Utilizing Changes in Human Brain Connectivity to Establish a Dose-Response Relationship Involved in the Therapeutic Actions of Prefrontal Brain Stimulation on Depression Symptoms

Protocol Number: 54909

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Principal Investigator: Nolan Williams, MD

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Summary of Changes from Previous Version (Version 3.4):

Affected Section(s)	Summary of Revisions Made	Rationale
-	Table of Contents page numbers	Organization
1.3	Removed HDRS-17 from Screening Visit. Visit Names. Removal of NTF for screening sharing	Update
2.3.1	Referenced 8.3.3.3 for all AEs	Clarification
4.1	Added clarification around goal of treatment and explanation around depth corrections and 120% RMT limit	Clarification
4.3	120% RMT limit clarification added.	Clarification
5.1 - 5.2	Visit naming. Moved timepoint table for eligibility criteria	Clarification
5.4	Visit naming	Clarification
5.5	Removed Mandarin from recruitment materials	Clarification
6.1.2	90 - 120% RMT limit clarification added	Clarification
6.3	added link to recent RCT	
7.2	Clarification that subjects may withdraw at any time	Clarification

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8.1	**Updated screening process to reflect current practices. Removed HDRS 17. Re-named visits.**	**Clarification**
8.2	**Added emergency escalation language**	**Update**
8.3.3.3	**Compiled list of AEs**	**Clarification**
8.3.5	**Updated frequency to bi-annual DSMB**	**Update**
9 - 9.4.5	**Clarified language**	**Clarification**
9.4.9	**Updated exploratory analyses**	**Update**
10.1.1.2	**Clarified plan for review of consent documents**	**Clarification**
10.1.5	**Updated study psychiatrist contact info. Removed outdated advisory committee info.**	**Update**
10.1.7	**Clarified Dr. Spiegel is medical monitor.**	**Clarification**
10.1.8	**Referenced Quality Management SOP**	**Update**
10.1.9.1	**Added documentation of blood draws**	**Update**
10.3	**Removed GRID/HAMD17**	**Update**
10.4	**Added updates from Protocol 3.4**	**Update**
11.0	**Added citations**	**Update**

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

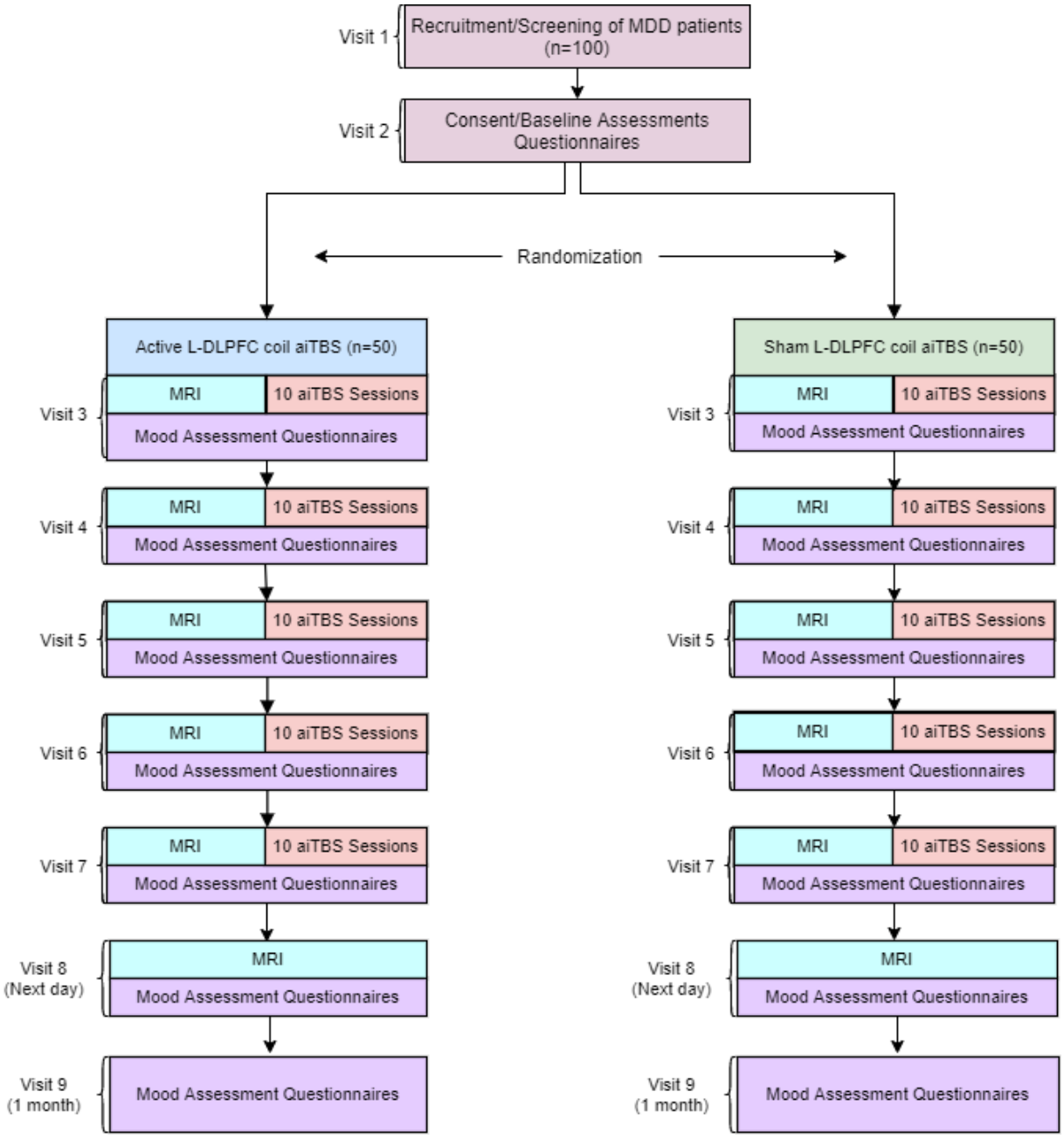
1.1 SYNOPSIS

Title:	Utilizing Changes in Human Brain Connectivity to Establish a Dose-Response Relationship Involved in the Therapeutic Actions of Prefrontal Brain Stimulation on Depression Symptoms
Study Description:	This double-blind, randomized, sham-controlled mechanistic study aims to establish a resting-state functional connectivity (RS FC) dose-response curve associated with the therapeutic actions of our accelerated intermittent theta-burst stimulation (aiTBS) neuromodulation approach in outpatients diagnosed with Major Depressive Disorder (MDD) as defined by DSM-5 criteria. We will accomplish this by applying the aiTBS protocol (10 daily interventions of aiTBS to a customized target within the left dorsolateral prefrontal cortex identified with fMRI for five consecutive days) and measuring RS FC between the subgenual anterior cingulate cortex (sgACC) and the default mode network (DMN) after each day of intervention. We then will determine the relationship between changes in the RS FC and both changes in clinical symptoms of depression (24 hour version of the Montgomery-Asberg Depression Rating Scale-Self Report [MADRS-S]) and acute mood state (Immediate Mood Scaler [IMS-12]). Our mechanistic hypothesis is that the nature of the improvement in mood will be linear, related to session number (accumulated pulse dose), and correlated with degree of change in RS FC between the sgACC and DMN.
Objectives:	Primary Objective:

Endpoints:	The primary objective of this investigation is to establish a resting state functional connectivity (RS FC) dose-response curve of active versus sham aiTBS. Secondary Objectives: (a) To determine the relationship between active versus sham aiTBS-induced clinical improvement (as measured by reduction of the MADRS-S) and changes in RS FC between the sgACC and DMN. (b) To determine the relationship between acute mood state (as measured by the IMS-12) and changes in RS FC between the sgACC and DMN in those receiving active versus sham aiTBS. Primary Endpoint: Change in RS FC between the sgACC and DMN over 5-days of active aiTBS as compared to sham aiTBS. Secondary Endpoints: (a) Relationship between clinical improvement (measured by the MADRS-S) and RS FC between the sgACC and DMN. (b) Relationship between acute mood state (measured by the IMS-12) and RS FC between the sgACC and DMN.
Study Population:	We will enroll 100 participants and employ a two-arm design with 50 subjects per arm. The target population is adults of all genders and ethnicities who are between 22 and 65 years of age with treatment-resistant major depressive disorder and who are otherwise in good general health. Participants must be without contraindications to Magnetic Resonance Imaging (MRI) or transcranial magnetic stimulation (TMS) and must be able to attend all study visits.
Phase:	This is a mechanistic study similar to a phase 2 trial.
Description of Sites/Facilities Enrolling Participants:	**1. The Stanford Department of Psychiatry and Behavioral Sciences:** This study will be conducted within the Stanford Department of Psychiatry and Behavioral Sciences outpatient building. The outpatient building will provide both the office space and infrastructure for the screening / enrollment of participants, the delivery of study intervention, and the assessment of clinical outcomes. **2. The Stanford Center for Cognitive and Neurobiological Imaging:** The Stanford Center for Cognitive and Neurobiological Imaging (CNI) provides a research-dedicated 3T MRI scanner, a GE Ultra-High Performance MR system that will be used with a Nova Medical 32-channel head and neck coil. Improvements in fMRI technology are implemented from time to time at

	major academic research environments. Fidelity to sequences used at the beginning of the study will be maintained to ensure that changes in signal-to-noise ratio do not take place during the course of the study.
Description of Study Intervention:	We will deliver both active and sham study intervention via a MagPro X100 (MagVenture, Skovlunde, Denmark) TMS device equipped with a Cool-B65 A/P coil. The intervention paradigm consists of 10 daily sessions (50 total over 5 days) of intermittent theta-burst (iTBS) stimulation (3-pulse 50-Hz bursts at 5-Hz for 2-second trains, with trains every 10 seconds), delivered with 50-minute inter-session intervals (10-minute sessions, 50-minutes in between sessions). Stimulation will be delivered at 90% of the resting motor threshold (with depth correction to account for the distance between the scalp and cortex). An operator entered code (derived from randomization) will instruct the device to deliver active or sham magnetic stimulation. Custom MATLAB scripts will be used to identify the portion of the left DLPFC maximally anticorrelated with the sgACC, which will serve as the target for both active and sham stimulation. The intervention target will be located via a Localite TMS Navigator (Localite, Bonn, Germany). More specifically, the left dorsolateral prefrontal cortex is located according to routine, FDA-approved clinical rTMS practice. Our scripts then further discern functional subregions within the left DLPFC.
Study Duration:	The entire project is estimated to take approximately 60 months, broken down into 48 months of data collection and an additional 12 months of analysis.
Participant Duration:	The total duration for each participant from the time of baseline assessment to study completion is approximately 6-weeks.

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

Assessment	Visit 1 - Remote Screening*X	Visit 2 - In-person Screening 2 / Baseline*	Visit 3-7 MRIs & Intervention	Visit 8 Next day	Visit 9 1 Month Follow Up*
Screening Informed Consent	x				
Demographics	x				
MRI Safety Screening Form	x		x		
TASS[1]	x				
Medical History	x				
ATHF-Current[2]	x				
ATHF-Lifetime	x				
Current Medications	x	x	x	x	x
Study Informed Consent		x[a]			
Capacity to Consent		x[a]			
Urine Drug Screen		x[a]	x	x	
Urine Pregnancy Test		x[a]			
Inclusion/ Exclusion Criteria	x	x			
Enrollment/ Randomization		x			
COVID19 screener		x	x	x	
Stimulation log			x		

Motor threshold		x[a]	x		
Adverse Events			x	x	x
SCID Overview	x				
MINI	x				
BPD diagnostic module from SCID for DSM-5[Ψ]	x				
MSM[3]	x				
MSSI[#4]	x	x[a]			
Columbia-Suicide Screening Rating Scale (C-SSRS)	x				
MADRS-S	x	x[a]	x (Visit 3)		x
MADRS-S (24-h version)			x (Visits 4-7)	x	
YMRS	x	x[a]	x		
MADRS-CR	x				
IMS-12[5]		x	x	x	
Pittsburgh Sleep Quality Index (PSQI)	x	x			
FIGS^		x			
CTQ^		x			
MRI Scan			x	x	
Blood Draw^		x		x	x

[*]Indicates a participant encounter that occurs remotely via video call and/or online data capture.
[Ψ]If a participant meets the screening threshold on the self-reported BPD prescreening questions from SCID for DSM-5, then the diagnostic module will be performed
[#]The MSSI will be administered at screening and baseline and then as part of any clinical consultation with the study psychiatrist or their designee at any other study visit if a participant scores a 3 on item 9

of the MADRS-S ('Zest for life' item).
^Only applicable if participants opts into optional additional blood marker substudy
[X]- Subjects who have provided screening data for another study in Stanford University's Brain Stimulation Laboratory (BSL) may have their screening data used for this study. Additionally, screening data used in this study may be used in another study in BSL. The purpose of this is to reduce unnecessary burden to the subject. The discretion of which screening data is accepted is up to the discretion of the investigator. Data will be manually re-entered within BSL's RedCap system from the previous study into the new study.
[a]Refers to measures required to confirm study eligibility that occur at the In-person Screening 2 / Baseline (Visit 2).
*Study activities associated with Visits 1, 2, and 9 may or may not occur on the same day.

2 INTRODUCTION

2.1 STUDY RATIONALE

The Challenge (Clinical): Treatment-Resistant Depression (TRD) is an escalating problem in the US. Major depressive disorder (MDD) is the leading cause of disability worldwide[6,7] with lifetime prevalence in the US >20%[8]. Combating TRD will require improvements in treatments; however, treatment development is limited by insufficient understanding of the mechanism of disease remission. Discovering the network-level mechanisms of remission has been particularly challenging due to treatments requiring weeks-to-months to induce remission[9], limited effectiveness of available treatments[10,11], and potential disease heterogeneity[12,13].

