**STUDY PROTOCOL**

**Study protocol for evaluating the clinical efficacy and neurobiological correlates of sequential treatment with tDCS primed iTBS and ECT in treatment-resistant depression**

[version 1; peer review: 1 approved with reservations]

Preeti Sinha\(^1\)*, Umesh Shreekantiah\(^2\)*, Nishant Goyal\(^2\)*, Vanteemar Sathyarayana Sreeraj\(^1\), Shyam Sundar Arumugham\(^1\), Subham Samantaray\(^2\), Ashok Jammigumpula\(^3\), Gopala Krishna Kadarapura Nanjundaiah\(^4\), Sudhir Venkataramaih\(^4\), Kandavel Thennarasu\(^5\), Chandramouli Roy\(^2\), Abhiram Narasimhan Purohith\(^3\), Sonia Shenoy\(^3\), Channaveerachari Naveen Kumar\(^1\), Venkataram Shivakumar\(^6\), Kaviraj Udupa\(^7\), Kesavan Muralidharan\(^1\), Ganesan Venkatasubramanian\(^1\), Jagadisha Thirthalli\(^1\), Samir Kumar Praharaj\(^3\), Urvakhsh Meherwan Mehta\(^1\)

\(^1\)Department of Psychiatry, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, 560029, India
\(^2\)Central Institute of Psychiatry, Ranchi, Jharkhand, 834006, India
\(^3\)Department of Psychiatry, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, Udupi, Karnataka, 576104, India
\(^4\)Department of Neuroanaesthesia and Neuro Critical Care, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, 560029, India
\(^5\)Department of Biostatistics, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, 560029, India
\(^6\)Department of Integrative Medicine, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, 560029, India
\(^7\)Department of Neurophysiology, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, 560029, India

* Equal contributors

---

**Open Peer Review**

**Approval Status**

\(\text{v1} \) version 1 30 Sep 2022

---

**Abstract**

**Background:** Treatment-resistant depression is a burdensome condition. Intermittent theta burst stimulation (iTBS) of the left dorsolateral prefrontal cortex is considered a treatment option in early course of resistance with a proportion of such patients responding to it. Preliminary evidence suggests a role of priming iTBS stimulation with preconditioning using cathodal transcranial direct current stimulation (tDCS). This protocol describes a double-blind randomized sham-controlled study to evaluate the clinical efficacy and tolerability of tDCS-primed iTBS in the treatment of resistant...
depression. Non-responders to this trial will be offered open-label electroconvulsive therapy. All participants will undergo neurobiological investigations that will enable the identification of potential response predictors and mechanisms.

**Methods:** Three hundred and fifty consenting patients with treatment resistant depression will be randomly assigned to receive 20–30 daily sessions of true-tDCS or sham-tDCS primed iTBS over left dorsolateral prefrontal cortex at three study centers. After this blinded sham-controlled trial, non-responders to the intervention will be offered open-label true ECT. Clinical assessments, neurocognitive assessments and multimodal investigations (magnetic resonance imaging, electroencephalography, heart rate variability, investigative transcranial magnetic stimulation-transcranial direct current stimulation, gene polymorphisms) will be conducted at baseline and repeated after the end of the trial, as well as open-label ECT course. The trial will evaluate the improvement in depressive symptoms (Hamilton depression rating scale) between the two groups as the primary outcome measure.

**Keywords**
primed neuromodulation, rTMS, tDCS, meta-plasticity, electroconvulsive therapy, treatment-resistance, depression
Corresponding authors: Samir Kumar Praharaj (samir.kp@manipal.edu), Urvakhsh Meherwan Mehta (urvakhsh@nimhans.ac.in)

Author roles: Sinha P: Conceptualization, Methodology, Resources, Writing – Review & Editing; Shreekantiah U: Funding Acquisition, Methodology, Resources, Supervision, Writing – Review & Editing; Goyal N: Funding Acquisition, Investigation, Methodology, Validation, Writing – Review & Editing; Seeraj VS: Investigation, Methodology, Project Administration, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation; Arumugham SS: Formal Analysis, Funding Acquisition, Resources, Software, Writing – Original Draft Preparation; Samantaray S: Investigation, Methodology, Writing – Review & Editing; Jammigumpula A: Methodology, Writing – Review & Editing; Nanjundaiah GKK: Methodology, Writing – Review & Editing; Venkataramaiah S: Methodology, Writing – Review & Editing; Thennarasu K: Formal Analysis, Methodology, Writing – Review & Editing; Roy C: Investigation, Methodology, Writing – Review & Editing; Purohith AN: Methodology, Writing – Review & Editing; Shenoy S: Supervision, Writing – Review & Editing; Kumar CN: Methodology, Writing – Review & Editing; Shivakumar V: Investigation, Methodology, Writing – Review & Editing; Udupa K: Investigation, Writing – Review & Editing; Muralidharan K: Funding Acquisition, Methodology, Resources, Supervision, Writing – Review & Editing; Thirthalli J: Conceptualization, Funding Acquisition, Methodology, Resources, Supervision, Writing – Review & Editing; Praharaj SK: Funding Acquisition, Methodology, Resources, Supervision, Writing – Review & Editing; Mehta UM: Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome/DBT India Alliance [IA/CRC/19/1/610005]. It comprises a committee of technical experts who will periodically review the trial progress, research practices, outputs and accountable utilization of funds. The clinical research center for neuromodulation in psychiatry consists of the three participating institutes (NIMHANS, Bengaluru; CIP, Ranchi; and KMC, Manipal). The heads of the three institutes are the sponsors. The funders and sponsors will support the project through expert suggestions through the technical committees but have not be involved in the study design and they will not be involved in the collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. The investigators hold the final authority on taking these decisions.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Sinha P et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Sinha P, Shreekantiah U, Goyal N et al. Study protocol for evaluating the clinical efficacy and neurobiological correlates of sequential treatment with tDCS primed iTBS and ECT in treatment-resistant depression [version 1; peer review: 1 approved with reservations] Wellcome Open Research 2022, 7:242 https://doi.org/10.12688/wellcomeopenres.18192.1

First published: 30 Sep 2022, 7:242 https://doi.org/10.12688/wellcomeopenres.18192.1
**Introduction**

