the effect of D-Cycloserine on the TMS-SR, in those classified as 'non-responders'. Robust replication of these effects is required to more fully understand the interaction between inter-individual responses to stimulation and both repeated spaced iTBS and D-Cycloserine. Repeated-spaced iTBS is proposed to improve effects of iTBS on treatment outcomes via the enhancement of NMDA-R-involved LTP-like neuroplastic effects [12, 36]. Combining iTBS and D-Cycloserine improves stimulation effects on treatment outcomes in major depressive disorder [23]. It might be possible to further enhance these effects by combining repeated-spaced iTBS and D-Cycloserine. However, it is equally possible that combination of these plasticity-inducing protocols would result in non-linear effects on synaptic plasticity [49]. Further exploration of the effects of combined repeated-space iTBS and D-Cycloserine supplementation on synaptic plasticity in non-motor networks is required.

Some limitations of the experiment must be acknowledged. Visual inspection of the changes in 140%/120% ratio (Fig. 2) suggests that the effects of the second iTBS may be more stable in the presence of D-Cycloserine; however, our experiment was not significantly powered to detect this effect. In addition, we used a biphasic pulse to examine the TMS-SR properties. Whilst this decision possibly provided some insight into the mechanisms of action of D-Cycloserine, it also limited our ability to examine the true slope of the stimulus-response curve, which may have provided more insight into the effects of repeated spaced iTBS. Finally, though our design was informed by previous literature, we can not dissociate late changes that occur after a single iTBS from the effects of the second iTBS and this would require sham iTBS to definitively address.

CONCLUSION

The present results add to the growing body of literature supporting a repeated spaced stimulation paradigm for iTBS TMS. Our data demonstrate that agonism of the NMDA-R enhances the effects of repeated spaced iTBS, particularly using the TMS-SR metric of corticospinal excitability. Together, our data indicate that pharmacological adjuncts to stimulation, such as those targeting the NMDA-R, may be a method to enhance the effects of repeated spaced iTBS. However, our data were collected in healthy participants, and therefore it remains to be determined how pharmacological adjuncts impact repeated spaced iTBS plasticity in clinical populations and treatment protocols.

DATA AVAILABILITY

Data from this study is available upon reasonable request from the corresponding author.

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AUTHOR CONTRIBUTIONS

JGW, JC, MNS, and AM made substantial contributions to the study conception, data acquisition and analysis and interpretation of the work. All authors were involved in the drafting and final approval of the manuscript and agree to be accountable for all aspects of the work.

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COMPETING INTERESTS

JGW, JC, and MNS have no competing interests. AM has a provisional method of use patent application for the combination of D-Cycloserine with intermittent theta-burst stimulation for depression and obsessive-compulsive disorder.

ADDITIONAL INFORMATION

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