Depression as a Condition of Dysfunctional Emotion Regulation Networks: Some of the earliest MDD neuroimaging studies reported reduced activity in the left dorsolateral prefrontal cortex (L-DLPFC) of patients with MDD[14–16], which is restored with effective treatment[17–20]. This early data suggested a major role of top-down dysfunction in the pathophysiology of depression[21,22]. Similarly, concomitant increases in activity in subgenual anterior cingulate cortex (sgACC) long have been observed in patients during depressive episodes[23], and this has been hypothesized to be causally related to reductions in L-DLPFC activity[24]. This hypothesis has been reinforced by observations that antidepressants lead to reductions in sgACC activity[17,18,25–30]. This led to the conception of depression as dysregulation in corticolimbic systems[22]. In recent years, this conceptualization has evolved to include neural networks and their interactions. Several lines of evidence suggest dysfunction of emotion regulation (ER) in MDD[31] with neural correlates observed with fMRI[32,33]. The neural basis of ER includes emotional reactivity: amygdala and striatum; explicit control of emotion – central executive network (CEN): DLPFC, ventrolateral PFC (vlPFC); and implicit ER – default mode network (DMN), salience network (SN) and insula. Dysfunction within the DMN, CEN, and SN has repeatedly been reported in MDD[34–38].

Depression as a Condition of Dysfunctional *Connectivity within and between* Emotion Regulation Networks: Numerous resting-state functional connectivity (RS FC) MRI studies have demonstrated that individuals with MDD have altered within network and cross network connectivity[39,40]. The hyper activity/connectivity within DMN has been considered as evidence of dysfunction of rumination and self-

referential processing, and treatment is hypothesized to normalize DMN activity/connectivity[36,41–44].Depression is associated with increased fc between DMN and sgACC[34,35,39,45–49]. The degree of treatment-resistance has been correlated with the degree of sgACC-DMN connectivity where higher connectivity is associated with more treatment resistance[34]. Depression is also associated with increased fc between DMN and amygdala[50–54]; DLPFC and amygdala[55]; and, PFC and striatum[56–59]. Increased DLPFC-striatum fc correlates with increased depression severity and fc between DLPFC-striatum connectivity decreases following treatment[56]. There are also alterations in salience network (SN) fc in MDD and more specifically insular fc with multiple relevant nodes[39,60,61]. Insula has a well-known role in interoception[62,63], and insula activity is correlated with abnormal interoception in MDD[64]. Increased SN fc has been reported to be associated with decreased anticipated pleasure[65] and MDD[66]. Individuals with depression have reduced fc between amygdala-precuneus[67] and DLPFC-insula[68].

Excitatory repetitive transcranial magnetic stimulation (rTMS) applied to the L-DLPFC is a therapeutic non-invasive brain stimulation technique for TRD: Excitatory rTMS (≧5 pulses/second) increases cortical excitability over a cortical target[69,70]. Following the emotional dysregulation hypothesis of depression, stimulation of the CEN at the L-DLPFC node via rTMS[1–3] has been hypothesized to produce antidepressant effects via enhanced regulation of emotion. This is in line with the observation that increased activity in CEN results in suppression of DMN[71], and this has led others to hypothesize that improved accuracy of targeting these networks might produce better treatment efficacy[72–77]. This fits well with the hypothesis that L-DLPFC has reduced activity in depression, preventing suppression of sgACC activity, and that restoring L-DLPFC activity with treatment inhibits sgACC activity. When applied to the L-DLPFC, this approach was originally conceived to normalize hypoactivity in L-DLPFC[78], reduce sgACC hyperactivity and strengthen indirect inhibitory connections between the L-DLPFC and sgACC. For some patients, rTMS to L-DLPFC has been demonstrated to be an effective and durable[79] antidepressant treatment which has been FDA-approved for TRD[80]. Traditional rTMS produces its antidepressant effect during the later part of a treatment course which involves 90,000 pulses applied at 3000 pulse sessions five days per week for 6 weeks. Remission rates from depression with conventional rTMS remain low at 35%[81,82].

Properties of rTMS Dosing:

Session Dosage and Total Dosage: Total dose of stimulation appears to be important for rTMS therapeutic effects[83]. 60,000-90,000 pulses are used for the 6-week standard high frequency rTMS course[81,82]. In cases where higher pulse dosages have been used, there is evidence of greater efficacy[84,85]. There also is evidence that there are a number of responder trajectories that are related to the level of TRD[86]. In the case of the FDA-approved iTBS protocol[87], 600 pulses are applied during each session such that 18,000 pulses are delivered over a six-week treatment course. While there had been a question as to whether >600 pulses per session might result in a reversal in the cortical excitability of a prefrontal target[88], there is now evidence in depressed[89,90] and normal healthy controls that 1800 pulses of iTBS per session ($iTBS_{1800}$) produces long-lasting effects in the intended direction[91]. In fact, $iTBS_{1800}$ is the only pulse dose that has yielded a positive result in a blinded TBS trial[92–95] and that has been shown to produce the intended resting-state effects[96]. Additionally, the 1800 pulse dose has been shown to produce long-lasting changes in cortical excitability[97] and resting-state functional connectivity[96] .

Stimulation Intensity Adjustments for Differential Prefrontal Cortical Atrophy and Induced E-Field: Intermittent theta-burst stimulation applied <100% rMT produces the optimal change in prefrontal cortical excitability[98] and resting state changes[99,100] when compared to suprathreshold stimulation. This convergent data suggests that unlike traditional rTMS[101], there appears to be an inverse U-shaped curve with TBS intensities requiring an optimal intensity directly over a target. This finding would explain why,

of the blinded iTBS trials for TRD, the only positive trials were found in those studies utilizing subthreshold %MT[93], while those trials utilizing >100% %MT did not separate from sham[95].

Furthermore, with age there is differential atrophy of PFC and PMC[102]. Depth correction[103] historically has been applied to the resting motor threshold to adjust for differences in the cortical depth of the individual's functional target compared to the primary motor cortex in order to consistently achieve the intended rMT at the target. In the OPT TMS trial it was determined that a standard %MT could be used (120% avg of depth adjustment), but with traditional HF rTMS[104] there is no inverted U-shaped curve[101] like there may be with iTBS[98,100]. Accordingly, it is very unlikely that an average multiplier can be used with iTBS given its principles.

Patterning:

Pattern of Stimulation Session (Intermittent Theta-Burst Stimulation (iTBS)): Theta-burst stimulation (TBS), a patterned form of rTMS modeled after endogenous hippocampal discharge patterns[105,106], has been shown to be more efficient than standard rTMS in modulating cortical excitability[107] and last ~60 min[108]. Recent clinical trials indicate non-inferiority of 600 pulses/day of iTBS given over 3 min versus 3,000 pulses/day of conventional rTMS given over 37 min in treating depression (now FDA-approved)[80] and notable efficacy in treating TRD[90]. What is unknown is the ideal timing of when each session should be applied, and currently 24 hours is the default[109]. It is unclear if this approach is ideal[110].

Pattern of stimulation course (Intersession interval (ISI)): An accumulation of TBS effects can occur when TBS stimulation is applied in a neurobiologically-informed, patterned manner (compared to en-masse stimulation)[111] allowing for a dose-response curve to be constructed[112]. Previous work has demonstrated that iTBS sessions delivered to hippocampal slices with inter-session intervals of 50-90 min have a cumulative effect on dendritic spine enlargement, a process involved in synaptic strengthening. In comparison, iTBS sessions delivered with inter-session intervals of ≦40 min do not have a cumulative effect on dendritic spines[113–115]. Two iTBS sessions delivered to the motor cortex[110,116] or prefrontal cortex[117] with a 15-minute inter-session interval have been shown not to result in an incremental increase in cortical excitability over a single iTBS session, while two applications of iTBS with a 30-min ISI does result in an increase in cortical excitability[110,116]. When 1800 pulses of iTBS is applied at a subthreshold %MT at the 15 min post time point, there is an initial increase in fc between ACC and DMN (in the pathological direction). At the 30 min time point, there is a decrease in fc between ACC and DMN and these changes are maximal at the 50 min time-point. Interestingly, Nettekoven et al. similarly reported that the 30 min time point does result in changes in RS FC[110,116], but the 50 min time point is optimal for engaging the entire mood regulatory network. This could explain the limited efficacy of a previously reported accelerated iTBS protocol that used an inter-session interval of only 15 min[118,119]. This work provides further support for an ISI that is ≧50 min[96]. Studies that have applied TBS to cortical targets have shown that two spaced stimulation sessions produce greater[110,120,121] and longer lasting[121,122] changes in cortical excitability than single sessions. This finding holds for other cortical targets in diseased[123] and normal subjects[124]. Recent attempts at this have demonstrated that multiple sessions of iTBS per day increase efficacy compared to single applications/day both clinically[125] as well as physiologically[105].

Network-Level Targets and Stimulation Effects:

Effective Treatment Requires Precise Targeting: Neuroimaging data suggest the higher the anti-correlation between the stimulated region of the L-DLPFC & sgACC, the better the clinical outcome[77]. This finding has since been confirmed in other cohorts[72,77]. A recent TMS-fMRI study used resting-state fMRI to target the region of the L-DLPFC which showed greatest fc with the sgACC and showed stimulation propagated to the sgACC in all participants[126]. In comparison, another study which targeted L-DLPFC using

anatomical MRI coordinates (the border of BA9 & BA46), stimulation propagated to the sgACC in only 44% of participants[127]. These data suggest that using resting-state fMRI to identify and stimulate the region of the L-DLPFC which is most anti-correlated with the sgACC in each individual could increase the efficacy of TMS protocols[96,128] and may explain why attempts at accelerated iTBS (without fc targeting) may have not have demonstrated efficacy from sham[96,118,119].

Prestimulation Connectivity is Predictive of Post-Stimulation Efficacy: The effects of rTMS/iTBS on cortical excitability are linked to its effects on resting state functional connectivity[110,116]. Past studies have found that fc between stimulation site L-DLPFC & striatum[129,130] and between downstream targets (ACC/VMPFC, dACC & l-insula)[131,132,132] predicts TMS efficacy, and several groups have reported that fc between stimulation site & sgACC that is more negative results in greater reductions in depressive symptoms with rTMS[72,74,77].

rTMS/TBS is a Functional Connectivity (FC) Modulator: Stimulation of L-DLPFC can result in fc changes. rTMS to L-DLPFC reduces fc between DMN & striatum in normal healthy controls (NHCs)[128,133]. Improvement in depression is correlated with more reduction in L-DLPFC-striatum fc after therapeutic L-DLPFC rTMS[134,135]. Excitatory rTMS to L-DLPFC also has been shown to modulate sgACC activity/fc[41,42,133] and reduce fc between sgACC & DMN when targeted to the anticorrelated fc[91] and increase fc between DLPFC & sgACC when no fc targeting is used[136] in NHCs. iTBS to L-DLPFC reduces fc/effective connectivity (ec) between L-DLPFC & r-insula[137,138] with baseline fc/ec between L-DLPFC & r-insula predicting degree of network changes[137,138]. iTBS/rTMS to L-DLPFC potentiates upstream neural circuits[77] to normalize sgACC-DMN connectivity[36,43,44,76,139]. Prior studies have reported significant reductions in fc between sgACC & 1-2 nodes of DMN[36,42–44,140] as well as FPN fc[139] after active but not sham rTMS/iTBS[139].

Properties Required for iTBS-induced Network-Level Changes:
First, the intensity in which iTBS is applied affects its modulation of fc[98,100]. Second, the network configuration (amount of pre-stimulation fc between target pairs) of the pre-stimulation targeted network is associated with post-stimulation efficacy[120,132,138,141–143]. Third, when applying multiple stimulation applications, the ISI must be sufficient for changes in fc to occur[99,144] or there is no incremental change[98,144]. Fourth, in order to achieve consistent target engagement, the cortical & downstream target must be identified and targeted[126,145,146] or the stimulation will not be consistently distributed into the downstream target[127]. Fifth, the position of the rTMS coil must be superimposed over the cortical target[147,148] to exert intended effects. Sixth, the intensity of the stimulation must be adjusted to differential depth of prefrontal target[103] as under/over stimulation results in no change[98,100].

The Challenge (Methodological):
Our lab's accelerated intermittent theta-burst stimulation (aiTBS) protocol is a form of neuromodulation that induces remission in 90% of individuals with TRD within 1-5 days[149]. This study addresses our current limitations of understanding the MOA of aiTBS's dramatic therapeutic[149] and network-level effects[150]. This study will provide an opportunity to iteratively assess changes in RS FC after each day of stimulation (i.e., every 18,000 pulses - which is equivalent to an entire conventional 6-week course of iTBS[80]) and thereby establish a dose-response relationship between stimulation and change in RS FC at our target (sgACC-DMN)[86]. This study is the only way to accomplish this because if one assumes that the rate of decrease is linear, recent data demonstrate a 12 point per day decrease in the HDRS-17[86], and given that the high end of the scale is a 52, it would take 5 days to reduce the score from 52 to remission range. To apply 7.5 months (5X dose of traditional) of once per day of rTMS would be infeasible from a clinical study standpoint. Otherwise, there would be a high expected regression to the mean of a sample receiving 5 times the conventional 6-week course (7.5 months long duration of therapy if given by conventional

means)[151]. We recently demonstrated a significant reduction in mean sgACC-DMN connectivity after aiTBS with significant reductions in fc between sgACC and 3 of 4 DMN nodes[150]. Individual changes in fc magnitude showed that participants with the lowest magnitude reductions in fc between sgACC and midline DMN experienced the best clinical improvements. This makes sense in the context of the longitudinal fc changes induced by ECT where an early fc decrease significantly predicted a later increase in fc[152]. These findings may suggest that less or more sessions of aiTBS would result in differential fc/clinical outcomes and illustrate the need for this study to construct an fc-based dose-response curve.