**Background and rationale for the study**

Depression is a leading cause of global disability and about one-third of the individuals with depression who receive treatment fail to respond adequately (Rush et al., 2006). Treatment guidelines recommend in-depth clinical research to arrive at evidence-based treatment options for such individuals and non-invasive brain stimulation or neuromodulatory therapies are one such treatment avenue. Among the available newer neuromodulatory therapies, intermittent Theta Burst Stimulation (iTBS), a form of Transcranial Magnetic Stimulation (TMS) has been shown to be equally effective as conventional high-frequency TMS in the treatment of resistant depression. Transcranial direct current stimulation (tDCS) on the other hand is effective in treating depression patients but may not be as effective as medications (Brunoni et al., 2017). Electroconvulsive therapy (ECT), a conventional neuromodulation therapy, is considered as the gold-standard treatment option for treatment-resistant depression (Goldberg, 2021). TMS and tDCS are newer non-invasive methods of neuromodulation that have focal action and are much safer in comparison to ECT. Like other treatment modalities, ECT, TMS and tDCS show variable treatment responses with some patients improving and others not improving. Methods to enhance treatment response are being actively investigated, especially in patients with treatment-resistant depression.

Preconditioning of target sites with tDCS is shown to modulate the after-effects of magnetic stimulations in different brain regions (Lang et al., 2004; Santos-Pontelli et al., 2016). This modulatory effect was also observed to alter the experimental pain threshold (Moloney & Witney, 2013). Priming the target motor regions with tDCS amplifies the effects of rTMS on motor evoked potentials (excitatory or inhibitory) (Hasan et al., 2012). A pilot study in depression attempted priming high frequency repetitive TMS (rTMS) with tDCS (Loo et al., 2009). They reported improvement in cathodal but not anodal tDCS preconditioned patients and a dramatic response with a single session in one of the patients was encouraging, suggesting a possibility of a faster response. There have been earlier attempts at using low intensity, high-frequency rTMS to prime low-frequency stimulation of right Dorsolateral Prefrontal Cortex (DLPFC) in depression (Fitzgerald et al., 2008; Giustiniani et al., 2020). Indeed, theta burst was used in one of the studies to enhance the high-frequency right-DLPFC stimulation (Nongpiur et al., 2011). These studies have been promising by demonstrating the feasibility of priming protocols. However, they have not been able to demonstrate definitive superiority over conventional protocols owing to methodological factors like small sample size. Moreover, the left DLPFC stimulation is found to be equal if not superior to bilateral DLPFC stimulation and better than right DLPFC stimulation (Lefaucheur et al., 2020). The effect of priming on left DLPFC stimulation in depression is not reported yet.

Induction of synaptic long-term potentiation (LTP) using intermittent theta bursts (iTBS) is affected by the membrane potential of the postsynaptic neuron through the mechanisms of metaplasticity. Cathodal tDCS increases the resting membrane potential and enhances the excitatory response of neurons to iTBS (Hasan et al., 2012). The combination of tDCS in a study did not accentuate the downregulation of stress response by iTBS at left DLPFC. However, anodal and not cathodal tDCS was used over the target site (De Smet et al., 2021). Hence, we posit that priming of iTBS by cathodal tDCS over left DLPFC would enhance the therapeutic effects of iTBS in depressive disorders. In a first-of-its-kind study, we plan to specifically examine the incremental clinical efficacy of cathodal transcranial Direct Current Stimulation (c-tDCS) primed intermittent Theta Burst Stimulation (iTBS) delivered to the left DLPFC in patients with treatment-resistant depression.

As a step-2 extension, non-responders to the step-1 treatment will be offered open-label electroconvulsive therapy (ECT). ECT is an effective treatment in depression and remains effective even in severe treatment resistant conditions (Fitzgibbon et al., 2020; Khalid et al., 2008). The mechanism of such effect is not clearly understood (Subramanian et al., 2022). In this background, the study uses neurobiological investigations to understand the mechanisms of neuromodulatory therapies and explore the biomarkers for their potential to predict treatment response.

This study aims to evaluate the clinical efficacy and tolerability of the tDCS-primed iTBS protocol. It also has an overarching aim of studying the neurobiology of treatment-resistance and treatment response in depression. The current paper focuses on the first aim and a separate paper would describe the methodology of neurobiological study of treatment-resistance in depression (as well as schizophrenia).

**Objectives and hypothesis**

**Primary**

To examine the incremental clinical efficacy of cathodal transcranial Direct Current Stimulation (c-tDCS) primed intermittent Theta Burst Stimulation (iTBS) delivered to the left Dorsolateral Prefrontal Cortex (DLPFC) in patients with treatment-resistant depression

**Hypothesis:** True c-tDCS primed iTBS will demonstrate a greater clinical efficacy (change in Hamilton Depression Rating Scale (HAMD) scores) compared to sham c-tDCS primed iTBS

**Secondary**

- To compare the neurobiological profile of patients with treatment-resistant depression with matched healthy controls using multi-modal data (brain imaging, electroencephalogram (EEG), TMS-tDCS perturbation study metrics, heart rate variability (HRV) and neuroplastic gene polymorphisms).
- To examine the clinical utility of the proposed sequential treatment algorithm for treatment-resistant depression.
- To evaluate the predictive utility of multi-modal data (brain imaging, EEG, TMS-tDCS perturbation study metrics, HRV and neuroplastic gene polymorphisms).
in identifying clinical response to the proposed sequential treatment algorithm for treatment-resistant depression.

- To examine the differential effect of true c-tDCS primed iTBS versus sham c-tDCS primed iTBS and ECT on the neurobiological profile of patients with treatment-resistant depression using multi-modal data (brain imaging, EEG, TMS-tDCS perturbation study metrics, HRV) and potential interactions with neuroplasticity gene polymorphisms.

**Protocol**

**Trial design**
We employ a parallel-arm double-blinded randomized sham-controlled design with an allocation ratio of 1:1 to determine the differential efficacy of cathodal tDCS primed iTBS and sham tDCS primed iTBS in treatment-resistant depression.

