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D-cycloserine blunts motor cortex facilitation after intermittent theta burst transcranial magnetic stimulation: A double-blind randomized placebo-controlled crossover study

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Introduction

Synaptic transmission can be manipulated using trains of electrical stimulation, revealing lasting changes in synaptic connection strength. These are termed long-term potentiation (LTP) when neural responses are facilitated and long-term depression (LTD) when attenuated, which are reproduced when using transcranial magnetic stimulation (TMS). Intermittent theta-burst stimulation (iTBS) with TMS of the motor cortex has revealed lasting facilitation of motor evoked potentials, and this appears to be, at least in part, dependent on the *N*-Methyl-D-Aspartate receptor (NMDAr).

The NMDAr is a glutamate receptor with ionotropic and metabotropic roles in plasticity. At low dose, the partial NMDAr agonist D-cycloserine improves several forms of human learning including probabilistic [1], visuospatial [2], and declarative memory [3]. Similarly, low dose cycloserine augments and stabilizes facilitation following electrical brain stimulation using transcranial direct current stimulation [4]. Consistent with these findings, iTBS [5] or tDCS [6] in conjunction with NMDAr antagonists reduces plasticity.

Yet, a preliminary crossover study involving 6 participants examined motor cortex iTBS in conjunction with 100mg of cycloserine and reported sustained attenuation of responses, whereas in the placebo arm the expected facilitation was observed [7]. To our knowledge, there have been no attempts to replicate this finding. Here, we re-examine NMDAr partial agonism using D-cycloserine and intermittent TBS (iTBS) in the motor cortex of healthy individuals using a randomized, double-blind, placebo-controlled crossover design.

Methods

Twelve healthy participants (7F/5M, 29.66 ± 6.37 years of age) were recruited for this double-blind randomized, placebo-controlled crossover trial (NCT03432689). It was approved by our clinical research ethics board and Health Canada approved the importation of cycloserine (D-Cycloserine; Purdue GMC Centre, IL), repackaged as 100mg capsules and microcrystalline cellulose capsules. We used atmospheric noise to generate a random number sequence. Treatment allocation was concealed.

Exclusion criteria were pregnancy, lactation, epilepsy, previous stroke, renal disease, liver disease, current alcohol use disorder, allergy to antibiotics, use of isoniazid, ethionamide, or bupropion, current psychiatric concerns, history of bipolar disorder, or intracranial implants.

Adverse events were elicited and recorded. To control for pre-stimulation somatic symptoms, participants completed the Toronto Side Effects Scale before iTBS and the following day for both study arms.

Using a MagPro X100, Cool-B70 coil (MagVenture, Denmark), and Visor2 TMS neuronavigation software (ANT Neuro, Germany), we recorded surface EMG to collect motor evoked potentials (MEPs) from the "hotspot" of the first dorsal interosseous muscle (FDI). We determined the resting motor threshold (RMT), defined as the lowest stimulator intensity that produced ≥5/10 MEPs with >50 μV amplitude. We collected MEPs at defined intervals in sets of 20 at 120% RMT at 0.2Hz. We also characterized a stimulus response curve (SRC) by averaging 4 MEPs at each intensity in intervals of 10% between 100 and 150% RMT, presented in random order. These SRCs were acquired immediately pre-iTBS and at +90min. As previous data involving electrical stimulation in conjunction with cycloserine revealed an effect lasting until the following day [4], we included a 16hrs time point where we acquired an SRC after once again determining the RMT.

The iTBS theta-burst protocol involved 20 trains of ten 3-pulse bursts at 50 Hz delivered at 5 Hz, with an 8 second inter-train interval. iTBS was administered at 80% RMT.

Participants ingested their capsules 30 minutes prior to entering the experimental suite. They were then explained the experimental protocol *in situ*, electrodes attached, template MR brain registered, the FDI "hotspot" identified, RMT determined, and baseline MEPs collected. Accordingly, iTBS was delivered ≥60 minutes elapsed after capsule ingestion when plasma cycloserine levels would have reached a steady state [8].