Innovation:

This study is **innovative** technologically in its approach to rapidly modulate the neural circuitry underlying depression and measure the functional connectivity changes resulting from that modulation using precision medicine. This efficacy and rapidity is achieved via several technological innovations over conventional rTMS: 1) The number of pulses given per day (18,000) is higher than any other known application, and this may address potential under dosing of rTMS in treatment of depression[84,90]. 2) iTBS is more efficient than rTMS at a pulse ratio of 1:5 with 1 pulse of iTBS equivalent to 5 10Hz rTMS pulses and at a time ratio of 1:12.3 with every 1 min of iTBS being equivalent to 12.3 min of 10Hz rTMS[81]. 3) We space iTBS sessions at 60-min intervals, which may be optimal for producing long-term potentiation via iTBS as shown by studies of synaptic potentiation[153]. Notably, theta-burst trains that are spaced by 60 min result in over twice the magnitude of potentiation as trains spaced by 30 min, and trains spaced by 60 min induce the same magnitude of synaptic potentiation as those spaced by 90 min[153]. 4) We account for the significant variability in head anatomy[154]. 5) We employ an automated personalized targeting approach based a hierarchical clustering algorithm to identify functional subregions of the L-DLPFC & sgACC. A separate decision-making algorithm then evaluates the size, shape, and the functional connectivity relationships between each L-DLPFC subregion and each sgACC subregion in order to identify the optimal L-DLPFC target with an existing anti-correlation functional connectivity relationship with the sgACC. The advantages of aiTBS also include more rapid delivery of treatment, more rapid improvement in symptoms, and fewer needed visits; thus, likely enhancing retention and treatment completion. aiTBS provides a new opportunity to begin investigating the network alterations underlying MDD remission. Fc changes that occur with aiTBS may reflect generalized network-level changes associated with remission from depression. Given that we found that fc significantly decreases between several of these regions following aiTBS-induced remission from MDD, the magnitude of fc change often correlating with symptom improvement, and the pre-treatment connectivity between several regions to be predictive of the magnitude of this improvement, this study will allow for an opportunity to study the nature of the trajectory of the fc changes in relation to clinical changes[5,86].

aiTBS is a reimagination of the original engineering conditions of the first rTMS depression experiment[78]. In constructing aiTBS, we understood that multiple variables were being modified concurrently[155], but we decided to construct a stimulation methodology which retained those variables demonstrated to be correct: [target (DLPFC)[82,156–160], laterality (left)[82,157–159,161,162], direction of stimulation (excitation[82,157,159,162,163], biological and clinical efficacy of iTBS[81,164]] while simultaneously addressing those variables which continue to be uncertain (intersession interval (ISI)[109,144,165], targeting within DLPFC[166], nature[100] of pulse dose[84,85]). We are emulating the approach taken by all those who have successfully developed an innovative new therapeutic intervention[167–170]. Three parameters of particular note are: within node (L-DLPFC) targeting[99,128,171], dose (amount[84,85] and nature[110,116]), and pattern[110,116]. We independently conceptually addressed these variables and, after careful consideration, we constructed a stimulation strategy that reflects the modern understanding of the neuroscientific principles of neuromodulation[172]. Targeting the placement of neuromodulation devices is crucial[16,168], and given recent advances in fc-based targeting, we decided to utilize the only candidate brain target for rTMS therapy for TRD (L-DLPFC-sgACC)[72,77,99,128,175] – all others are skull targeting approaches resulting in a variably positioned

brain target[104,171,176], which may have resulted in previous failed attempts[119,177]. Further, the doses of rTMS applied per day are several orders of magnitude less than other more effective forms of neuromodulation[178] (600-3,000 pulses/day[87] versus 500,000 pulses/day for DBS[179]). Additionally, new data suggest that the intensity of the dose may be subthreshold[98,100]. We sought to produce as rapid-acting of effects as possible, as conventional rTMS studies are plagued by the potential for individuals receiving sham to experience regression to the mean[151] due to the extended timeframe of the therapy[180]. Further, an ISI (as conventionally applied) is 24 hrs which has no known physiological evidence[109] and other investigators have utilized ISIs[119,177] which are not consistent with known physiology[96,98,110,115,128]. Hence, an accelerated protocol makes sense from a clinical trial design perspective as well as the basic neuroscience perspective.

2.2 BACKGROUND

Preliminary Data

aiTBS acute efficacy: Numerous past reports have found iTBS of the L-DLPFC to be an effective treatment for depression[87,164,177]. In our first open-label study of aiTBS for treatment of the most severe, treatment-resistant depression (MSM score of every patient was 14)[181,182], we found a 76% reduction in depression symptoms (HDRS-17) with 5/6 participants showing >50% reduction in symptoms and 4/6 remitting from depression after 5 days of aiTBS, despite having failed ECT, conventional rTMS, numerous medications (including ketamine), and psychotherapy.

We recently published our open-label follow-up study[149]. We have treated 31 participants with highly treatment resistant depression (Maudsley Staging Method score ≥11) with only 1 drop-out during the 5-day treatment course. We observed an extremely high rate of remission (28/31, 90%, MADRS <10) in less than 5 days of treatment (mean days to remission = 2.96±1.48). After 5 days of aiTBS there was an 88% reduction in mean MADRS with scores from 37.71±7.24 to 4.65±6.46. Notably, our results are nearly identical for the subset of 22 patients with major depressive disorder (MDD, the 9 other patients had bipolar depression or Parkinson's-related depression), as this MDD sample is more reflective of the population we plan to target in this study. Mean Hamilton Depression Rating Scale-6 (HDRS-6) scores decreased with each additional day of stimulation over the entire 5 day stimulation course. None of the participants who had previously not responded to an FDA-approved rTMS treatment course responded after the first day of aiTBS, when the equivalent amount of stimulation as an FDA-approved treatment course was administered. However, 76.92% of the rTMS non-responders in our study did respond to aiTBS, suggesting that FDA-approved protocols may be underdosing. Mean HAMD-17 significantly decreased from 26.280 (±4.909, range 20-35) at baseline to 4.722 (±4.599, range 0-16) after treatment ($t_{(17)}$=11.275, p<.001). Mean MADRS significantly decreased from 33.220 (±5.208, range 27-44) at baseline to 4.833 (±5.813, range 0-19) after treatment ($t_{(17)}$=14.915, p<.001). Baseline HAMD-17 and MADRS scores were highly correlated (r=.817, p<.001), as were the percent-change scores from baseline to post-treatment (r=.948, p<.001). MADRS was used as the primary clinical outcome for all fc analyses, and all results are equivalent for HAMD-17.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Immediate Risks:

aiTBS: Seizure risk is generally the most significant immediate concern associated with rTMS, at approximately 1:30,000 sessions for traditional high frequency rTMS[183]. However, there are no reported seizures with the iTBS (excitatory) parameters that will be used in this study[184]. Only one case of TBS has resulted in seizure, and this case was associated with the use of continuous (inhibitory) TBS at >/=100% resting motor threshold[184,185], whereas we will use iTBS at 90%. Any seizure that theoretically could occur would likely be self-limited and lead to no residual neurological impairment. Seizures induced by rTMS are rare, brief and don't result in sequelae, with no events of status epilepticus ever being reported[183].

Another potential immediate risk is that of induction of mania. Although there have been no such cases of mania induced by iTBS, there have been at least 13 cases of rTMS-induced mania reported from fixed-frequency rTMS[186]. The rTMS-induced mania rate reported by Xia et al was 0.84% as compared to 0.73% for sham, which were not significantly different from each other. Most of the reported cases of rTMS-induced mania were in individuals with bipolar disorder, a population that will not be included in the current study. Nevertheless, it is possible that patients could develop symptoms of hypomania or mania following stimulation. Patients' symptoms will be monitored carefully by both members of the research team and clinical staff. The YMRS will be administered daily to check for mania symptoms. If patients show hypomanic/manic symptoms, no further stimulation will be administered and the patient's clinician will be notified.

Another potential risk is mild pain at the site of stimulation, which occurs during stimulation only, and which wanes with each session. There is also a risk of psychological distress related to this pain, should it occur, though discomfort associated with rTMS is typically mild, and not distressing when participants are reassured it is not dangerous.

Finally, expected AEs have been reported in association with iTBS in a recent large RCT[187], and a full list of expected AEs can be found in Section 8.3.3.3.

MRI: There are some contraindications that may prevent a participant from having a scan; however, all participants will be screened prior to scanning to evaluate these risk factors[188]. Due to the strength of the magnetic field, contraindications include participants with heart pacemakers, metallic foreign bodies in their eye, and brain aneurysm clips, as the magnetic field may dislodge the metal leading to injury[184,188] Possible side effects of undergoing an MRI are listed in 8.3.3.3.

Lidocaine: Possible side effects associated with Lidocaine are mentioned in 8.3.3.3.

Confidentiality: A risk of all clinical studies is that they collect personal information that may be seen by individuals not associated with the study. This could lead to psychological, social, cultural and legal risks. To protect participants' confidentiality, a numerical system will be used as unique identifiers, and identifying information will not be stored with the data. Electronic data will be stored on shared computer drives and in REDCap, a HIPAA compliant electronic data capture software, maintained by the university under rigorous firewall and password protection. Secured information linking participant's ID with protected health information will be separated from the unidentified data.

Long-range Risks: No long-range risks associated with iTBS or routine MRI have been reported, and none are anticipated for this study.

Alternative Treatments and Procedures: There are several other treatments for severe depression; however, none carry the optimal low risk/benefit ratio or quick action of aiTBS. The most realistic alternatives would be electroconvulsive therapy, conventional rTMS, and/or medications. Although the current FDA-approved form of conventional rTMS is effective, a typical treatment course takes 6 weeks to complete and the open label efficacy rates are lower then this approach and participation in this protocol does not preclude a subsequent traditional rTMS[82] . Similarly, an acute course of electroconvulsive therapy takes typically 1 month to complete, carries greater risks than rTMS[189], and cultural acceptance is low[190] . Medications take months to begin working and their efficacy is severely limited compared to ECT or rTMS[9,191]. Esketamine/Ketamine could also be considered given their rapid onset of action, but their effects are short-lived[192].

2.3.1 Known Potential Benefits

Based on our previous open-label studies[172,193], many of the research participants who receive active aiTBS are likely to achieve rapid improvements in their depression symptoms, with associated improvement in their social and occupational functioning. Identifying an efficacious treatment for a subject's depression may have long-range benefits as well, in that treatment effects may be sustained and the subject will have identified an effective potential treatment after multiple treatment failures.

Furthermore, the information gained from this study will inform further optimization and personalization of rTMS therapy for TRD. Specifically, this study will provide invaluable data on the transition from the depressed network configuration to the remission network configuration and how this transition relates to aiTBS and depression symptoms. Our results might provide a neurobiological basis for L-DLPFC inhibition of sgACC as a causative factor in the development of TRD, allowing for potentially more effective mood control.

2.3.2 Assessment of Potential Risks and Benefits

Given the high degree of safety of MRI and rTMS[183], there is a low risk to high benefit ratio for this intervention. rTMS will be performed and monitored according to widely accepted principles that maximize safety[183]. Based on our previous open-label studies[172,193], many of the research participants who receive active aiTBS are likely to achieve rapid improvements in their depression symptoms, with associated improvement in their social and occupational functioning.

Furthermore, the information gained from this study will inform further optimization and personalization of TMS therapy for TRD. Specifically, this study will provide invaluable data on the transition from the depressed network configuration to the remission network configuration and how this transition relates to aiTBS and depression symptoms. Our results might provide a neurobiological basis for L-DLPFC inhibition of sgACC as a causative factor in the development of TRD, allowing for potentially more effective mood control. Hence, we believe that the risks associated with this study are minimal compared to the knowledge gained.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To establish a resting state functional connectivity (RS FC) dose-response curve of active versus sham aiTBS.	Change in RS FC between the sgACC and DMN over 5-days of active aiTBS as compared to sham aiTBS. RS FC will be measured on the morning of each intervention day and the day following intervention.	We hypothesize that participants (n=50) receiving active aiTBS applied to DLPFC-sgACC will demonstrate a significant attenuation of RS FC between sgACC and DMN compared to sham (n=50) after each day of stimulation and these effects will accumulate linearly over the course of the 5 days of aiTBS. The central, consistent change in neurocircuitry observed in depression is hyperconnectivity between the SCC and DMN[43] and our preliminary data involving RS FC changes from aiTBS demonstrates highly significant changes[194].
Secondary		
(a) To determine the relationship between active versus sham aiTBS-induced clinical improvement (as measured by reduction of the MADRS-S) and changes in RS FC between the sgACC and DMN. (b) To determine the relationship between acute mood state (as measured by the IMS-12) and changes in RS FC between the sgACC and DMN in those receiving active versus sham aiTBS.	(a) Relationship between clinical improvement (measured by the MADRS-S) and RS FC between the sgACC and DMN. MADRS-S will be collected at baseline, the end of each intervention day, the day following intervention, and 1-month after intervention. RS FC will be measured on the morning of each intervention day and the day following intervention. (b) Relationship between acute mood state (measured by the IMS-12) and RS FC between the sgACC and DMN. IMS-12 will be collected at baseline, on the morning of each intervention day, and the day following intervention. RS FC will	Our mechanistic hypothesis is that active versus sham aiTBS-induced improvement in mood will be linear, related to session number (accumulated pulse dose), and will be associated with (accumulated) decrease in sgACC- DMN resting state functional connectivity, as compared to sham.

	be measured on the morning of each intervention day and the day following intervention.	
Exploratory		
(a) To determine the relationship between active versus sham aiTBS-induced clinical improvement (as measured by reduction of the MADRS-S) and changes in RS FC in an unbiased, whole-brain analysis. (b) To determine the relationship between acute mood state (as measured by the IMS-12) and changes in RS FC in an unbiased, whole-brain analysis in those receiving active versus sham aiTBS.	(a) Relationship between active versus sham aiTBS-induced clinical improvement (as measured by reduction of the MADRS-S) and changes in whole-brain RS FC. MADRS-S will be collected at baseline, on each intervention day, and the day following intervention. Whole-brain RS FC will be measured on the morning of each intervention day and the day following intervention. (b) Relationship between acute mood state (as measured by the IMS-12) and changes in whole-brain RS FC in those receiving active versus sham aiTBS IMS-12 will be collected at baseline, on the morning each intervention day, and the day following intervention. Whole-brain RS FC will be measured on the morning of each intervention day and the day following intervention.	Our hypothesis is that greater network segregation will be detected for better clinical outcomes and that a combination of the functional connectivity features involving emotion regulation will have better prediction accuracy for clinical outcomes. This alternative approach will allow us to identify other RS FC changes with especially large effect sizes, which would be important candidates for further investigation in future studies.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Hypothesis: Participants (n=50) receiving active aiTBS applied to DLPFC-sgACC will demonstrate a significant attenuation of RS FC between sgACC and DMN compared to sham (n=50) after each day of stimulation and these effects will accumulate linearly over the course of the 5 days of aiTBS.