**Study setting**
This will be a hospital-based study. The study will be conducted on patients with treatment-resistant depression at three mental healthcare and research institutes in India - National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru; Central Institute of Psychiatry (CIP), Ranchi; and Kasturba Medical College (KMC), Manipal. The study will be centrally coordinated at NIMHANS.

**Subjects**

**Selection of patients**
Patients with Major Depressive Disorder (DSM-5), who have antidepressant treatment resistance or intolerance (as per the selection criteria listed below) will be screened and recruited for this research study from the clinical services of the three institutes.

**Inclusion criteria**
1. Major Depressive Disorder diagnosis (DSM-5) (American Psychiatric Association, 2013);
2. Age 18–60 years;
3. Any sex;
4. Right-handed (Edinburgh Handedness Inventory) (Oldfield, 1971);
5. Hamilton Depression Rating Scale (HAMD-17) score ≥ 18 (Hamilton, 1960);
6. No clinical response to an adequate dose of two antidepressant medications (based on antidepressant treatment history form score) OR unable to tolerate at least two separate trials of antidepressants of inadequate dose and duration. Adequate antidepressant doses in the current episode would be considered if a score of three or more in Antidepressant Treatment History Form - Short Form (ATHF-SF) for dose equivalence and duration (i.e., four weeks) is noted with an absence of evidence for substantial non-adherence. Clinical global impression-improvement (CGI-I) score of “Minimally improved”/“No change”/“Worsening”/“Improved but relapsed on medications” would be considered for resistance (Sackeim et al., 2019);
7. Currently on a stable antidepressant regimen (no more than 25% of variation in antidepressant dose) for at least four weeks before initiating intervention;
8. Capacity to consent for research studies as per the assessment using the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC) (Jeste et al., 2007);
9. Written informed consent (Sreeraj et al., 2022).

**Exclusion criteria**
1. Resistant to ≥3 antidepressants or to an adequate ECT trial.;
2. Suicidal intent (HAMD suicide item score of 3 or 4)/any psychiatric emergency (Hamilton, 1960);
3. Bipolar depression;
4. Psychotic disorder or psychotic symptoms;
5. Neurological/clinical evidence of other medical comorbidity that could substantially influence depression;
6. Unstable medical illness;
7. Co-prescribed BZD >1 mg clonazepam equivalents or anti-epileptic drugs;
8. Severe general medical/neurological comorbidity that precludes ECT or has an effect on cognition and behaviour;
9. Current psychoactive substance dependence (except caffeine or nicotine);
10. Pregnancy or postpartum period;
11. Any contraindication for Magnetic Resonance Imaging (MRI);
12. Any contraindication for Transcranial Magnetic Stimulation (assessed using TMS adult safety screen (Keel et al., 2001));

**Interventions in step 1**
True cathodal transcranial Direct Current Stimulation (c-tDCS) primed intermittent Theta Burst Stimulation (iTBS)
The cathode will be placed over the left DLPFC and the anode over the right orbitofrontal cortex, with a stimulation intensity of 2mA, for 20 min, with 20 s fade-in and fade-out phases. Conductible rubber electrodes inside 0.9 mmol saline wetted 5 × 7cm pouches (EASYpad™, Soterix Medical Inc) will be used for delivering tDCS (Soterix 1x1 CT device with HD-Adaptor, Soterix Medical Inc).

This will be followed within 5 min by T1-weighted MRI guided iTBS delivery to the left DLPFC as triplets of 50 Hz bursts, repeated at 5 Hz; 2 s on and 8 s off; 600 pulses per
session; duration of ~3 min delivered at 120% of resting motor threshold (RMT) as used in a recent study (Blumberger et al., 2018). The stimulation localisation will be based on an anatomical landmark on the subject’s T1 MRI. The anterior boundary of the middle frontal cortex will be identified at the level of appearance of the left olfactory sulcus in the anterior-most coronal slice. The posterior boundary of the middle frontal gyrus will be defined by the precentral gyrus. The junction of anterior 1/3rd and posterior 2/3rd of the middle prefrontal cortex will be considered as left DLPFC for stimulation (Mylius et al., 2013). Three-dimensional T1-weighted MRI based neuronavigation (Brainsight® TMS navigation, Brainbox and Visor®, ANTNeuro) will be used for delivering iTBS. If T1-weighted MRI/neuronavigation is unavailable, BeamF3 software, BA9/BA43 locator version (Beam et al., 2009), that relies on head measurements in computing the F3 location of 10–20 EEG system, will be used for target localisation. This location has high concordance in localising the MRI-directed anatomical localisation (Fitzgerald, 2021).

**Sham c-tDCS primed iTBS**

Sham stimulation uses the same method of administration, but the direct current will be delivered only during the initial ramp-up phase to 2 mA in 20 s, followed by ramping down for the next 20 s. No current will be delivered during the duration of the session. This will be followed by the iTBS treatment as mentioned above.

**Intervention in step 2**

**Electroconvulsive therapy (ECT)**

Right unilateral modified ECTs (d’Elia position on the right side) will be administered thrice weekly using the Nivique ECT device (Model: Niviqure – INT 5, Medtech India Pvt., Ltd., Bengaluru) with two lead EEG monitoring. Brief-pulse square-wave stimulation with a constant current at 800 mA, 75 bidirectional pulses per second with a pulse width of 1 ms will be implemented. The titration method will be used to determine the seizure threshold during the first ECT session. Subsequent ECTs will be administered at six times this threshold. If, in a subsequent session, the participant does not have a seizure, then the charge will be increased by 25–50%.

ECT ( thrice weekly) will be administered for a minimum of six sessions. Subsequently, ECT will be continued at the same frequency unless a decision is made to reduce the frequency of sessions to two per week because of cognitive adverse effects. The ECT course will be terminated if there is no change in the clinical status from the previous session for three consecutive sessions (defined using Sheehan’s Clinical Global Impression-21, SCGI-21) after the sixth ECT. Irrespective of the number of sessions, ECT will be terminated in any of the following situations: (1) complete remission of symptoms; (2) significant cognitive impairment (as decided clinically) despite reducing the frequency to twice weekly; (3) medical conditions precluding continuation of ECT; (4) on request from the participant; (5) if participants develop suicidality (though this may happen very rarely) amounting to HAM-D suicide item > 2, then they will be terminated from the study and their treatment will be discussed with the treating team.

