Brain Stimulation 15 (2022) 1372–1374

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

A replication study of NMDA receptor agonism sufficiency to enhance 10-Hz rTMS-induced motor cortex plasticity

Dear Editor,

Clinical applications of repetitive transcranial magnetic stimulation (rTMS) have advanced without complete understanding of its mechanisms of action. Pharmacology can help unravel the neuronal-level actions of rTMS in humans by blocking or targeting molecular mechanisms and examining the impact on neurophysiology measures such as motor-evoked potentials (MEPs). However, such studies are scarce and underpowered, necessitating replication studies to confirm these foundational findings (reviewed in Refs. [1,2]). We sought to replicate our own experiment, which found a significant effect of n-methyl-D-aspartate (NMDA) receptor agonism on rTMS-induced plasticity, despite only 10 subjects and effect size of only 0.3 (Power = 0.42). We therefore sought to test the reproducibility of our previously low-powered study while retesting the role of the NMDA receptor in 10-Hz rTMS [3]. We hypothesized that 10-Hz rTMS strengthens affected networks by reinforcing synaptic connections through long-term potentiation (LTP), based on animal studies and our previous findings [4,5]. We therefore predicted that a single dose of DCS, relative to placebo, would enhance the magnitude of MEPs otherwise potentiated by 20 minutes of 10-Hz rTMS.

We used similar methods to the study performed at the Medical University of South Carolina (MUSC) [3] by again recruiting ten healthy right-handed, non-smoking adults (6 female) from 21 to 39 years old (28 ± 6.0) into a randomized, double-blind, crossover study approved by the Butler Hospital Institutional Review Board. All participants provided informed consent prior to any research procedures, and we excluded those with brain disorders, or who were actively taking neuropsychotropic medications. We randomly assigned participants to a single dose of either 100 mg D-cycloserine or identical microcrystalline cellulose capsules (Tidewater pharmacy, Mt. Pleasant, SC) in a blinded random manner over two separate visits, at least 1-week apart (Fig. 1A).

A PowerMAG EEG100 power unit and PMD70-pCool Coil (Mag&More, Germany) were used to stimulate the left motor cortex "hotspot". MEPs were recorded from the right first dorsal interosseous (FDI) muscle with surface electromyography (EMG) electrodes (Cardinal Health, USA). The raw signal was amplified and filtered by CED 1902 and 1401 microprocessors and analyzed with Signal software (Cambridge Electronic Devices, UK). Pulses were kept within 0.5 mm of target with neuronavigation performed with a Brainsight 2 System (Rogue Research, Quebec, Canada).

Approximately 60 minutes following drug administration, we measured baseline assessments including: resting motor threshold (rMT), one bin of 40 single-pulses (SP) at 120% rMT, and one SP at every percent intensity from 20% to 100% of maximum machine stimulator output in randomized order fit to a Boltzmann sigmoidal recruitment curve (RC). Pulses were jittered at 4–7 second intervals. Paired-pulse measures including short intracortical inhibition (SICI) and intracortical facilitation (ICF) were collected as previously described [4]. Long intracortical inhibition (LICI) was produced with pulses separated by 100 ms at 120% rMT. These were followed by an rTMS 'plasticity protocol' (20 min of 10-Hz stimulation; 1.5 sec on/58.5 sec off at 80% rMT) two-hours after drug administration. rTMS parameters were based on Jung et al. (2008) [6]. We then conducted the same assessments over 1-h after the plasticity protocol, with bins of 40 SP collected every 15 minutes (see Fig. 1A for details). Baseline measures were collected after drug administration in order to directly test the effect of DCS on rTMS, rather than on baseline excitability, which DCS has been previously shown to have no effect [7].

All data were analyzed with R software (version 4.2, R Core Team, Vienna, Austria). We analyzed SP data over a 1-h time course with a general linear mixed model for continuous outcomes with a random effect for repeated measures using the package lme4. We fit a series of models testing the effects of drug, time, and the drug x time interaction. ANCOVA was used to test potential order effects. Raw RC data was fit to a Boltzmann sigmoidal function using Levenberg-Marquard nonlinear least-mean squares algorithm. Paired student's t-tests were used to compare RC slope, intercept, and height averages before and after rTMS, as well as percent change associated with rTMS between drug conditions. The level of significance was set at $p < .05$. All subjects completed the study and reported no adverse effects. Subjects correctly guessed which pill they received 4/20 times.