Phase: This is a mechanistic study similar to a phase 2 trial.

Trial Design: Randomized, sham-controlled, double-blinded mechanistic study.

Methods to Minimize Bias: This study will achieve robust and unbiased results via several design features including: explicit inclusion/exclusion criteria; randomization of intervention condition; the use of a validated sham control; blinding; use of validated laboratory and interview/self-report measures and methods; explicit hypotheses and corresponding planned statistical analyses; power estimates; planned handling of retention/attrition and missing data; and, careful consideration of potential confounds. All experimental details will be reported in a fully transparent manner to support replication**.**

Dose Escalation: TMS stimulation dose will be individually determined according to 90% rMT plus depth correction, and then the aiTBS protocol will be administered with a consistent dose across all participants receiving active stimulation of 18,000 pulses/day and 90,000 pulses/course.

The goal of treatment in this study is to ensure the correct amount of treatment (90% of motor threshold) is received in the target location (L-DLPFC) rather than under-stimulating and under- treating participants. Depth correction accounts for the variable distances between the scalp and motor hotspot and scalp and treatment target from person to person. In other words, this is a personalized treatment intensity that takes into account individual variability. However, to ensure safety we never treat higher than 120% of the person's resting motor threshold, which is the standard TMS treatment intensity in routine clinical practice.

Study Arms: Two arms, active vs sham in 50:50 randomization

Site(s): Single Site (Department of Psychiatry & Behavioral Sciences, Stanford University)

Name of Study Intervention: Accelerated intermittent theta-burst stimulation (aiTBS).

Interim Analysis: N/A

Stratification: We will attempt to assess the role of gender in our analyses to determine the potential utility of additional follow-up projects that focus on any discovered gender-based observations. However, it may not be feasible to run sub-group analyses in this study because of potentially insufficient sub-group sample sizes.

Sub-Studies: None

4.2 Scientific Rationale for Study Design

This is a double-blinded, randomized, controlled mechanistic study using a 1:1 ratio between active and sham intervention. During the blinded period, the investigator, participants, clinical assessors, and treaters will be blinded to active or sham intervention delivery. Participants with a higher level of treatment resistance, who will be the focus of this study, tend to be less susceptible to placebo effects of iTBS therapy[90]. Nevertheless, the use of a sham controlled group is essential to ensuring that our outcomes of interest can be properly attributed to active aiTBS.

The aiTBS intervention will be administered with an 'A/P' coil (MagVenture, Denmark) which has both an active and sham side that are identical in appearance. The intervention condition is determined based on a code assigned at randomization. Based on the assigned code the magnetic stimulation is either directed towards or away from the head (i.e., when the randomization code assigns sham stimulation then the magnetic stimulation ends up being directed away from the head). During sham stimulation, however, the magnet still activates. Accordingly, the sound, appearance, and sensation of sham stimulation operation is the same as it is for active stimulation. Thus, there is no way for the operator to know if the patient is getting sham or active stimulation. Similarly, raters will have no knowledge of which ID corresponds to which intervention. This type of sham has been demonstrated to be indistinguishable from real TMS, has been well tolerated[195,196], and successfully used in clinical trials[157,197].

4.3 JUSTIFICATION FOR DOSE

Total dose of stimulation appears to be important for rTMS therapeutic effects[83]. 60,000-90,000 pulses are used for the 6-week standard high frequency rTMS course[81,82]. In cases where higher pulse dosages have been used, there is evidence of greater efficacy[84,85]. There also is evidence that there are a number of responder trajectories that are related to the level of TRD[86]. In the case of the FDA-approved iTBS protocol[87], 600 pulses are applied during each session such that 18,000 pulses are delivered over a six-week treatment course. While there had been a question as to whether >600 pulses per session might result in a reversal in the cortical excitability of a prefrontal target[88], there is now evidence in depressed[89,90] and normal healthy controls that 1800 pulses of iTBS per session ($iTBS_{1800}$) produces long-lasting effects in the intended direction[91]. In fact, $iTBS_{1800}$ is the only pulse dose that has yielded a positive result in a blinded TBS trial[92–95] and that has been shown to produce the intended resting-state effects[96]. Additionally, the 1800 pulse dose has been shown to produce long-lasting changes in cortical excitability[97] and resting-state functional connectivity[96] .

Stimulation Intensity Adjustments for Differential Prefrontal Cortical Atrophy and Induced E-Field:
Intermittent theta-burst stimulation applied <100% rMT produces the optimal change in prefrontal cortical excitability[98] and resting state changes[99,100] when compared to suprathreshold stimulation. This convergent data suggests that unlike traditional rTMS[101], there appears to be an inverse U-shaped curve with TBS intensities requiring an optimal intensity directly over a target. This finding would explain why, of the blinded iTBS trials for TRD, the only positive trials were found in those studies utilizing subthreshold %MT[93], while those trials utilizing >100% %MT did not separate from sham[95].

Furthermore, with age there is differential atrophy of PFC and PMC[102]. Depth correction[103] historically has been applied to the resting motor threshold to adjust for differences in the cortical depth of the individual's functional target compared to the primary motor cortex in order to consistently achieve the intended rMT at the target. In the OPT TMS trial it was determined that a standard %MT could be used (120% avg of depth adjustment), but with traditional HF rTMS[104] there is no inverted U-shaped curve[101]

like there may be with iTBS[98,100]. Accordingly, it is very unlikely that an average multiplier can be used with iTBS given its principles.

Patterning:

Pattern of Stimulation Session (Intermittent Theta-Burst Stimulation (iTBS)):

Theta-burst stimulation (TBS), a patterned form of rTMS modeled after endogenous hippocampal discharge patterns[105,106], has been shown to be more efficient than standard rTMS in modulating cortical excitability[107] and last ~60 min[108]. Recent clinical trials indicate non-inferiority of 600 pulses/day of iTBS given over 3 min versus 3,000 pulses/day of conventional rTMS given over 37 min in treating depression (now FDA-approved)[80] and notable efficacy in treating TRD[90]. What is unknown is the ideal timing of when each session should be applied, and currently 24 hours is the default[109]. It is unclear if this approach is ideal[110].

Pattern of stimulation course (Intersession interval (ISI)):

An accumulation of TBS effects can occur when TBS stimulation is applied in a neurobiologically-informed, patterned manner (compared to en-masse stimulation)[111] allowing for a dose-response curve to be constructed[112]. Previous work has demonstrated that iTBS sessions delivered to hippocampal slices with inter-session intervals of 50-90 min have a cumulative effect on dendritic spine enlargement, a process involved in synaptic strengthening. In comparison, iTBS sessions delivered with inter-session intervals of ≦40 min do not have a cumulative effect on dendritic spines[113–115]. Two iTBS sessions delivered to the motor cortex[110,116] or prefrontal cortex[117] with a 15-minute inter-session interval have been shown not to result in an incremental increase in cortical excitability over a single iTBS session, while two applications of iTBS with a 30-min ISI does result in an increase in cortical excitability[110,116]. When 1800 pulses of iTBS is applied at a subthreshold %MT at the 15 min post time point, there is an initial increase in fc between ACC and DMN (in the pathological direction). At the 30 min time point, there is a decrease in fc between ACC and DMN and these changes are maximal at the 50 min time-point. Interestingly, Nettekoven et al. similarly reported that the 30 min time point does result in changes in RS FC[110,116], but the 50 min time point is optimal for engaging the entire mood regulatory network. This could explain the limited efficacy of a previously reported accelerated iTBS protocol that used an inter-session interval of only 15 min[118,119]. This work provides further support for an ISI that is ≧50 min[96]. Studies that have applied TBS to cortical targets have shown that two spaced stimulation sessions produce greater[110,120,121] and longer lasting[121,122] changes in cortical excitability than single sessions. This finding holds for other cortical targets in diseased[123] and normal subjects[124]. Recent attempts at this have demonstrated that multiple sessions of iTBS per day increase efficacy compared to single applications/day both clinically[125] as well as physiologically[105].

Intervention Regimen: We will employ a spaced, intermittent theta-burst technique that is capable of providing a significant change in neural activity: 10 minutes of stimulation followed by a 50 min inter-sessional interval with no stimulation. In total, participants will receive 10 sessions of this 10-minute aiTBS per day for a total of 100 minutes of aiTBS stimulation per day.

Name and Justification: The aiTBS protocol was developed to utilize the accelerated neuromodulatory application of 90,000 pulses of intermittent theta-burst stimulation at 90% of the resting motor threshold (rMT), as this has been demonstrated to be effective in producing a prolonged change in cortical excitability[124] as well as safe[198]. The %rMT will be adjusted based on skull-to-cortex distance. The 50-minute inter-sessional interval is essential, as it has been demonstrated that prolonged stimulation approaches without spacing in between can cause a reversal of intended effect, as described above[88]. This spacing also has been utilized in animal models of LTP/LTD induction[199].

Route: The spaced theta-burst stimulation approach will be applied to the left dorsolateral prefrontal cortex (L-DLPFC) in the region of the L-DLPFC that has the highest functional anticorrelation with the subcallosal cingulate cortex (SCC). This sub-region of the L-DLPFC will be identified by utilizing neuronavigation hardware and coupling this with the latest in cutting-edge rTMS targeting methodology[147].

Dosage: 90% of the resting motor threshold adjusted to target depth will be utilized as the dose of aiTBS because this dose has been demonstrated effective in modulating the desired cortical target across several studies, as described above[121,124]. 1800 pulses of iTBS with 3-pulse 50 Hz bursts given at 5Hz every 200 ms will be utilized, as this is the optimal approach for producing stimulation[199]. This study approach will utilize 60 stimulation trains of 30 pulses with 800 ms spacing in between each train of iTBS, as has been reported previously[121,124]. This stimulation approach will be applied for 10 minutes every hour for 10 sessions a day. Each participant will receive a total of 50 sessions (of 10-minute aiTBS) by the end of the 5 stimulation days for a total of 90, 000 pulses of aiTBS. To ensure safety we never treat higher than 120% of the person's resting motor threshold, which is the standard TMS treatment intensity in routine clinical practice.

A depth-adjusted aiTBS treatment dose >65% of maximal stimulator output is an exclusion criterion. The rationale is that at doses >65% of maximal stimulator output, a phenomenon known as magnetic roll-off may occur, such that the actual iTBS stimulation dose delivered may be less than the intended dose.

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3. Here, the end of the study is defined as completion of visit 9 as shown in the SoA.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Male or Female, between the ages of 22 and 65 at the time of screening (Visit 1).

2. Able to read, understand, and provide written, dated informed consent prior to screening. Proficiency in English sufficient to complete questionnaires / follow instructions during fMRI assessments and aiTBS interventions. Stated willingness to comply with all study procedures, including availability for the duration of the study, and to communicate with study personnel about adverse events and other clinically important information.

3. Currently diagnosed with Major Depressive Disorder (MDD) and meets criteria for a Major Depressive Episode, according to the criteria defined in the Diagnosis and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5).

4. Medical records confirming a history of moderate to severe treatment-resistance as defined by a score of 7-14 on the Maudsley Staging Method (MSM[3]).

5. MADRS score of ≥20 at screening (Visit 1).

6. TMS naive.

7. Access to ongoing psychiatric care before and after completion of the study.

8. The dose of the primary antidepressant medication (if applicable) must be stable for 6 weeks prior to baseline (Visit 2), and participant must agree to continue at this dose throughout the study period.

9. In good general health, as evidenced by medical history.

10. For females of reproductive potential: use of highly effective contraception for at least 1 month prior to baseline (Visit 2) and agreement to use such a method during study participation.