**Adherence to intervention**

All sessions will be provided at the hospital by the team of researchers. The financial burden to the participants will be mitigated by covering the incidental expenses and reasonable compensation for their time and other expenses. Adverse effects will be diligently monitored and any discomfort to participants will be addressed. In case of intolerance, the highest tolerable dose of iTBS below 120% RMT will be administered. The research team will clarify any study-related concerns by being easily accessible (through mobile phone, email etc.). These measures will enhance intervention adherence and study retention.

**Concomitant care**

Deviation from the study guidelines due to unforeseen situations will be documented and reported. A change in antidepressant dose by more than 25% from the dose at the initiation of the intervention or a change in antidepressant or other neuromodulatory interventions will be considered as a dropout. All ongoing antidepressant medication and psychosocial interventions concomitant to the study intervention will be allowed. No new interventions will be initiated during the study.

**Outcome**

The primary outcome will be measured as the change in scores from baseline to the end of 4–6 weeks of the trial. The primary outcome will be the change in HAM-D score at the end of 20th treatment session. A HAMD-17 score reduction of ≥50% from baseline will be considered as a response for prediction analysis and a post-treatment HAMD-17 score of <13 will be considered as remission (Paykel et al., 1999).

Secondary outcome measures include broader clinical symptoms, adverse effects and neurobiological parameters (The detailed protocol of neurobiological parameters will be described in a separate paper). Clinical symptoms will be measured by the Inventory of Depressive Symptomatology and Self-Report (IDS-SR) (Rush et al., 2000), Hamilton Anxiety Rating Scale (HAM-A) (Thompson, 2015), and Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) will be applied at baseline and every week till the end of the intervention. Cognitive and functional abilities will be assessed using the Brief Assessment of Cognition in Schizophrenia (BACS) (Keefe et al., 2004). This tool has been used in earlier transdiagnostic studies in patients with depressive disorders (Huang et al., 2020; Pu et al., 2017). Groningen Social Disabilities Schedule (GSDS) (Wiersma et al., 1988) and Social and Occupational Functioning Assessment Scale (SOFAS) (Rybarczyk, 2011) will be used at baseline and after each step of the intervention.

Safety of the intervention sessions will be other secondary outcome measures: the tDCS-related adverse effects questionnaire (Brunoni et al., 2011; Chhabra et al., 2020), TMS-related adverse effects questionnaire (Giustiniani et al., 2020) and ECT side effect checklist will be used to monitor adverse effects.
after each intervention session. In the case of participants in step-2 of the study, B4ECT-ReCoDe – Battery for ECT Related Cognitive Deficits (Viswanath et al., 2013) will be administered every week till the end of the intervention.

Neurobiological assessments will be performed at baseline and after the end of the RCT. All the clinical (except intervention-specific tools) and neurobiological assessments will be repeated in the same frequency as in the open-label arm of the study (step-2 described below).

Participant timeline (Figure 1)
Step-1: Participants meeting the selection criteria will be enrolled in a sequential neuromodulation treatment study. All treatments will be added on to ongoing pharmacotherapy. In step-1 (randomized controlled trial), participants will be randomized to receive iTBS (sham tDCS-primed or true c-tDCS primed) for 20 days. Participants with an improvement in HAMD-17 score of more than 30% from baseline, but who do not achieve remission (HAMD-17 score <8), will receive 10 more treatment sessions.

Step-2: Non-responders to the intervention in step-1 will be offered open-label modified ECT on alternating days for a minimum of six ECTs and continued till plateauing of SCGI-21 scores in three consecutive ECTs. Written informed consent will be obtained separately prior to the initiation of both steps.

Sample size
The sample size is determined for the primary outcome in step-1 of the sequential study, i.e., change in HAM-D scores with the two RCT arms. The National Institute of Health and Care Excellence has specified a threshold of three points on the HAM-D scale to determine a clinically meaningful difference between active pharmacotherapy and placebo (National Institute of Health and Care Excellence, 2009). Assuming a standard deviation of nine rating points between the two arms (Blumberger et al., 2018), a difference of three points changes on the HAM-D with the tDCS primed iTBS protocol in comparison to sham primed iTBS, yields an effect size of 0.33 magnitude. A sample of 146 in each group would be able to detect an effect of 0.33 with an error probability of 0.05 and a power of 80% on an independent sample t-test for post-treatment scores. Anticipating a dropout rate of ~20%, we aim to recruit 175 participants in each study arm (total n = 350). For comparison studies between patients and healthy controls, a sample size of at least 300 healthy subjects would offer optimal power as per the sample size of previous neurobiological studies in this area.

Allocation
Sequence generation
Consenting patients will be randomly assigned using computer-generated codes to true/sham tDCS. Random allocation of two treatments (active c-tDCS and sham tDCS) for the three study centres with unequal strata sizes of 175, 88 and 87 will be

Figure 1. Depiction of the study procedure. In step-1 of the study, the participants with treatment-resistant depression will be randomized to receive 20 (four weeks) of iTBS primed with active cathodal tDCS or sham tDCS. In participants with a partial clinical response by the end of 20 sessions, interventions will be continued for 10 more days. Non-responders will be offered open-label true-ECT in step-2 of the study. Neuroimaging and neurophysiological tests will be done at the baseline and end of each step. Blood samples will be collected for genetic polymorphism assays at the baseline. SZ: schizophrenia, ECT: electroconvulsive therapy, MRI: magnetic resonance imaging, EEG: electroencephalogram, TMS/tDCS: Transcranial Magnetic Stimulation/Transcranial Direct Current Stimulation, BDNF: brain-derived neurotrophic factor, COMT: catechol-O-methyltransferase.
carried out in Stata v15.1 (Stata, Stata, C; RRID:SCR_012763). This procedure will provide a sequence of treatments (active c-tDCS & Sham tDCS), which will be randomly permuted in blocks of varying sizes and order. An independent statistician will manage the randomisation and allocation concealment. A set of device manufacturer designed random six-digit numeric codes will be used. Entering a predesigned code from this list will deliver the active or sham tDCS as per the parameters described above. These codes will be assigned to the statistician-generated sequential list of randomisation tables by an independent researcher. The allocation sequence generation will be centrally coordinated at NIMHANS.