Raw EMG traces were extracted and MEPs analyzed using SPSS v24 (Chicago, IL). We normalized MEP timecourse to the mean of −10min and -5min MEPs. One outlier was identified using Tukey's Fences and replaced with the mean of nearby points. We utilized

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repeated measures two-way ANOVA, with LSD paired-samples post-hoc comparisons to explore significant interactions. We utilized Cohen's d to calculate effect size. Significance was set at $\alpha \leq 0.05$.

Results

No adverse events occurred. Cycloserine + iTBS did not significantly increase side effects compared to placebo + iTBS (Cohen's $d = 0.02$). Seven of twelve (58.3%) participants correctly guessed their treatment allocation. Daily RMTs did not significantly differ between cycloserine and placebo arms.

We observed blunted plasticity after iTBS in the cycloserine arm as measured by MEPs (Fig. 1A; Time $F(10,220) = 2.44$, $p = 0.0089$; Treatment $F(1,220) = 1.50$, $p = 0.23$); Time*Treatment $F(10,220) = 2.01$, $p = 0.033$). Post-hoc comparisons reveal a significant attenuation of MEPs immediately after iTBS in the cycloserine arm (0.88 ± 0.24 vs 1.19 ± 0.42; $t(11) = 2.31$, $p = 0.041$), 5-min after iTBS (1.08 ± 0.42 vs 1.47 ± 0.51, $t(11) = 2.41$, $p = 0.034$), but potentiation 60-min after iTBS (1.34 ± 0.38 vs 1.09 ± 0.37; $t(11) = 2.82$, $p = 0.017$). No other time points statistically differentiated cycloserine and placebo treatment arms.

SRCs at baseline were comparable (Fig. 1B), and there was no statistical change 90 minutes after iTBS (Fig. 1C). However, 16 hours following placebo + iTBS MEP amplitude was overall decreased (Fig. 1D; Intensity $F(5,110) = 1.98$, $p = 0.086$, Treatment $F(1,110) = 4.30$, $p = 0.049$, Treatment*Intensity $F(5,110) = 1.33$, $p = 0.25$).

Discussion

Our data suggests that cycloserine blunts early synaptic changes after iTBS. Though previous data revealed a sustained attenuation of responses after iTBS + cycloserine [7], in this larger sample we observed only brief attenuation followed by a transient delayed potentiation. We first considered whether this merely reflected heterogeneity in the effects of iTBS + cycloserine on MEP time courses, with some participants demonstrating LTD and others LTP. Indeed, 3/12 participants had an MEP amplitude suppression that returned to baseline at 60 minutes after iTBS + cycloserine, similar to the aggregate data reported by Teo et al. [7]. These participants did not account for the initial suppression and delayed transient potentiation, as the pattern remained after removing these participants from analyses. While our data is at odds with the preclinical literature demonstrating enhanced plasticity with D-cycloserine, we note that anodal tDCS + cycloserine initially marginally blunted facilitation relative to anodal tDCS + placebo, and that the aggregate data similarly revealed a delayed potentiation between 20 and 60 minutes [4].

The mechanism whereby low dose D-cycloserine, and accordingly NMDAr agonism, in conjunction with iTBS changes physiological adaptation is unclear. TBS involves a summation of inhibitory and excitatory effects [2], and therefore the biphasic relationship we observe could be interpreted as cycloserine differentially impacting neuronal subpopulations at different phases of plasticity. Preconditioning by cycloserine is another potential mechanism [11,12], and indeed glycine and cycloserine result in rapid NMDAr