11. Agreement to adhere to Lifestyle Considerations (see section 5.3) throughout study duration.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Pregnancy
2. Primary psychiatric condition other than MDD requiring treatment except stable comorbid anxiety disorder
3. History of or current psychotic disorder or bipolar disorder
4. Severe borderline personality disorder.
5. Diagnosis of Intellectual Disability or Autism Spectrum Disorder
6. Current moderate or severe substance use disorder or demonstrating signs of acute substance withdrawal
7. Urine screening test positive for illicit substances
8. Active suicidal ideation (defined as an MSSI > 8) or a suicide attempt (as defined by the C-SSRS) within the past one year
9. Any history of ECT (greater than 8 sessions) without meeting responder criteria
10. Recent (within 4 weeks of any clinical effect) or concurrent use of rapid acting antidepressant agent (i.e., ketamine or a course of ECT)
11. History of significant neurologic disease, including dementia, Parkinson's or Huntington's disease, brain tumor, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma
12. Untreated or insufficiently treated endocrine disorder.
13. Contraindication to receiving rTMS (e.g., metal in head, history of seizure, known brain lesion)
14. Contraindication to MRI (ferromagnetic metal in their body)
15. Treatment with another investigational drug or other intervention within the study period
16. Depth-adjusted aiTBS treatment dose > 65% maximum stimulator output (MSO)

17. Unstable symptoms between screening (Visit 1) and baseline (Visit 2) as defined by a > 30% change in MADRS-S score.
18. Any other condition deemed by the PD to interfere with the study or increase risk to the participant

For clarity, the visit(s) that each eligibility criterion is determined are provided in the following table:

Inclusion or Exclusion Criterion #	Form Name	Assessment Timepoint
Inclusion Criteria		
1	Demographic Information	Remote Screening Visit 1
2	Capacity to Consent	Remote Screening Visit 1
3	MINI DSM5	Remote Screening Visit 1
4	Maudsley Thase Rush	Remote Screening Visit 1
5	MADRS-C	Remote Screening Visit 1
6	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
7	Demographic Information	Remote Screening Visit 1
8	ATHF	Remote Screening Visit 1
9	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
10	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
11	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
Exclusion Criteria		
1	Urine Pregnancy Screen	In-Person Screening 2/Baseline - Visit 2
2	MINI DSM5	Remote Screening Visit 1
3	MINI DSM5; YMRS	Remote Screening Visit 1;

		In-Person Screening 2/Baseline - Visit 2
4	SCID For DSM-5 Borderline Personality (Clinician Module)	Remote Screening Visit 1
5	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
6	SCID Overview	Remote Screening Visit 1
7	Urine Toxicity Screen	In-Person Screening 2/Baseline - Visit 2
8	C-SSRS - Baseline/Screening; MSSI	Remote Screening Visit 1; In-Person Screening 2/Baseline - Visit 2
9	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
10	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
11	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
12	Stanford Medical And Psychiatric Review	Remote Screening Visit 1
13	Stanford Medical And Psychiatric Review; TASS TMS Safety Form	Remote Screening Visit 1
14	Stanford Medical And Psychiatric Review; MRI Safety Form	Remote Screening Visit 1
15	Main Study Consent	In-Person Screening 2/Baseline - Visit 2
16	Log of Motor Thresholds	In-Person Screening 2/Baseline - Visit 2
17	MADRS-S	Remote Screening Visit 1; In-Person Screening 2/Baseline - Visit 2
18	All forms	All visits

The effects of rTMS on the developing human fetus are unknown[200]. Accordingly, we will not be enrolling pregnant women to this study. Women of childbearing potential must agree to use adequate contraception (hormonal / barrier method of birth control or abstinence) prior to study entry and for the duration of study participation. Females of childbearing-age will have a pregnancy test prior to starting the rTMS stimulation course. Should a woman become pregnant or suspects she is pregnant while participating in this study, she should inform study staff.

Participants taking certain psychoactive medications will be assessed for safety by the PD, due to potential for increase of seizure risk[182] and change in cortical excitability[200].

Individuals older than 65 years of age will be excluded for the following reasons:

1) Dementia can present as depression prior to cognitive symptoms (both vascular and neurodegenerative)[201], and a significant battery of neuropsychological tests would be required to

accurately discern cognitive dysfunction from depression from dementia[202]. Given that this is a mechanistic grant, inclusion would increase potential of false negatives.

2) Even though the study depth adjusts individuals with greater prefrontal over primary motor cortex atrophy, the safety limit is at 120% and some individuals require greater than 120% depth adjustment to reach PFC[102]. With this, there is evidence of reduced efficacy in those individuals with advanced age[203,204].
3) There is evidence of reduced plasticity in individuals with advanced age with a reduction in the duration of the effects of TBS[205,206] which may impact the response to the approach.

Screening (Visit 1 - Remote Screening) must be completed within 3 months of formal study enrollment and baseline assessment (Visit 2 - In-person Screening 2 / Baseline), otherwise screening measures will need to be repeated prior to moving forward with enrollment and baseline assessment. Inclusion criteria 8 + 10 are relative to the date of first treatment administration.

5.3 Lifestyle Considerations

During this study, participants are asked to:

- Abstain from becoming pregnant from the screening visit (Visit 1 - Remote Screening) until after the final study visit (Visit 9).
- Continue usual intake patterns of caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) without significant change for the duration of the study.
- Abstain from alcohol for at least 24 hours before the start of each MRI and TMS session.
- Participants who use tobacco products will be informed that use will be allowed only in between intervention sessions.

5.4 Screen Failures

Screen failures are defined as participants who sign the screening ICF and/or the study ICF and complete some or all of the V1 - Remote Screening and/or Visit 2 - In-person Screening 2 / Baseline visit(s) but are not subsequently enrolled in the study due to not meeting study entry criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) due to a specific modifiable factor may be rescreened at a later date once appropriate study criteria are met. Rescreened participants will be assigned a modified version of the same participant number as for the initial screening (e.g., s001, s001a, s001b, etc.).

In the case of screen failure, screening data of such patients will be electronically stored in our REDCap database.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Target Study Sample:

Racial Categories	***Ethnic Categories***				
	Not Hispanic or Latino		*Hispanic or Latino*		***Total***
	Female	***Male***	***Female***	***Male***	
American Indian/Alaska Native	*0*	*0*	*0*	*0*	***0***
Asian	*10*	*6*	*0*	*0*	***16***
Native Hawaiian or Other Pacific Islander	*4*	*2*	*0*	*0*	***6***
Black or African American	*6*	*4*	*0*	*0*	***10***
White	*60*	*40*	*24*	*26*	***150***
More than 1 race	*10*	*6*	*2*	*0*	***18***
Total	***90***	***58***	***26***	***26***	***200***

Anticipated accrual rate: Participants will be recruited through the current Stanford Brain Stimulation Laboratory clinical trial recruitment pipeline and the Stanford Depression Research Clinic (Dr. Williams is a member). Based on our random sampling via chart reviews, there are approximately >110 participants who meet inclusion criteria each year at Stanford. These numbers exceed our power analysis, which indicates a plan of recruiting 25-30 participants per year at Stanford.

Anticipated number of sites: We will have a single study site and anticipate enrolling 100 participants from the U.S.

Source of participants: Participants will be recruited from the general public as well as outpatient clinics at Stanford Healthcare, private practice clinics, and the student health center at Stanford University.

Recruitment venues & how potential participants will be identified and approached: Participants will be recruited through the Stanford Brain Stimulation Lab online recruitment pipeline (online portal for referrals) and the Stanford Depression Research Clinic (Dr. Williams is a member). Potential participants will then be invited via email or phone to consent to and participate in screening with a study team member (over zoom) for further assessment of eligibility.

Types of recruitment strategies planned: Recruitment strategies include social media campaigns and in-services with outpatient clinics at Stanford Healthcare's psychiatry department as well as private practices in the community. Patient advocacy groups will be targeted for recruitment such as National Alliance on Mental Illness.

Participant retention plan: Participants will provide best contact information including telephone, email, and up to two emergency contacts, all of which will be utilized as needed to ensure participant retention. Additionally, participants will be compensated $50 USD for study visits 2 to 8 (baseline to follow-up), for a total of $350 USD. They will be compensated at their last study visit.

Specific strategies that will be used to recruit and retain historically under-represented populations: We will make every effort to recruit an ethnically and racially representative sample who meet our study criteria, such as being depressed and able to undergo an fMRI scan and TMS interventions. The data in the Inclusion Enrollment Report are based on extrapolations from our random sample of patients who meet inclusion criteria who have undergone clinical rTMS at Stanford. Overall, 37% of our sampled patients who met inclusion criteria were non-White, and we will work towards staying as close as possible to these diversity numbers. We expect a similar proportion of non-White patients. Our staff will be trained and sensitive to the issues related to recruitment and retention of diverse populations. Culturally sensitive retention of participants in the study for all of the assessments will be practiced. We have three decades of experience in conducting such research studies and have trained our research staff to provide warm and caring interactions with our research participants, expressing ongoing appreciation for their participation, and responding immediately and supportively to any questions or issues that emerge from them or their families. In our experience, this approach has improved ongoing research participant adherence to protocols. We make every effort to ensure that our research participants feel respected for their important contribution to our research.

In order to facilitate recruitment of diverse populations, recruitment materials will be made available in English and Spanish. The Stanford Hospital and Clinics have 24hr per day access to interpreters – in person, by video, or by phone – and this will be available to all of our participants. We will also leverage the expertise in the Office of Community Engagement in the School of Medicine to target diverse patient populations.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Active aiTBS Intervention: We have previously discussed this stimulation approach with the FDA and the FDA deemed the approach nonsignificant risk (NSR). aiTBS will be delivered similarly to our past[172207] aiTBS studies using the MagVenture MagPro X100 (Skovlunde, Denmark) TMS device equipped with a Cool-B65 A/P coil. The L-DLPFC functional target will be localized for each participant using a Localite TMS Navigator (Localite, Bonn, Germany). Participants will then be treated with 1,800 pulses of iTBS (3-pulse 50-Hz bursts at 5-Hz for 2-second trains, with trains every 10 seconds) per session at 90% resting motor threshold with

depth-adjustment to the personalized functional target[103]. Each session will last 10 min followed by a 50-minute intersession interval. Ten sessions will be applied per day (18,000 pulses/day) for 5 consecutive days (90,000 total pulses)**.**

Sham aiTBS Intervention: Intervention will be administered with an 'A/P' coil (MagVenture, Denmark) which has both an active and sham side that are identical in appearance. The intervention condition is determined based on a code assigned at randomization. Based on the assigned code the magnetic stimulation is either directed towards or away from the head (i.e., when the randomization code assigns sham stimulation then the magnetic stimulation ends up being directed away from the head). During sham stimulation, however, the magnet still activates. Accordingly, the sound, appearance, and sensation of sham stimulation operation is the same as it is for active stimulation. Thus, there is no way for the operator to know if the patient is getting sham or active stimulation. Similarly, raters will have no knowledge of which ID corresponds to which intervention. This type of sham has been demonstrated to be indistinguishable from real TMS, has been well tolerated[194,195], and successfully used in clinical trials.

6.1.2 Dosing and Administration

The goal of treatment in this study is to ensure the correct amount of treatment (90% of motor threshold) is received in the target location (L-DLPFC) rather than under-stimulating and under- treating participants. Depth correction accounts for the variable distances between the scalp and motor hotspot and scalp and treatment target from person to person. In other words, this is a personalized treatment intensity that takes into account individual variability. However, to ensure safety we never treat higher than 120% of the person's resting motor threshold, which is the standard TMS treatment intensity in routine clinical practice.

In scenarios where the cortical target depth is more superficial than the hotspot depth, the intensity will only be adjusted upwards. In no case will the stimulation be below 90% of the resting motor threshold.

Following the baseline MRI scan, participants will present to the clinic for the first of their 5-day course of aiTBS interventions (Visits 3-7). We will deliver both active and sham study intervention via a MagPro X100 (MagVenture, Skovlunde, Denmark) TMS. The intervention paradigm consists of 10 daily sessions (50 total over 5 days) of intermittent theta-burst (iTBS) stimulation (3-pulse 50-Hz bursts at 5-Hz for 2-second trains, with trains every 10 seconds), delivered with 50-minute inter-session intervals (10-minute sessions, 50-minutes in between sessions). Stimulation will be delivered at 90% of the resting motor threshold (with depth correction to account for the distance between the scalp and cortex). aiTBS protocol will be administered with a consistent dose escalation across all subjects receiving active stimulation of 18,000 pulses/day and 90,000 pulses/course. An operator entered code (derived from randomization) will instruct the device to deliver active or sham magnetic stimulation.

Custom MATLAB scripts will be used to identify the portion of the left DLPFC maximally anticorrelated with the sgACC, which will serve as the target for both active and sham stimulation. The intervention target will be located via a Localite TMS Navigator (Localite, Bonn, Germany). More specifically, the left dorsolateral prefrontal cortex is located according to routine, FDA-approved clinical rTMS practice. Our scripts then further discern functional subregions within the left DLPFC.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The intervention device has been purchased by the investigative team, and its use will be closely monitored by the PD and Co-I's.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Because we will use the same device and coil for both active and sham study intervention (but merely change the coil side based on active or sham stimulation, as described in 6.1.1), the two study interventions will be indistinguishable.

6.2.3 Product Storage and Stability

The study equipment will be stored in the Brain Stimulation Lab space, with routine and any additionally needed maintenance completed per manufacturer standards by the manufacturer themselves (through the purchase of a 'platinum warranty').

6.2.4 Preparation

The MRI, TMS, and neuronavigation devices will be used according to the manufacturer's instructions.

6.3 Measures to Minimize Bias: Randomization and Blinding

In this study, participants will be randomized to active vs. sham aiTBS in a 1:1 ratio in randomly selected block sizes of 2, 4, or 6 as based on a random number generator and without stratification. Blinding will be achieved via a sham rTMS coil (Cool-B65 A/P) used with the device (MagPro X100, MagVenture, Skovlunde, Denmark). This coil can deliver either active or sham iTBS in a manner that is randomized by the system itself and therefore blinded to the treater. The sham setting on this coil looks and sounds identical to the active setting but has a hidden aluminum plate blocking actual stimulation. The MagVenture TMS device holds a blinded key code that is kept by the individual that holds the blind. During the rTMS setup, the operator is instructed to flip the coil to correspond with the key code, but the operator is not able to discern the active versus sham stimulation.

For additional blinding of the operator:
- The participant wears a mouth-guard to prevent potential teeth grinding and/or audible noises from the sound of his/her mouth moving from active stimulation.
- Participants will wear headphones that generate the sound of the TMS machine as well as earmuffs to mask the actual noise of the TMS machine.
- The operator will apply lidocaine numbing gel before sessions 1, 3, 5, 7 and 9 to the scalp where the TMS coil is being placed to alleviate active stimulation discomfort.
- The operator will not touch the surface of the coil, as one side may be hotter than the other; knowing which side is hotter or colder can potentially unblind him/her.

- The operator will hang a blinding sheet/cloth over the side of the TMS coil to act as a barrier between him/her and the participant. This prevents the operator from being able to see if there are facial muscle movements, which could indicate active stimulation.

This protocol has been used to successfully blind participants in our recent RCT.

The blinded randomization code will be kept in the individual case report form (CRF), and the key linking the code to the participants condition will be kept separately in an encrypted file, stored outside of the lab and able to be accessed only by a person who has no direct or indirect interaction with participants or data collection. The blind will be maintained until the completion of the study and data-lock is achieved, after which planned unblinding will take place.