Concealment mechanism
The person administering tDCS will enter the specific code assigned to the participant for all the subsequent sessions. Sequentially recruited participants will be allotted the codes from the preassigned list of codes at the time of the first intervention session. Neither participant, nor administrator or researcher involved in participant assessment will be aware of the tDCS stimulation type assigned to these codes.

Blinding (masking)
The participants, their caregivers, investigators, treatment administrators, outcome and safety assessors, treating psychiatrists, data managers and data analysts will be blinded to the allocated study arm. To maintain rigorous blinding, assessors will remain independent of administrators.

Emergency unblinding
Serious adverse events (SAE) will be reported to the trial management group (DSMB), which will assess for suspected unexpected serious adverse reaction (SUSAR); the participant, caregiver, clinical team and the investigators may be unblinded at their discretion. Assessment of blinding efficacy will be done on participants and outcome assessors after the last (scheduled/terminated) blinded intervention session using a five-point Likert scale.

In addition to the above discussed screening tools and outcome tools, all participants will be assessed using a comprehensive semi-structured proforma to collect the socio-demographic and clinical details (Sreeraj et al., 2022). Clinical ratings will be performed by mental health professionals. They will be trained in administering the clinical rating scales, and inter-rater reliability assessments will be performed for all outcome measures. Table 1 describes all the clinical measurement tools and Figure 2 depicts the assessment timepoints.

Multi-modal neurophysiological data (MRI, EEG, cortical perturbation using Transcranial Magnetic Stimulation/Transcranial Direct Current Stimulation, heart rate variability) would be acquired before and after interventions to identify predictive factors and understand the mechanistic basis of the ECT outcome. Clinical and neurobiological evaluations will be repeated even after the end of the open-label phase. Also, neuroplasticity gene polymorphisms (Brain-Derived Neurotrophic Factor (BDNF) and catechol-O-methyltransferase (COMT) genes) will be assessed alongside clinical and neurophysiological data to identify the predictors of clinical response. At least 300 subjects matched (as a group) for age, sex, education, handedness, and socio-economic status will be chosen for the comparative analyses of neurobiological studies. They will undergo a one-time baseline assessment of clinical and neurobiological parameters.

Neurobiological investigations
MRI studies
MRI data will be acquired in 3-Tesla scanners (Philips Ingenia 3.0T CX scanner, Philips N.V.). Structural MRI, resting-state functional MRI and Diffusion Tensor Imaging will be acquired as per the established protocol (Parekh et al., 2021). Magnetic resonance spectroscopy (MRS) studies will be done to measure glutamate levels (Fan et al., 2017; Younis et al., 2020) in the left DLPFC.

Cortical reactivity and plasticity: Motor cortical reactivity will be determined by employing single and paired-pulse TMS-EMG investigational approaches to determine the motor threshold, silent period, and intracortical inhibition/facilitation. We will also employ perturbation-based motor cortical plasticity experiments to quantify the magnitude and direction of change in motor evoked potentials with a single session of iTBS/cTBS/c-tDCS. The type of perturbation protocols used will mimic those used in the actual treatment protocols (Mehta et al., 2019).

EEG: Continuous resting-state EEG will be acquired for 20 minutes. Sixteen minutes of eyes-closed resting-state EEG would be acquired in two separate time blocks of 12 min and four min with an intervening four-min duration of an eyes-open EEG. EEG data will be acquired by a 64-channel amplifier with the electrode montage placed based on the international 10/10 system. All data will be referenced against an electrode centred on the midline between Fz and Cz, and sampled at 5 kHz (Barr et al., 2007)

HRV: Fifteen min resting ECG will be obtained to determine the beat-to-beat variability of heart rate, and the multitude of both time (SDNN, RMSSD, PNN50, triangular index) and frequency domain (low and high frequency) measures of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Blood assay
Blood samples will be collected from the ante-cubital vein into K$_3$ EDTA vacutainer tubes (Becton & Dickinson, U.S.A). DNA extraction will be carried out using the commercial spin column method (Qiagen, Inc.). Extracted DNA will be checked for quality assurance and stored at -80°C for later use. PCR amplification for SNP assays of selected functional polymorphic variants of COMT & BDNF (Chhabra et al., 2018) genes will be performed.

Further details of the neurobiological data acquisition protocol will be described in a separate paper.
Table 1. Clinical measurement tools and their timeline in the assessment of participants.