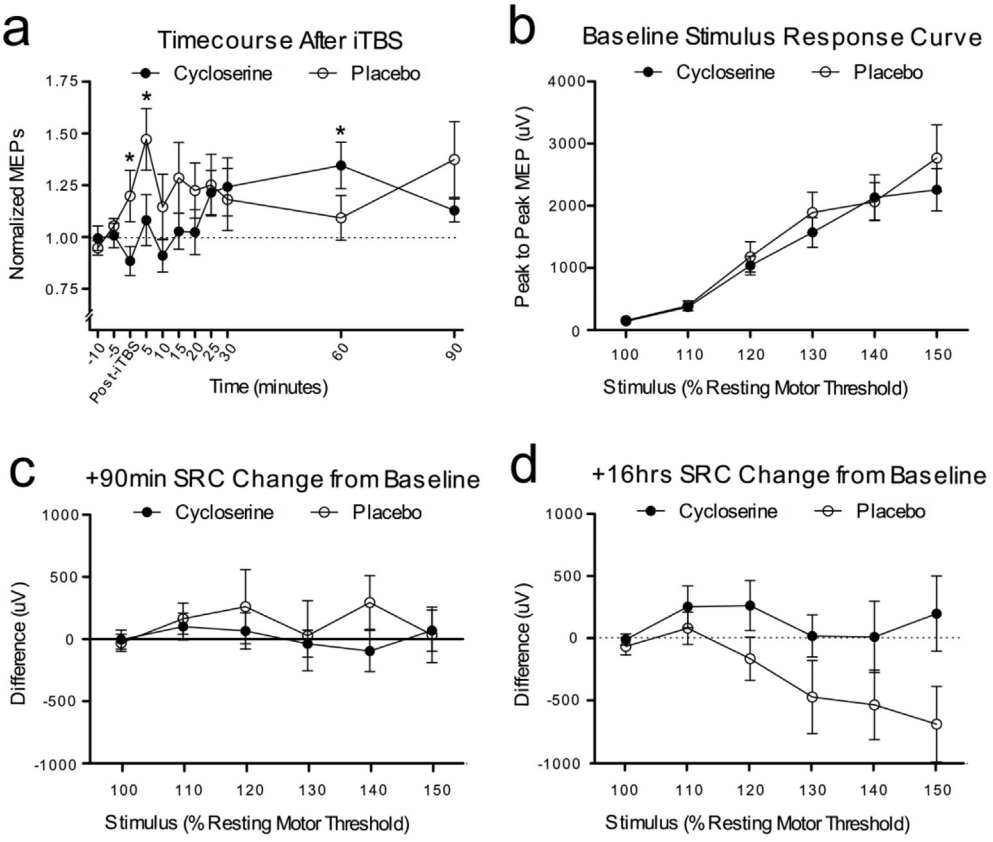

Fig. 1. a) Mean timecourse of MEP evolution in cycloserine and placebo arms. * indicates $p < 0.05$. While the timecourse continued into the following day, we do not present this data due to changes in RMT unrelated to treatment allocation. **b)** Mean SRCs prior to iTBS. **c)** Mean change in SRC from baseline at 90 minutes following iTBS (positive values indicate an increase in MEP at 90 minutes). **d)** Mean change in SRC from baseline at 16 hours following iTBS. Error bars indicate standard error of mean.

internalization [10] that may alter the relative balance of NMDAr and non-NMDAr dependent contributions after iTBS. This, either independently or in conjunction with gating [13] or other homeostatic mechanisms [9] may account for the blunted plasticity observed in our study.

Finally, our data suggests that iTBS results in adaptive changes that are persistent, evident in a shift in the stimulus response curve the following day. These lasting changes are not evident when iTBS is delivered in the presence of low-dose cycloserine, however our data can not inform whether this is related to the immediate disruption of plasticity or to its systemic presence during the process of consolidation. Our findings extend previous work revealing increased excitability after iTBS [11] to suggest homeostatic changes evident the following day, and future studies should employ long- and short intracortical inhibition paradigms to further characterize these late effects.

Additional research is required to understand the molecular mechanisms of iTBS, in order to enhance network level changes and improve treatment outcomes when using these modalities in neurological and psychiatric conditions.

Conflicts of interest

Authors BS, FPM, AK, and AM declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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