Our 1-h time-course data revealed a similar trend to our previous single-pulse results, but did not reach significance (Fig. 1B, $F = 2.19$, $p = .12$). Drug order had no effect on MEP amplitudes ($p = .52$). Our nonsignificant results with $n = 10$ emphasize the need for larger sample sizes. Power analysis with our effect size of 0.3 suggests 19 subjects would be required. Nevertheless, facilitatory changes in RC supported our central hypothesis. We found a significant difference in the degree of rTMS-mediated change (before vs after rTMS) in intercept between placebo and DCS conditions (Fig. 1D, $p = .04$). We further observed a decrease in intercept exclusively in the DCS condition from pre to post rTMS, indicating a left (excitation) shift (Fig. 1E, $p = .03$). rTMS had no significant effect on the RC slope nor height for either drug despite visual differences seen in plateau (Supplemental Fig. 1) and there was also no difference in the degree of pre-to post-change between drug conditions

https://doi.org/10.1016/j.brs.2022.09.014

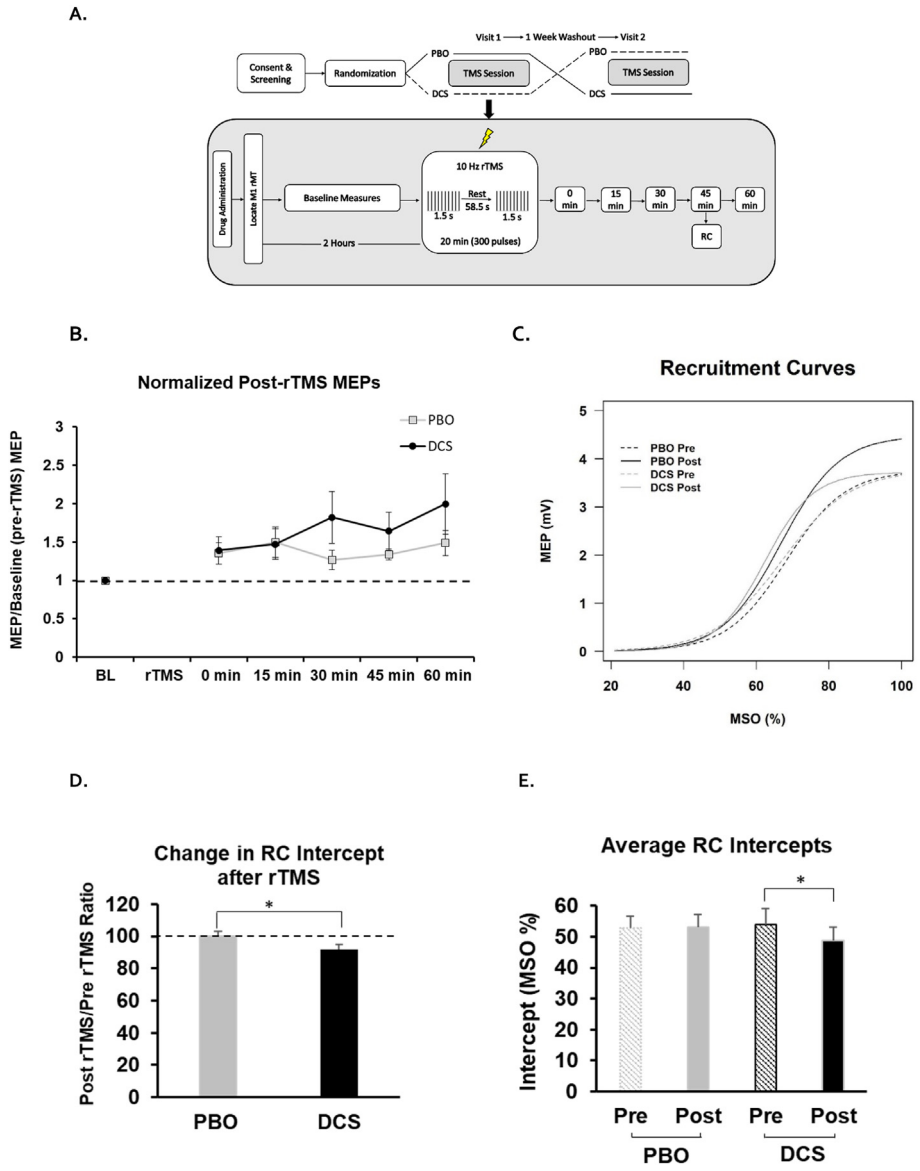

Fig. 1. NMDA receptor partial agonist, D-cycloserine, enhances 10-Hz rTMS facilitation of single-pulse MEPs. (A) Study design. Top: Overview of two-week experiment. Below: Individual TMS session protocol. Baseline measures are performed approximately 60 minutes after drug ingestion and include SP, PP, and RC. PEST MT and SP bins were recorded at every 15 minute post-rTMS time points. **(B)** SP time course: Averaged normalized (to baseline) MEP values and SEM (error bars) for each time point after 10-Hz rTMS: ns, $p = .12$ overall, $\eta^2 p = .03$, CI [-0.60, 0.67] (average across timepoints) no effect of drug condition or time. 0 min: PBO (1.35 ± 0.14) DCS (1.39 ± 0.18); 15 min: PBO (1.50 ± 0.20) DCS (1.47 ± 0.20); 30 min: PBO (1.27 ± 0.13) DCS (1.82 ± 0.34); 45 min: PBO (1.34 ± 0.07) DCS (1.64 ± 0.2560); 60 min: PBO (1.49 ± 0.16) DCS (2.00 ± 0.39). **(C)** Fitted recruitment curves before and after rTMS by drug condition. **(D)** Degree of RC intercept change after rTMS: Averaged percent change and SEM (error bars) for each drug: $p = .04$. PBO (100.56 ± 2.31) DCS (91.51 ± 3.13). **(E)** Mean RC intercept values and SEM (error bars) before and after rTMS: $p = .03$ before and after with DCS. PBO Pre (52.9 ± 3.9) PBO Post (53.2 ± 4.11) DCS Pre (53.9 ± 5.19) DCS Post (48.7 ± 4.50). M1 = Primary Motor Cortex; rMT = resting motor threshold; PEST= Parameter Estimation by Sequential Testing; BL= Baseline; rTMS = repetitive Transcranial Magnetic Stimulation; MEP = motor evoked potential; PBO= Placebo; DCS = D-cycloserine; SP = single pulse; PP= Paired-Pulse; RC = recruitment curve; SEM = standard error of the mean.

for either measure (slope: PBO 1.18 ± 0.64, DCS 1.12 ± 0.45, $p = .78$; height: PBO 1.26 ± 0.52, DCS 0.94 ± 0.28, $p = .19$).