<table>
<thead>
<tr>
<th>Clinical measure</th>
<th>Clinical measurement tool</th>
<th>Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Structured Clinical Interview for DSM-5 disorders, Clinician Version (SCID-CV)</td>
<td>Screening</td>
</tr>
<tr>
<td>Handedness</td>
<td>Edinburgh Handedness Inventory</td>
<td>Screening</td>
</tr>
<tr>
<td>Capacity to consent to participate in the research</td>
<td>University of California, San Diego Brief Assessment of Capacity to Consent (UBACC)</td>
<td>Screening and Pre-step-2</td>
</tr>
<tr>
<td>Safety screening for Transcranial magnetic stimulation (TMS)</td>
<td>TMS Adult Safety Screen (TASS)</td>
<td>Screening</td>
</tr>
<tr>
<td>Safety for magnetic resonance imaging (MRI)</td>
<td>MRI-safety checklist</td>
<td>Screening</td>
</tr>
<tr>
<td>Clinical details</td>
<td>Comprehensive semi-structured proforma</td>
<td>Screening/Baseline</td>
</tr>
<tr>
<td>Personality assessment</td>
<td>Mini International Personality Item Pool (Mini-IPIP)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Response to past antidepressant trials</td>
<td>Antidepressant Treatment History Form-Short Form (ATHF-SF)</td>
<td>Screening/Baseline</td>
</tr>
<tr>
<td>Objective depression rating</td>
<td>Hamilton Depression Rating Scale (HAM-D)</td>
<td>Screening/Baseline and weekly during the trial period</td>
</tr>
<tr>
<td>Subjective depression rating</td>
<td>Inventory for Depressive Symptomatology Self-Rated (IDS-SR)</td>
<td>Screening/Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>Anxiety symptoms rating</td>
<td>Hamilton Anxiety Rating Scale (HAM-A)</td>
<td>Screening/Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>tDCS and TMS side effects</td>
<td>TMS-related adverse effects questionnaire and tDCS-related adverse effect questionnaire</td>
<td>Daily after each session of tDCS and iTBS</td>
</tr>
<tr>
<td>Post-ECT Time-to-Reorientation</td>
<td>5-item time-to-reorientation questionnaire</td>
<td>Before and every 10 minutes post-ECT till 60 minutes/completely reoriented</td>
</tr>
<tr>
<td>ECT-related immediate and delayed adverse effects</td>
<td>ECT-side effect checklist</td>
<td>After each ECT</td>
</tr>
<tr>
<td>ECT-related cognitive deficits</td>
<td>Battery for ECT-Related Cognitive Deficits (B4ECT-ReCoDe)</td>
<td>Baseline and weekly during the trial period (after 24 hours of previous ECT session)</td>
</tr>
<tr>
<td>Neurocognitive assessment</td>
<td>Montreal Cognitive Assessment (MoCA)</td>
<td>Baseline and weekly during the trial period (during step-2 after 24-hours of each ECT session)</td>
</tr>
<tr>
<td>Global clinical severity</td>
<td>Clinician Global Impression-Severity (CGI-S)</td>
<td>Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>Global clinical improvement</td>
<td>Sheehan-Clinical Global Improvement Scale 21 (S-CGI-21)</td>
<td>After 24 hours of each ECT during step-2</td>
</tr>
<tr>
<td>Disability and functioning</td>
<td>Groningen Social Disability Schedule (GSDS)</td>
<td>Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>Functional ability</td>
<td>Social and Occupational Functioning Assessment Scale (SOFAS)</td>
<td>Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>Neurobiological tests</td>
<td>MRI/MRS/EEG/Perturbation studies/HRV</td>
<td>Baseline, post-step-1 and post-step-2</td>
</tr>
<tr>
<td>Genotyping</td>
<td>Blood sampling</td>
<td>Baseline</td>
</tr>
</tbody>
</table>

*Weekly assessments during the course of treatment would be done every week even if the frequency of ECT is reduced to 2/week.

Data management
The multi-level data acquired from the enrolled participants will be quality-checked and anonymised so that subject identifiers are removed. These data will be stored with a coded identification. Different streams of data (e.g. clinical, MRI, EEG etc.) will be linked using codes to facilitate seamless retrieval of different data types.
Figure 2. Gantt chart of the assessment timeline in patients. Grey boxes are scheduled timepoints of respective assessments. *Weekly assessments during the course of treatment will be done every week even if the frequency of ECT is reduced to two per week.

Data analysis plan
Anonymized, coded data will be used for analysis. Analysis will be done in the R (version 4.2.0 or later; RRID:SCR_001905) environment for statistical computing and graphics. The primary outcome will be measured as a change in HAM-D scores from baseline to post-treatment using a two-sample t-test. The last observation carried forward method will be used for handling the missing data of those participants who prematurely terminate the intervention. The percentage of responders between the two groups will be compared using logistic regression with estimates of adjusted odds ratio. Further, a comparison of the change in symptoms scores between the two groups at the end of the interventions will be done through linear mixed effect model analysis. Time to response will be compared between the two groups using survival curves. Adverse effects and dropout rates will also be compared between the two groups. Suitable imputation methods will be used for the assessment of categorical outcome.

The response rate of right unilateral ECT in patients who do not respond to rTMS will be calculated. Further, mechanistic and predictive modelling of the therapeutic responses with these neuromodulation modalities will be performed using the neurobiological data. This will be in addition to the baseline comparison of biomarkers with healthy controls.

Data monitoring

Formal committees
The data safety monitoring board (DSMB), an independent advisory body, assesses data during the course of a study contributing to the scientific and ethical integrity of the study. It will periodically review and evaluate data on clinical efficacy and safety collected during the study and assess reports on cumulated SAE. It consists of a biostatistician, psychiatrist, and ethicist with a specialization in psychiatry. The board, based on the DSMB charter, will provide a recommendation report on the continuation, modification, suspension, or termination of the trial to the study sponsor. The sponsor will submit the report to the ethics committees and trial management group (TMG) for deciding further course of action.

The TMG will comprise a group of investigators from the three collaborating institutes. It will monitor the day-to-day execution of the trial. It will take cognizance of trial participant dropout in the event of withdrawal of consent, non-compliance with study guidelines, and worsening of symptoms. It will interact with the DSMB and IEC. It will review the reports and recommendations of these oversight/monitoring committees and plan appropriate actions. It will decide on trial terminations and any trial modifications of the study procedures. It will also oversee the submission of modification, premature termination (if any),
and final (completed) trial summary report to the IEC, trial registries, and funding agency.

The data management committee (DMC) comprises the principal investigators of the three institutes. It will be dedicated to reviewing the data archival, management, and sharing. It will execute the periodic data auditing and audit of the research practices related to the studies. It will ensure anonymization and de-identification of data. It will monitor the storage of data in the encrypted data storage servers. It will oversee the sharing of de-identified data upon reasonable request from collaborators or the research community. Public access will be granted as per the policy of the DBT/Wellcome Trust India Alliance and international good research practices in strict compliance with the ethics guidelines for biomedical research in India.

Safety and harm
At no point in the study, will the participants be exposed solely to placebo treatment. That is to say, both groups will receive an active evidence-based treatment during step-1 and the step-2 would entail treatment with ECT in an open-label fashion. Safety considerations have been thoroughly evaluated and a scientific rationale for the stimulation parameters within safety norms has been proposed. Also, during the course of any of these interventions (tDCS and iTBS) both the subject and the doctor can talk to each other. Very rarely, some people may feel uncomfortable. In case, if the subject develops any significant discomfort during any of the assessments/interventions, the procedure will be immediately aborted.

Intermittent theta burst stimulation (iTBS)
Intermittent theta-burst stimulation is a safe and well-tolerated treatment as evidenced by recent large clinical trials (Blumberger et al., 2018). When administered within existing safe delivery practices (Blumberger et al., 2018; Oberman et al., 2011; Li et al., 2014) and guidelines (Rossi et al., 2021; Rossi et al., 2009), the risk of minor (scalp discomfort) and major (seizures) adverse events is considered less than minimal. In this study, we will be using the stimulation parameters whose safety has been established in an earlier study by Blumberger et al. (2018). Participants will be screened using TMS Adult Safety Screen (TASS) (Keel et al., 2001) before recruitment and TMS related adverse effects will be monitored after each session using a questionnaire (Giustiniani et al., 2020).