Unplanned unblinding will only take place in the unlikely events that either: a) the independent DSMB requests unblinding for concerns over adverse events that are more serious / frequent than expected, or; b) on an individual basis should knowing the randomized condition for an individual participant result in improved clinical decision making in an emergency setting. In the case of unplanned unblinding, both the Stanford IRB and the Program Officer assigned to the project at NIMH will be informed.

We do not anticipate unintentional unblinding; however, in the event a member of the study team becomes unblinded, we will track this event in the CRF, and all study-team members know to not unblind other study-team members.

6.4 Study Intervention Compliance

The study intervention is procedural in nature and, accordingly, study adherence will be directly observed. There are pre-specified places within each participant's CRF where notes to file can be logged, and we plan on documenting any missed aiTBS / MRI sessions in this location. In our experience with other investigations using aiTBS, retention is high and missed sessions are low. We subsequently do not expect to have many instances of non-adherence.

6.5 Concomitant Therapy

Participants will be required to continue all psychotropic medications throughout the study period, as they were at the time of screening. Any ongoing psychotherapy that is in a maintenance phase (i.e., not skills training) may be continued, but no new psychotherapy may be initiated during the course of the study.

To ensure adequate sleep each evening prior to TMS intervention (a safety requirement), certain sleep medications (e.g., zolpidem, zaleplon, eszopiclone, quetiapine) may be prescribed by the PD or study MD immediately prior to or during the course of TMS. These medications may be administered up to the night prior to the stimulation session (until midnight), but not during the morning of the sessions or at any time during the intervention day. The use of alternative hypnotics or the short term use of anxiolytic medications (e.g., hydroxyzine, propranolol) during an intervention day requires prior approval from the PD or study MD.

As per clinical standard of care with rTMS, participants are encouraged to bring OTC acetaminophen or ibuprofen for prophylactic use if they are concerned about the potential emergence of adverse events

that are commonly experienced with rTMS. Such use would be considered as standard of care. Short-term treatments for other headaches, mild aches/pains, or seasonal allergies will be allowed during the study provided the medications utilized have no established psychotropic effects that might confound interpretation of study outcome measures. These medications may include non-sedating, over-the-counter analgesics or antihistamines.

6.5.1 Rescue Medicine

No rescue medications will be used.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation from aiTBS does not mean discontinuation from the study, and remaining study procedures will be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be documented as an adverse event (AE), according to the AE protocol.

Discontinuation from aiTBS can be classified as temporary or permanent depending on the timing in which it occurs. If the participant has not completed visit 3 as defined in the ***Section 1.3, Schedule of Activities (SoA)***, they may qualify for temporary study discontinuation. Temporary discontinuation may result from any COVID-19 related reason or other qualifying reason. This may be, but is not limited to, temporary study discontinuation while a participant quarantines or receives COVID-19 care. After receiving health clearance, they may resume the study at visit 2 if they continue to meet study criteria.

If aiTBS discontinuation occurs after the completion of visit 3, as defined in **Section 1.3** SoA, or if a participant chooses to discontinue the study intervention, this will be classified as a permanent study discontinuation.

The data to be collected at the time of permanent study intervention discontinuation will include (if possible) the following:

- A final MRI scan (if not yet completed).
- Questionnaires about mood and well being.
- Questions about any adverse events or health problems since the last visit.

Subjects may be withdrawn from the study by the Protocol Director if a subject:

- Experiences a seizure
- Is non-adherent with study procedures
- The randomization code is broken for the subject

The Protocol Director may also withdraw a subject if he/she believes that for safety reasons it is in the best interest of the subject to be withdrawn.

Discontinuation information [e.g., date and the reason(s) for discontinuation] will be recorded in the subject's CRF (i.e., Study Completion Form) and relevant forms on REDCap.

As this is a mechanistic study, subjects withdrawn from the study may be replaced if withdrawal occurs any time prior to study completion. Should a participant fail to return for required visits or drop out of the study, effort will be made to determine why. This information will subsequently be recorded on the subject's CRF and relevant forms on REDCap.

Subjects will be encouraged to remain adherent with all expected study visits. Non-adherence to expected study visits will be documented and may result in removal from the study. This will be clearly discussed during the informed consent process and reinforced throughout the study through regular screening for issues with compliance.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy.
- Significant study intervention non-compliance.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Disease progression which requires discontinuation of the study intervention.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Participant unable to receive aiTBS for the entire 5 days.
- If an incidental finding is discovered upon imaging that either the study staff (physicians) and/or CNI assigned radiologist deems unsafe or requires immediate medical followup.

The reason for participant discontinuation or withdrawal from the study will be recorded on the REDCap eCRF. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form and are randomized and receive the study intervention and subsequently withdraw or are withdrawn or discontinued from the study before completing all required MRI scans as defined in the SoA, may be replaced.

Subjects may withdraw voluntarily from the study at any time.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for one scheduled visit and is unable to be contacted by the study staff.

We will take the following actions if a participant fails to return for a required study visit:

- We will attempt to contact the participant and reschedule the missed visit within 24 hours and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or his designee will make every effort to regain contact with the participant (where possible, 3 telephone calls or emails and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). We will record these contact attempts in the participant's designated REDCap eCRF.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with the primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Screening process:

Participants will be recruited through the Stanford Brain Stimulation Lab online recruitment pipeline (online portal for referrals) and the Stanford Depression Research Clinic (Dr. Williams is a member). Potential participants will then be invited to consent to and participate in screening with a study team member (over zoom) for further assessment of eligibility. Screening and enrollment info can be pulled from the REDCap study database. Screening and enrollment info will include consent dates, subject IDs, and outcomes . When a remote consent is conducted, electronic signature of consent will be obtained via Adobe Sign.

Our target population is rTMS-naïve participants with major depression and moderate-high treatment-resistance (on MSM). In-depth screening will include demographics information; psychiatric and medical history; current and past medications with adequacy of trials assessed by the Antidepressant Treatment History Form (ATHF) Current/Lifetime[208]; psychiatric diagnoses via the Overview/History section of the Structured Clinical Interview for DSM-5 (SCID) and the Mini-International Neuropsychiatric Interview for DSM-5 (MINI v.7.0.2); borderline personality disorder diagnostic module from SCID for DSM-5 (if a participant meets the screening threshold on the self-reported prescreening questions); the Montgomery-Asberg Depression Rating Scale-Self Report (MADRS-S), and the Montgomery-Asberg Depression Rating Scale-Clinician Rated (MADRS-CR); suicidal ideation and behavior with the Modified Scale for Suicidal Ideation (MSSI) and the Columbia-Suicide Screening Rating Scale (C-SSRS); acute mood state with the Immediate Mood Scaler (IMS[209]-12); manic symptoms will be screened for with the Young Mania Rating Scale (YMRS); severe insomnia will be screened for with the Pittsburgh Sleep Quality Index (PSQI). Participants will also be asked specifically about any contraindications to receiving rTMS such as metal in or near the head using the TMS Adult Safety Screen (TASS); any significant neurologic disease, including dementia, Parkinson's or Huntington's disease, brain tumor, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma; previous exposure to any rTMS approach; history of dementia or cognitive impairment; active suicidal ideation or a suicide attempt within the past one year; and whether they are receiving or planning to receive any other investigational interventions during this study. A motor threshold (MT) will be obtained (at Visit 2 - In-person Screening 2/Baseline as a screening measure) to confirm it does not exceed the threshold for study eligibility. Screening will be performed by a consistent group of qualified study staff who have been trained and certified by the lab's Psychological Director to ensure fidelity to assessment procedures.

Screening (Visit 1 - Remote Screening) must be completed within 3 months of formal study enrollment and baseline assessment (Visit 2 - In-person Screening 2 / Baseline), otherwise screening will need to be repeated prior to moving forward with enrollment and baseline assessment. Visit 2 In-person Screening 2/Baseline is separated from the screening visit (Visit 1 - Remote Screening) to allow initial remote screening and to confirm stability of symptoms (stability is defined as a change in MADRS-S score < 30% from screening). Accordingly, a potential subject still may be considered a screen failure even if they participate in Visit 2. Screen failures are defined as participants who sign the screening ICF and/or the study ICF and complete some or all of the Visit 1 and/or Visit 2 visit(s) but are not subsequently enrolled in the study due to not meeting study entry criteria.

Subjects who have provided screening data for another study in Stanford University's Brain Stimulation Laboratory (BSL) may have their screening data used for this study. Additionally, screening data used in this study may be used in another study in BSL. The purpose of this is to reduce unnecessary burden to the subject. The discretion of which screening data is accepted is up to the discretion of the investigator. Data will be manually re-entered within BSL's RedCap system from the previous study into the new study.

Please refer to the Schedule of Activities in Section 1.3 of the MOP understand the screening measures required to confirm study eligibility that occur at the In-person Screening 2 / Baseline (Visit 2)

Efficacy assessments related to primary and secondary objectives:

- ***Radiographic or other imaging assessments:*** All participants will undergo magnetic resonance imaging (MRI) evaluations at: baseline (morning prior to first TMS session) and each subsequent morning throughout the five-day TMS course (visits 3-8). MRI acquisitions will include T1-weighted structural and resting state functional magnetic resonance imaging sequences. Participants will be instructed to keep their eyes open and concentrate on a fixation cross for the duration of the resting state fMRI scan and to lie as still as possible. The fixation cross will be presented via a projector system and visualized through a mirror on the head coil. Subjects will confirm verbally during setup that the fixation cross is centered within their visual field, which can be adjusted via the head coil mirror angle. The image quality of the baseline scan will be evaluated for quality assurance, it is important that these are assessed, as they are the bases for our personalized targeting generation, as well as subsequent determination of a dose-response curve. We anticipate little to no group differences in functional connectivity between the active and sham groups at the time of baseline acquisition due to random allocation; however, this will be confirmed statistically post study completion. Following the establishment of fMRI baseline, we hypothesize that we will observe functional connectivity changes that are linearly correlated with the effects of the 5-day active TMS intervention.
- ***Administration of questionnaires or other instruments:*** The clinical active versus sham aiTBS-induced effect will be tested using either the standard (72-hour) version or a 24-hour version of the MADRS-S depending on time point (clinical improvement) and IMS-12 (acute mood state). **On the morning after the 5th day of stimulation (visit 8), participants will receive the IMS-12, MADRS-S and YMRS at the same visit as their MRI scan.**

- ***Additional optional blood draw study:***

An additional optional procedure consisting of three blood draws will be available to participants in this trial. These blood draws will occur on the In-person Screening 2/Baseline (Visit 2), the next day (Visit 8), and one-month follow up visit (Visit 9).

This procedure aims to establish how peripheral levels of the mitochondrial metabolite acetyl-L-carnitine (LAC) are predictive of the anti-suicidal response to accelerated intermittent theta burst stimulation (aiTBS) repetitive transcranial stimulation (rTMS) in patients with treatment resistent depression. Recent evidence suggests that peripheral LAC levels may be a biomarker of depression[210]. No studies to date have investigated the influence of antidepressant rTMS treatments and peripheral LAC levels. The relationship between baseline levels of LAC and response to rTMS are unknown. Further, the effects of rTMS on peripheral levels of LAC is also unknown. This additional study seeks to investigate these questions. Blood will be drawn by a certified phlebotomist at the time points noted above. A total of 30 mL of blood will be collected for each blood draw (10 mL in a heparinized tube and 20 mL in EDTA tubes). Additionally, Childhood Trauma Questionnaire (CTQ) and Family Interview for Genetic Studies (FIGS) assessments will be administered during the baseline evaluation to those who consent for this optional study. Additional biomarkers of depression may also be assayed by our group or our collaborators at NYU and/or University of Antwerp. A portion of the de-identified plasma samples will be sent to our collaborators at NYU and/or University of Antwerp for analysis. Another portion of the de-identified plasma samples will be stored at Stanford along with buffy coat samples for subsequent analyses of depression biomarkers including telomere length.

8.2 Safety and Other Assessments

All patients will undergo a clinical study visit with the study psychiatrist (or their designee) as part of the assessment for suitability in the study. This visit will include assessment of current psychiatric symptoms, psychiatric history (including past diagnoses, hospitalization, suicide attempts, past medication trials), current medical concerns [if any], past medical history, current medications, allergies, family psychiatric history, and social history. A mental status exam will be performed. If assessed by clinical judgment to be needed, vital signs and certain studies (e.g., ECG, basic labs) will be obtained. Follow up clinical study visits will be performed as needed.

- ***Emergency Escalation***
  - A study MD is always available and is the first point of contact in the case of an emergency.
  - In the event of a significant safety concern or medical emergency, staff will call emergency medical services (911 from a cell phone or 9-911 for University Police from a campus phone) or the study MD.

- ***Radiographic or other imaging assessments.*** Participants will be screened for MRI contraindications prior to enrollment in the study (at the screening visit). The Stanford CNI MRI safety form will be completed by the participant prior to each imaging session to confirm no changes in medical history occurred relevant to MRI contraindications. This safety screening tool includes questions regarding occupational/hobby risks (metal workers), surgical procedures

involving implants and devices, as well as questions about tattoos and piercings. The investigator or qualified study staff will review MRI risks and the MRI safety form in detail when signing the study informed consent and before each imaging session. Finally, a hand-held metal detector will be used to confirm the participant has removed all metal from their person (hair clips, jewelry, etc). If an incidental finding is discovered upon imaging that either the study staff (physicians) and/or CNI assigned radiologist deems unsafe or requires immediate medical followup, the participant will be alerted to the need for appropriate clinical follow up according to the protocol that has been created by the Stanford Center for Cognitive and Neurobiological Imaging (CNI) (https://cni.stanford.edu/wiki/Operations#Incidental_Findings).

- MRI Safety Screening Form: This is a participant self-report instrument. Participants will fill out a MRI Safety Screening Form to determine if the participant is safe to receive an MRI. We will utilize this screening tool to make sure that the participant has no contraindications for MRI that would exclude the participant from participating.