Our pharmaco-rTMS replication study suggests that NMDA activation may be sufficient to enhance 10-Hz rTMS-induced facilitation as captured by excitatory left-shift in RC (Fig. 1C), and produced trend-level MEP enhancement in response to single-pulses over a 1-h time-course, consistent with results published previously (Fig. 1B) [3]. Our trend-level results may be the result of insufficiently powered experiments. Our original study was likewise underpowered, although the results were significant. Many of the foundational studies elucidating the mechanism of TMS have likewise had very small sample sizes. These results demonstrate the need to adequately power future experiments to ensure correct mechanistic conclusions.

10-Hz rTMS lowered the average intercept (before and after, $p = .03$) after taking DCS, but not placebo ($p = .80$). Differential proportion of cortical involvement (compared to spinal) may be one reason that recruitment curves appeared to be more sensitive to NMDA agonism on rTMS effects than set stimulus MEPs. In fact, even when compared with intracortical excitability assays, recruitment curves have been reported to be more sensitive to receptor modulation [8]. Thus, acute pharmacologic NMDA receptor

activation was *sufficient* to enhance rTMS-induced potentiation, demonstrating a more specific role of NMDA receptors in rTMS facilitation (compare with necessity of NMDA receptors demonstrated by Huang et al., 2007 [9]). Taken together, these results and other human data [3,4,9], combined with prior animal data [5] support the notion that 10-Hz rTMS-induced motor plasticity may work through LTP-like mechanisms.

To date, no studies have examined whether NMDA receptor augmentation is *necessary* to enhance the faciliatory effects of 10-Hz rTMS in humans, though parallel work with intermittent (i) TBS demonstrates their necessity [9]. DCS has been found to reverse the excitatory effects of iTBS [10] and blunt responses in a replication study [11] in healthy human subjects. Interestingly, DCS was sufficient to stabilize iTBS effects in depressed subjects over a 24-h period [12]. Moreover, preliminary data from a randomized controlled study found that DCS enhanced iTBS antidepressant effects by more than 2-fold [13]. These results emphasize the therapeutic potential inherent in rTMS-mediated modulation of key brain networks through leveraging our developing knowledge of its neuronal mechanisms. Our ultimate goal is to understand how TMS works clinically. It is therefore important to note that our motor protocol differs from clinical protocols in pulse number, intensity, train duration, intertrain interval as well as cortical region. The prolonged clinical protocol could have opposing (homeostatic) effects [14]. The critical next step, therefore, is to test these NMDA receptor-mediated effects with rTMS in the appropriate population and cortical target.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Prayushi Sharma, Jee Won Kang, and Eric Tirrell for technical support and Nicole Armstrong, PhD for statistical support. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM130452, Center for Biomedical Research Excellence, Center for Neuromodulation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.09.014.

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13 July 2022
Available online 27 September 2022