Transcranial Direct Current Stimulation (tDCS)
Over the past years, as a part of earlier ongoing research studies that have been approved by the NIMHANS ethics committee, tDCS has been administered to patients with schizophrenia as well as patients with several other psychiatric diagnoses (e.g. OCD) (about 150+ patients have received tDCS over the past years with the total number of patient sessions being more than 2000) using standard equipment as per established guidelines with stringent safety measures with evaluation using a standard check after each session. These sessions were well tolerated and there were no significant adverse events (Chhabra et al., 2020). This is in tune with a systematic review that has examined data from about 209 studies and has concluded that tDCS is a safe technique (Salehinejad et al., 2021).

Electroconvulsive therapy (ECT)
ECT will be administered in the ECT suite by a team of trained psychiatrists, anaesthetists and paramedical staff. Standard anaesthetic procedures will be followed for the participants receiving ECT. These will be tailored to individual patients as determined by the attending anaesthesiologist. All the participants will be assessed after each ECT session using the Sheehan-Clinical Global Improvement Scale (S-CGI) for monitoring clinical changes. Patients will be closely monitored for any adverse effects using checklists and tests as described previously.

SAE will be reported to the trial management group (TMG) and DSMB, which will assess for suspected unexpected serious adverse reaction (SUSAR). In an unlikely event of a harm attributable to participation in research procedures, financial compensation will be provided as per the provisions of the National Ethical Guidelines for Biomedical and Health Research involving Human Participants by the Indian Council of Medical Research (ICMR) (ICMR, 2017).

Ancillary care and post-trial care
In the event of withdrawal of consent, non-compliance with study guidelines, and worsening of symptoms the concerned participant will be dropped from the trial. The best-practiced standard care at the respective institutes will be provided by the treating teams of the patients even after the trial.

Auditing
Periodic auditing of data and the research practices will be conducted by the trial management group and data management committee. They will ensure the quality of research and patient advocacy.

Ethics
The research protocol will be implemented in strict adherence to the National Ethical Guidelines for Biomedical and Health Research involving Human Participants (2017) by the Indian Council of Medical Research (ICMR) (ICMR, 2017). The team of investigators has rich experience in all the research procedures. We will implement all the research procedures taking utmost care to avoid any injury. In the unlikely event that the research subject suffers any injury attributable to participation in the research procedures of this project, the subject will be compensated financially as per the National Ethical Guidelines for Biomedical and Health Research involving Human Participants by the Indian Council of Medical Research. Required insurance coverage will be obtained using the research funds from the funding agency, DBT/Wellcome Trust India Alliance. The funding agency is in alignment with this policy of insurance coverage. The subject is free to withdraw at any time during the study without giving a reason. A decision to withdraw or not to take part, if the subject is a patient, will not affect in any way routine medical care provided at NIMHANS. All the tests and procedures carried out for the
Research purpose will be free of charge. The confidentiality of research data is ensured. In the case of data sharing, only de-identified data will be used as per the regulatory guidelines & international standards of practice. None of the researchers/ investigators affiliated with this project or clinical research centre has any financial or non-financial (personal, academic or political) conflict of interest to declare.

**Ethics approval:** Requisite clearances have been obtained from the ethics committees of all three institutes as well as from an ethics committee not affiliated with any of the three institutes (MS Ramaiah Medical College Ethics Committee). (IEC approval numbers: No.IEC/CIP/2020-21/337 dated 22/05/2021; NIMHANS/EC (BEH.SC.DIV) 23rd MEETING/2019-20 dated 07/04/2020; IEC:109/2020 dated 12/02/2020 and MSRM/ EC/AP-09/06-2020). The trial is registered in the Clinical Trial Registry of India (CTRI/2021/05/033784).

**Protocol modification:** Upon interim analysis or during the course of the study, any modification made to the study protocol will be reflected in the trial registries (CTRI & NIH), and would be intimated to the Institute Ethics Committee, India Alliance CPH Committee (Funding Agency) and Wellcome Open Research (where the study protocol has been published).

**Informed consent:** Trained health professionals will obtain written informed consent from the participants (Sreeraj et al., 2022). Participants will be assessed for their capacity to consent to the study, based on capacity to consent to research studies as per the assessment using the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC) (Jeste et al., 2007). Only such patients who can understand the implications of taking part in the trial will be recruited. Family members will be involved in the consenting process, but the final decision about participating in the study will be made by the participant him/herself. Written informed consent is again sought before initiating the ECT.

Additional written informed consent for using participant data for advanced analysis, future research studies and sharing of coded and de-identified data with interested national and international researchers/collaborators following the regulatory guidelines will be taken.

**Access to data**
The centralized data repository will be created and maintained at NIMHANS and it will archive de-identified and coded data from all three institutes. The data management committee of investigators of the three institutes will review data archival, management and sharing.

**Dissemination policy**
A clinical trial summary report will be prepared and provided to the institute ethics committees and trial registries within 12 months of the completion of the study. A final (completed) trial summary report will be submitted to the funding agency for perusal. De-identified data will be shared with the research community and public access will be granted as per the policy of DBT/Wellcome Trust India Alliance and international good research practices in strict compliance with the ethics guidelines for biomedical research in India. Encrypted data storage servers will host the multi-modal clinical and neurobiological data, including genetic data of all participants. The findings of the research will be disseminated primarily through publication in peer-reviewed journals. Research data may be presented at regional and international scientific fora. The study results and interpretation as a report will be shared with all major stakeholders. The team will engage in regular interaction with all stakeholders (psychiatrists in the community, patients and caregivers, policymakers, regulatory bodies, other psychiatry institutes and industry) for the dissemination of knowledge gathered from the trial.

Publications from the trial will acknowledge that the work has been carried out by all participating institutes (NIMHANS, CIP & KMC). The authorship will be in strict adherence to ethical and research publication guidelines (Resnik et al., 2016). There is no intention of using professional writers in any of these works.