- ***TMS safety:***
  - Transcranial Magnetic Stimulation Adult Safety Screen (TASS)[1]: This is a participant self-report instrument. Participants will fill out a TASS questionnaire to determine risk of seizure related to rTMS. Any identified seizure risk will disqualify the participant. This assessment is utilized in order to minimize the risk of seizure induction with rTMS.
  - As part of the clinical study visit, the study psychiatrist (or their designee) will review the participant's medical history to assess for any contraindications to receiving TMS.
  - Seizure monitoring: Treaters will be monitoring subjects for the potential development of seizures by continuous visual inspection during the course of each stimulation session (especially the more subtle signs and symptoms of frontal lobe seizures) and are trained in first responder management in the event of a seizure. In the event of a seizure or other medical emergency, EMS would be called for further support and management and to transport the participant to our local Emergency Department, which is located minutes away on the Stanford campus.
  - TMS adverse event monitoring: AEs will be monitored continuously by designated study staff, recorded in applicable subject-specific and study wide REDCap eCRFs, and reported to the Stanford IRB annually. Research staff will be trained to attend to signs of AEs and to report any potential unrelated or unexpected AE immediately to the PD or study psychiatrists. Follow-up of any AEs/SAEs will be performed as described in 8.3.4.

- ***Depression/Psychiatric Assessments:***

  - Young Mania Rating Scale (YMRS): This is an 11-item clinician-administered scale used to measure symptoms of mania. Each item is scored from 0-4 with scores of 4 indicating the presence of more severe mania. Participants who are currently experiencing a manic episode will not be enrolled in the study. This scale will be used to monitor symptoms of mania each day they receive aiTBS to make sure manic symptoms have not arisen during study enrollment. If patients develop hypomanic/manic symptoms (defined as YMRS ≥ 12), no further stimulation will be administered and the patient's clinician will be notified.
  - Item 9 on the Montgomery and Åsberg Depression Rating Scale - Self Report (MADRS-S - item 9): The 9-item self-report version of the MADRS has an overall score range from 0 to 27 with higher scores corresponding to higher levels of depression. We will use item 9 ('Zest for Life') as a safety measure in regards to suicide risk. A score of 3 on that item will trigger a consultation with the study psychiatrist or their designee which will include an

MSSI to further assess suicide risk and determine whether it is safe for the participant to continue in the study and whether any further intervention is indicated to maintain the participant's safety.

- CTQ and FIGS assessments will be given to those participants who consent for the optional blood draw study. CTQ responses will determine any correlation between LAC levels, aiTBS response, and childhood trauma. FIGS responses will be used to determine any correlation between LAC levels, aiTBS response, and family history of mental illness.

- All participants will receive a weekly phone call check-in from a CRC between Visits 8 and 9 for support and to ensure continuity. If any safety concerns are identified, the study MD will be alerted for further assessment and management, if indicated.

- CTQ and FIGS assessments will be given to those participants who consent for the optional blood draw study. CTQ responses will determine any correlation between LAC levels, aiTBS response, and childhood trauma. FIGS responses will be used to determine any correlation between LAC levels, aiTBS response, and family history of mental illness.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

*An **adverse event (AE)*** is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a person who has received stimulation with a TMS device or an MRI scan. The event need not be causally related to the TMS device or the MRI scan in order to be considered an AE.

An AE includes, but is not limited to:

- Any adverse finding including a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these.
- Any clinically significant worsening of a pre-existing condition;
- An AE that has been associated with a preexisting condition is a clinical condition (including a condition being treated) that is diagnosed before an informed consent form is signed and is documented as part of the subject's medical history.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or frequency will be used to determine whether an event is an intervention-emergent AE (IEAE).

An AE is considered to be intervention-emergent if [1] it was not present when the active phase of the study began and is not a chronic condition that is part of the subject's medical history, or [2] it was present at the start of the active phase of the study or as part of the subject's medical history, but the severity or frequency increased during the active phase. The active phase of the study begins at Visit 3.

For this study, the intervention follow-up period for adverse events is defined as 30 days following the last day of study intervention.

Follow up will be documented in the subject's study file.

8.3.2 Definition of Serious Adverse Events (SAE)

A Serious Adverse Event (SAE) is defined as an AE that:

- Results in death
  - Is life threatening
    - Life threatening refers to immediate risk of death as the event occurred or use or continued use of the device or other medical product might have resulted in the death per the reporter. A life-threatening event does not include an event that, had it occurred in a more severe form, might have caused death, but as it actually occurred did not create an immediate risk of death
- Requires inpatient hospitalization or prolongation of an existing hospitalization
  - Admission to or prolongation of an existing partial hospitalization program (PHP) or intensive outpatient program (IOP), both of which are outpatient programs, are not included in this definition
  - Hospitalization is defined only as an actual admission
  - Hospitalizations planned before the start of the study, for a preexisting condition that has not worsened, do not constitute an SAE (e.g., elective hospitalization for a total knee replacement due to a preexisting condition of osteoarthritis of the knee that has not worsened during the study)
- Results in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life
- Necessitates medical or surgical intervention to preclude such impairment
- Results in a congenital anomaly or birth defect

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered SAEs, based upon appropriate medical judgment.

An adverse event of special interest is a device-specific adverse event designated by the PD for transmission in the same time frame as an SAE, even if it does not meet serious reporting criteria. See section 8.3.8 for further details.

If there is any doubt whether the information constitutes an SAE, the PD will review the event and make a determination.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

All AEs will be assessed by the study clinician using the grading system defined below and recorded on designated REDCap subject-specific and study-wide AE logs.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Causal relationship options and definitions are as follows:

· Definitely related: Event can be fully attributable to administration of the TMS stimulation or the MRI device.

· Probably related: Event is most likely to be explained by administration of the TMS stimulation or MRI device, rather than the subject's clinical state or other agents/therapies.

· Possibly related: Event is as likely explained by administration of the TMS stimulation or MRI device, as by the subject's clinical state or other agents/therapies.

· Probably not related: Event is most likely to be explained by the subject's clinical state or other agents/therapies, rather than the TMS stimulation or MRI device.

· Definitely not related: Event can be fully explained by the subject's clinical state or other agents/therapies, rather than the TMS stimulation or MRI device.

When assessing the relationship between an investigational product/protocol and an AE, the following parameters are considered:

· Temporal relationship between the TMS device/protocol and the AE

· Biologic plausibility of relationship

· Subjects' underlying clinical state or concomitant agents/therapies

· Where applicable, whether the AE abates on discontinuation of the TMS device or MRI device (de-challenge)

· Where applicable, whether the AE reappears on repeat exposure to the TMS device or MRI device (re-challenge)

SAEs that are not TMS/MRI device-related may nevertheless be considered by the Protocol Director or the IMC to be related to the conduct of the study, i.e., to a subject's participation in the study.

8.3.3.3 EXPECTEDNESS

	Number of participants reporting each adverse event (%)*	
	10 Hz rTMS group (n=204)	iTBS group (n=208)
Headache	131 (64%)	136 (65%)
Nausea	22 (11%)	14 (7%)
Dizziness	8 (4%)	18 (9%)
Unrelated medical problem†	47 (23%)	46 (22%)
Fatigue	14 (7%)	16 (8%)
Insomnia	14 (7%)	10 (5%)
Anxiety or agitation	8 (4%)	9 (4%)
Back or neck pain	7 (3%)	6 (3%)
Unrelated accidents	2 (1%)	3 (1%)
Vomiting	1 (<1%)	1 (<1%)
Tinnitus	1 (<1%)	3 (1%)
Migraine aura	3 (1%)	4 (2%)
Abnormal sensations	2 (1%)	4 (2%)

rTMS=repetitive transcranial magnetic stimulation. iTBS=intermittent theta burst stimulation. *p>0·05 on Fisher's exact tests for each pair of proportions. †Predominantly common infections such as colds and flus.

Table 3: **Adverse events**

In Table 3 above, there is a list of common, expected AEs[87]. Worsening of any symptoms of depression or related to depression also is an expected AE, as could be the case for any clinical trial of a novel therapy, particularly when there is a sham control condition as in our case. Furthermore, any iTBS study has a risk of mania and hypomania as an expected AE. The study psychiatrist, in accordance with the listed risks on the study ICF, will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Additional expected potential AEs associated with the intervention and other study procedures include:

- Seizure
- Induction of mania
- Mild pain at site of stimulation
- Anxiety
- Panic
- Symptoms of claustrophobia (feeling fearful, sweating, chills, dizziness, nausea, dry mouth, high heart rate, and high blood pressure)
- Rash, itching, redness, irritation, pain, swelling, and blistering conditions

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate electronic case report form (eCRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or until medical stability as judged by the PD or his designee.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study psychiatrist will record all reportable events with start dates occurring any time after the first study intervention until 30 days following the last day of study intervention. At each study visit, study staff will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

AEs will be systematically screened for at the start and end of every visit beginning at the active phase of the study (Visit 3). They will be screened for via the following standardized questions that will be asked by research staff:

- Have you had any difficulty with the study interventions?
- Have you had any problems or other things bothering you since our last visit?
- Has anything bothered you enough to...
    - ...prevent you from doing anything you had planned?
    - ...take a prescription or over-the-counter medication or supplement?
    - ...see a medical professional?
    - ...modify the treatment of a pre-existing medical condition?
- Have you been admitted to a hospital or seen in an emergency / urgent care clinic?

Any positive response(s) to the above questions will be documented electronically and further investigated as appropriate. Any documented AE will be asked about at each subsequent AE assessment until it resolves or through the time frame specified above. If an unexpected AE occurs, or if a common AE occurs with severity greater than that expected, the participant will be further evaluated by a member of the study team with formal medical training.

In addition, although the risk of TMS-induced mania is low[211], it will be monitored using the YMRS scale as a solicited event. The scale will be administered at the end of each stimulation day.

8.3.5 Adverse Event Reporting

AEs will be monitored continuously by designated study staff, recorded in applicable subject-specific and study wide REDCap eCRFs, and reported to the Stanford IRB annually.

Research staff will be trained to attend to signs of AEs and to report any potential unrelated or unexpected AE immediately to the PD. If AEs occur, appropriate medical and/or psychiatric care will be facilitated for the research participant, and recorded in the appropriate electronic AE logs on REDCap. As per Stanford IRB requirements, AEs will be reported to the IRB annually. The NIMH Program Officer will be informed of any actions taken by the IRB as a result of any AEs within 7 business days of notification by the IRB (e.g. study modifications imposed by the IRB).

All subjects will have telephone and email contact information to reach the Protocol Director and the Stanford IRB, in case of any distress or adverse response to rTMS or other components of the study.

All AEs and SAEs will be recorded on the subject-specific and study wide eCRFs, which will be provided to the DSMB on a bi-annual basis. Signs and symptoms will be recorded using standard medical terminology.

The following AE information will be included (when applicable): the specific condition or event and direction of change; whether the condition was preexisting (i.e., an acute condition present at the start of the study or history of a chronic condition) and, if so, whether it has worsened (e.g., in severity and/or frequency); the dates and times of occurrence; severity; causal relationship to the TMS device; action taken; and outcome.

8.3.6 Serious Adverse Event Reporting

The PD or his designee will immediately report to the sponsor any serious adverse event (SAE), whether or not considered study intervention related, including those listed in the protocol or investigator brochure, and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are SAEs (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event. In that case, the investigator will immediately report the event to the sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems either the event is chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

In case of SAEs, the study investigator will complete an Unanticipated Serious Adverse Event Form and submit to the study sponsor and to the reviewing Institutional Review Board (IRB) as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect. The study sponsor will conduct an evaluation of an unanticipated adverse device effect and will report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRBs and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter, the

sponsor will submit such additional reports concerning the event as FDA requests. The Investigator will provide all relevant documentation pertaining to an SAE (e.g., additional laboratory tests, consultation reports, discharge summaries, postmortem reports, etc.) to the DSMB and NIMH in a timely manner. Reports relative to the subject's subsequent course will be submitted to the DSMB and NIMH until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

Designated study staff will be responsible for ensuring that unanticipated problems are reported to the IRB in compliance with their requirements for reporting serious and unexpected adverse events. Reporting will be conducted in compliance with guidelines specified by the Stanford University Research Compliance Office.

8.3.7 Reporting Events to Participants

At enrollment, participants will be asked whether or not they would like to be informed about study outcomes. We will compile a list of those participants who would like to know about outcomes, and following study completion they will be individually emailed a copy of the study's primary outcome paper using individual, encrypted email.

We will closely monitor AE's on a continuous basis and will involve the independent DSMB in assessing whether any AE is unexpected or more severe than expected (at a rate greater than what would be expected by chance). Should we identify any AE that meets one of those two criteria or should we have any unexpected SAE's, we will notify currently enrolled participants and change our consent to include the pertinent information.

Incidental findings are likely to occur in one of two categories including clinical diagnostic findings and radiographic abnormalities. Should a pertinent clinical diagnostic finding (for instance an interview highly suggestive of occult bipolar disorder), we will inform the participant's psychiatrist. Should an incidental radiographic abnormality be detected during MRI, we will send the imaging to Stanford neuroradiology (as further described in 8.2).

8.3.8 Events of Special Interest

An adverse event of special interest is a device-specific adverse event designated by the PD for transmission in the same time frame as an SAE, even if it does not meet serious reporting criteria. For this protocol, seizure should be reported as an adverse event of special interest. If there is any doubt whether the information constitutes an SAE, the information should be treated as an SAE for the purpose of this study.

Other reportable information that does not meet the definition of an SAE but is reportable to NIMH and the DSMB with the timeliness of an SAE includes:

- Seizure
- Pregnancy occurring during the study period in which the subject was exposed to the TMS device;
- Overdose (e.g., a dose higher than that prescribed by a healthcare professional for clinical reasons) with or without AEs;
- Abuse (e.g., use for non-clinical reasons) with or without an AE;

- Inadvertent or accidental exposure with or without an AE;
- Device malfunction that would likely result in death, serious injury or other significant adverse event.