**Study status**
The study is currently open to recruitment.

**Discussion**
Depressive disorder is one of the commonest debilitating psychiatric disorders with nearly 15% of the general population being affected annually. Antidepressants that are first-line agents do not exert therapeutic response in nearly 30–40% of these patients. Treatment resistance is often coupled with socio-occupational dysfunctions, increased mortality due to comorbid physical illnesses, increased mortality due to suicidality as well as increased utilization of health care resources. Depression disorders with poor or only partial response to two adequate antidepressant trials have been called treatment-resistant depression. Generally, such situations warrant combination pharmacotherapy or augmentation with other treatment modalities. A small subset of patients with depressive disorders might not tolerate the antidepressants at adequate doses and could benefit from non-pharmacological interventions.

Non-invasive neuromodulation techniques including rTMS and tDCS are emerging as therapeutic interventions filling this arena. Generally used as add-on treatments, these non-invasive brain stimulation (NIBS) techniques offer complementary mechanisms of action in bringing robust and rapid therapeutic response. The core therapeutic mechanism in depression is the modulation of the synaptic plasticity through the repeated generation of action potentials or altering the resting membrane potentials by rTMS and tDCS, respectively. These mechanisms can further be used in augmenting the effect of others when used in combination with temporal precision and optimal parameters to induce metabolicity. The addition of ECT in step-2 of the study will help in determining the role of ECT in treatment-resistant patients not responding to tTBS (Bennabi et al., 2019; Sackeim, 2017). Not only does this study evaluate the differential efficacy of tDCS primed tTBS compared with clinically proven regular...
iTBS, but the neurobiological evaluation in the study will also attempt to demonstrate the differential mechanisms behind them and perhaps the differential predictive factors in choosing a treatment (rTMS vs ECT) and individualising protocols (primed iTBS vs conventional iTBS, as well as the duration of treatment).

Although iTBS has been used in the treatment of depression it has been shown to be heterogeneous in response. This is due to multiple factors including the varied targets and failure to achieve optimal electrical field even when a uniform site is targeted on the cortical surface. Given the emerging safety data for rTMS, it has been shown that more pulses within a day at a higher intensity have better efficacy. Thus, the current protocol maintains homogeneity in the target using anatomical localisation and neuronavigation wherever feasible. The dose of 120% RMT for 600 pulses provides an equally optimal opportunity for all individuals to receive optimal intensity at the targeted cortical surface.

The study has an ecologically valid design where the duration of the trial is kept flexible. Patients will receive intervention for 20 sessions followed by a further extension for another 10 sessions in case of partial response. Thereby, the unnecessary exposure of stimulation to patients who promptly respond to the intervention and those who do not respond can be minimised. Similarly, the number and frequency of ECT sessions in step-2 will be determined by clinical factors such as clinical response and adverse effects to keep it non-deviant from regular clinical scenarios.

The current study will have certain limitations owing to the application of conventional tDCS with relatively limited focality and lack of neuronavigation in one of the study sites. However, given the cost-effectiveness and ease of administration of conventional tDCS in comparison to high definition tDCS, the study will have higher generalisability and higher translation value for wider clinical applications.

Conclusions
This experiment will shed light on the potential of augmenting iTBS with cathodal tDCS in the treatment of resistant depression. The systematic prospective evaluation of treatment response, assessments of neurobiological parameters, and the transition of non-responders to treatment with ECT will contribute to furthering the personalization of NIBS in depressive disorders.

Data availability
Underlying data
No data are associated with this article.

Extended data
Open Science Framework: Semi-structured proforma for ‘Study protocol for evaluating the clinical efficacy and neurobiological correlates of sequential treatment with tDCS primed iTBS and ECT in treatment-resistant depression’. https://doi.org/10.17605/OSF.IO/34KC6 (Sreeraj et al., 2022)

This project contains the following extended data:
- Supplementary file 1: 03-Consent-Project-03.pdf (Informed consent form for patients)
- Supplementary file 2: Semi-structured proforma.pdf

Reporting guidelines
Open Science Framework: SPIRIT checklist for ‘Study protocol for evaluating the clinical efficacy and neurobiological correlates of sequential treatment with tDCS primed iTBS and ECT in treatment-resistant depression’. https://doi.org/10.17605/OSF.IO/34KC6 (Sreeraj et al., 2022)

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

References


Chhabra H, Shivakumar V, Subbanna M, et al.: Gene polymorphisms and...
ICMR: National Ethical Guidelines for Biomedical and Health Research involving human participants. 2017. Reference Source
Sreeja VS, Sinha P, Umesh S, et al.: Study protocol for evaluating the clinical

Wellcome Open Research 2022, 7:242 Last updated: 11 OCT 2022 Page 14 of 17
efficacy and neurobiological correlates of sequential treatment with tDCS primed iTBS and ECT in treatment-resistant depression. Open Science Framework. (Dataset). 2022.  
http://www.doi.org/10.17605/OSF.IO/34KC6  
PubMed Abstract | Publisher Full Text  
PubMed Abstract | Publisher Full Text  
PubMed Abstract | Publisher Full Text  
PubMed Abstract  
PubMed Abstract | Publisher Full Text | Free Full Text
Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 10 October 2022

https://doi.org/10.21956/wellcomeopenres.20168.r52600

© 2022 Tendolkar I. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Indira Tendolkar

Department of Psychiatry, Radboud University Medical Center, Nijmegen, The Netherlands

I think this is a very relevant study but in the first chapter of the introduction it lacks current literature on profiles that may in particular respond to ECT but potentially not to rTMS and that is psychotic depression, for example. The picture of treatment resistant depression is not homogenous and it is important to understand for which patients other forms of NIBS that ECT might be beneficial. I do see that the authors exclude psychotic symptoms, which I fully support, yet this is a condition you have to name.

Likewise in explaining why the tDCS priming may be relevant, it could be important to include this article, Alkhasli et al. (2022)\(^1\).

Otherwise the methods are explained clearly except that the candidate neuroplasticity biomarkers fall a bit out of the blue, but I do not consider this a big burden for the patients.

References

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Clinical neuroscience in psychiatry with a focus on brain stimulation of TRD

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.