8.3.9 Reporting of Pregnancy

We will not include pregnant women in this study. If the participant is of childbearing potential and not already pregnant, the participant must agree to use adequate contraception (hormonal / barrier method of birth control or abstinence) prior to study and for the duration of the study participation. During the screening phase of this study, participants of childbearing potential will be administered a pregnancy test; a negative pregnancy test will be required to confirm eligibility for study participation and will be completed prior to their receiving any rTMS stimulation. If a participant becomes pregnant or suspects she is pregnant while participating in the aiTBS stimulation week of the study, she should inform the research staff immediately via in-person meeting, telephone call, or email. If a participant reports pregnancy occurring during the study period in which the participant was exposed to the rTMS stimulation, research staff will stop rTMS stimulation and then notify the Protocol Director.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PD's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) and/or result in unexpected deaths or life-threatening experiences related to the research will be reported to the IRB and to the Data Safety Monitoring Board (DSMB) within 10 days of the investigator becoming aware of the event.
- Any other UP will be reported to the DSMB within 30 days of the investigator becoming aware of the event and subsequently reported to the IRB within 10 working days from receiving assessment from the DSMB.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 30 days of the IRB's receipt of the report of the problem from the investigator.

An investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)). A sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.4.3 Reporting Unanticipated Problems to Participants

Should an unanticipated problem occur (such as identifying an AE that is more common or severe than expected, or a data breach of any sort), the investigators will inform those participants that have been affected of both the problem and any proposed solution. Should the unanticipated problem have the potential to affect future participants it will be added to the consent document.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

- Primary Mechanistic Endpoint:

We hypothesize that the reduction in resting-state functional connectivity between the subgenual anterior cingulate cortex and the default mode network from baseline through intervention day 5 will be significantly greater for participants receiving active as compared to sham aiTBS.

- Secondary Mechanistic Endpoints:

(a) We hypothesize that there will be a significantly greater association between the change in depression symptoms (as measured by the MADRS-S) and the change in resting-state functional connectivity between the subgenual anterior cingulate cortex and the default mode network from baseline to after intervention day 5 for participants receiving active as compared to sham aiTBS.

(b) We hypothesize that there will be a significantly greater association between acute mood state (as measured by the IMS-12) and the change in resting-state functional connectivity between the subgenual anterior cingulate cortex and the default mode network from baseline to after intervention day 5 for participants receiving active as compared to sham aiTBS.

9.2 Sample Size Determination

Sample size justification (power analysis):
Key Hypothesis of the study: A significant reduction in functional connectivity between sgACC and DMN. We hypothesize that the reduction in functional connectivity between the subgenual cingulate cortex and the default mode network from baseline to after intervention day 5 will be significantly greater for participants receiving active compared to sham aiTBS. Several past studies have found no significant changes in functional connectivity with sham rTMS targeted to DLPFC[136,212]. Using preliminary data from our open-label study of aiTBS for treatment of severe depression, there was an effect size of 1-1.47 (depending on DMN node) for change in connectivity between sgACC and DMN[150]. One potential approach for determining the power calculation would be that considering we will have 10 unique comparisons with each day's data be compared to every other day over 5 days, and that we will be comparing across 4 DMN nodes and 2 sgACC nodes, our target alpha needs to be 0.05/(10*4*2) = 0.000625. Thus, for an effect size of 1, alpha of 0.000625, and a power of 0.80, and powering for an independent-measures t-test, 40 participants will be required for each arm. Regarding our secondary mechanistic endpoints, we have previously found a significant correlation between change in FC between sgACC and DMN in participants receiving open-lab aiTBS, Pearson r = 0.51. For a point biserial model, a two-tailed 2 test, and a power target of 0.80, 25 participants will be required for each group.

Our recruitment goals are based on our primary mechanistic endpoint. Considering a ~25% attrition rate (combined participants lost to followup and unusable or missing scans), **we plan to recruit 50 participants in each arm,** as this is larger than the 18 per arm that is required to power for a significant reduction in IMS-12, and large enough to provide sufficient power for our secondary mechanistic endpoints**.** Given that there are ~1,000 new depressed patients at Stanford per year, >75% are TMS-naïve, there are many more than 100 patients per year that meet inclusion criteria.

9.3 Populations for Analyses

This protocol is designed to test the mechanistic hypothesis that aiTBS reduces functional connectivity between sgACC and DMN. Thus, these mechanistic analyses will include only a Per-Protocol Analysis Dataset. The Per-Protocol Analysis Dataset defines a subset of the participants who complied with the protocol sufficiently to ensure that these data would be likely to represent the effects of study

intervention according to the underlying scientific model (e.g., participants who took at least 80% of study intervention for 80% of the days within the maintenance period).

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Descriptive Statistics: Categorical data will be presented as percentages. Continuous data will be presented as means with standard deviations.

Inferential tests: Statistical significance will be set at $p<0.05$, two-tailed.

Covariates: Baseline demographic and clinical measures will be compared across intervention groups using standard statistical methods (i.e., categorical variables compared using Pearson chi square test of independence; continuous variables compared using univariate ANOVAs). Characteristics that display imbalance at the baseline measure or an association with the dependent variable will be assessed for inclusion as covariates in the final models.

Assumption checks: Continuous data will be analyzed using standard linear mixed-effects modeling in line with intent to treat analysis principles. If residuals of the standard linear models are non-normally distributed (Shapiro-Wilk), then data will be analyzed with generalized linear mixed models with Satterthwaite approximation of degrees of freedom and robust estimation of coefficients to handle violations of model assumptions. Autoregressive covariance structure will be used for all analyses except for cases in which the models fail to converge or converge on non-real solutions. In these cases, alternate covariance structures will be explored until the models converge on real solutions. All post-hoc pairwise comparisons will be Bonferroni-corrected for multiple comparisons.

General approach considerations: Primary analysis models will be reported both unadjusted and adjusted for significant clinical covariates. All statistical analysis will be conducted using SAS/STAT version 9.4 (SAS Institute Inc. 2015. SAS® 9.4 Statements: Reference, Fourth Edition. Cary, NC: SAS Institute Inc.). All statistical analyses will be overseen by our biostatistician Dr. Booil Jo, an associate professor in the Stanford department of psychiatry and behavioral sciences - Center for Interdisciplinary Brain Sciences Research. Dr. Jo holds a PhD in Applied Statistics from UCLA and is a leading expert in statistical analysis of mental health research.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Our primary hypothesis is that participants receiving per-protocol active aiTBS will demonstrate a significant attenuation of resting-state functional connectivity (FC) between subgenual anterior cingulate cortex (sgACC) and default mode network (DMN) compared to per-protocol sham intervention.

We will use fMRI to measure resting-state FC between sgACC & DMN. Participants will be treated for 5 consecutive days with 10 sessions per day of active or sham aiTBS. Participants have a baseline MRI scan prior to each day of aiTBS intervention as well as the day following the completion of intervention. After standard preprocessing of fMRI data, we will calculate FC as the correlation of the activity pattern across

time between the sgACC and DMN regions of interest. All correlation coefficients will then be transformed into Fisher's Z score for further analysis.

Baseline demographic and clinical measures will be compared across intervention groups using standard statistical methods (i.e., categorical variables compared using Pearson chi square test of independence; continuous variables compared using univariate ANOVAs). Characteristics that display imbalance at the baseline measure or association with the dependent variable will be assessed for inclusion as covariates in the final models. Differences in effects as a function of gender will be considered for all analyses.

FC data being a continuous variable, will be analyzed using standard linear mixed-effects modeling, with time and intervention group (active vs. sham) set as fixed factors. Time (throughout the intervention) will be set as the within-subject, repeated measure. The effects of intervention group, time, and their interaction will be included in the model. If residuals of the standard linear models are non-normally distributed (Shapiro-Wilk), then data will be analyzed with generalized linear mixed models with Satterthwaite approximation of degrees of freedom and robust estimation of coefficients to handle violations of model assumptions. Autoregressive covariance structure will be used unless the model fails to converge or displays variance inflation. In these cases, alternate covariance structures will be explored until the models converge on real solutions. All post-hoc pairwise comparisons will be corrected for multiple comparisons.

The null hypothesis (i.e. no difference in change in FC between active and sham intervention groups) will be rejected if the intervention group x time interaction has a p-value < 0.05. Data will be presented as least-squares means with standard errors.

9.4.3 Analysis of the Secondary Endpoint(s)

Our secondary hypotheses are:

(a) We hypothesize that there will be a significantly greater association between the change in depression symptoms (as measured by the MADRS-S) and the change in resting-state functional connectivity between the subgenual anterior cingulate cortex and the default mode network from baseline to after intervention day 5 for participants receiving active as compared to sham aiTBS.

(b) We hypothesize that there will be a significantly greater association between acute mood state (as measured by the IMS-12) and the change in resting-state functional connectivity between the subgenual anterior cingulate cortex and the default mode network from baseline to after intervention day 5 for participants receiving active as compared to sham aiTBS.

We will use fMRI to measure resting-state FC between sgACC & DMN. Participants will be treated for 5 consecutive days with 10 sessions per day of active or sham aiTBS. Participants have a baseline MRI scan prior to each day of aiTBS intervention as well as the day following the completion of intervention. After standard preprocessing of fMRI data, we will calculate FC as the correlation of the activity pattern across time between the sgACC and DMN regions of interest. All correlation coefficients will then be transformed into Fisher's Z score for further analysis.

MADRS-S will be collected at baseline, the end of each intervention day, and the day following intervention. IMS-12 will be collected at baseline, on the morning of each intervention day, and the day following intervention.

Normality in change in both assessments and change in FC from baseline to post-intervention will be assessed via Shapiro-Wilk. Appropriate correlational analysis (Pearson or Spearman) will be used to quantify the association between change in FC and change in each assessment for each treatment group. Correlation coefficients will be compared statistically by performing Fisher's R-to-Z transformation and the difference between the two Z-scores will be tested for statistical significance using a two-tailed Z-test with an alpha level of 0.05 ($p<0.05$). The null hypothesis, i.e. no difference in the association between change in FC and change in each assessment between groups, will be rejected if the Z-test has a p-value < 0.05.

9.4.4 Safety Analyses

We will inquire about AE's at each visit as described above in the Safety and Other Assessment section above (section 8.2). AE's will initially be inquired about by clinical research coordinators and may then be assessed by study physicians during the same visit. The study physician in charge of assessing a given AE will provide a thorough description of the event in the participant's CRF and the AE will be followed up at each visit (or more frequently if needed) until it has completely resolved or until 30 days from the participant's last study visit. The study physician will specifically report on start date, stop date, severity, relationship, expectedness, outcome, and duration, as well as whether or not the AE meets criteria for a SAE. AE's will be coded based upon Medical Dictionary for Regulatory Activities (MedDRA) criteria, coded only once per participant (e.g. should a participant have a headache at each visit only a single headache AE will be coded), and compiled continuously for DSMB and progress reports. AE's will specifically be presented in table format based on System Organ Class, and grouped based on severity, relationship, and expectedness. Adverse events leading to premature discontinuation from the study intervention and serious intervention-emergent AE's, should they occur, will be presented separately in regulatory reports and final publications in table format. Once data lock and unblinding has occurred, safety data will be compared between active and sham aiTBS using chi-squared tests (or Fisher's exact test if indicated).

9.4.5 Baseline Descriptive Statistics

Demographic data will be summarized for sham and active intervention groups separately using standard descriptive statistics. For continuous measures (i.e. age and years of education), means and standard deviations will be calculated, and any group differences will be identified using univariate ANOVAs. Categorical measures (i.e. sex and race/ethnicity) will be quantified by counts and percentages, and any group differences will be detected using Chi-squared tests. Characteristics that display imbalance at the baseline measure or an association with the dependent variable will be assessed for inclusion as covariates in the final models.

9.4.6 Planned Interim Analyses

Because of the anticipated low level of adverse events of aiTBS given our recently completed smaller RCT, interim analysis of data, protocol and adverse events is not anticipated to be necessary. Serious adverse events will be reviewed on a monthly basis, unless a more urgent review is requested. As described in section 7, only under extreme circumstances or if it were determined that a high level of side effects was

due to aiTBS, would the PD be charged with breaking the study mask. This study will be stopped prior to its completion if: [1] the intervention is associated with adverse effects that call into question the safety of the intervention; [2] difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; [3] any new information becomes available during the study that necessitates stopping the study; or [4] other situations occur that might warrant stopping the study.

9.4.7 Sub-Group Analyses

We are recruiting 100 participants of all gender, ethnic, and racial backgrounds. We will assess the role of gender in our analyses to determine the potential utility of additional follow-up projects that focus on any discovered gender-based observations. However, it may not be feasible to run sub-group analyses in this study because of potentially insufficient sub-group sample sizes.

9.4.8 Tabulation of Individual participant Data

All participant data will be directly exported from REDCap. The exported data will be listed in a long format, such that each row (i.e. observation) represents a specific time point for each subject. The individual variables collected will be represented by columns (i.e. measures). For reproducibility, data will be exported and tabulated using either the R or Python programming language.

9.4.9 Exploratory Analyses

Two additional analyses will be applied to exploring the intervention effect-related FC features in the whole brain: the brain networks' organization and the multivariate FC patterns.

The brain is thought to organize in the way that brain regions could work together to maximize the ability to respond to cognitive demands while minimizing the wiring cost. Accordingly, brain regions are found to be closely correlated within brain networks, but for the regions across brain networks the correlations are relatively weaker or even anti-correlated[213]. We will calculate brain network segregation[214] to explore the relationship between the organization of brain networks and intervention effect. We will use Power's parcellation[215] and extract data from: somatosensory-motor, default-mode, frontoparietal, ventral attention, dorsal attention, cinguloopercular and salience networks. In order to calculate the brain network segregation, the mean within network correlation (Rwithin) will be calculated by averaging the FCs for regions within each network, and the mean between network (Rbetween) correlation will be calculated by averaging the FCs for regions from two different networks. Only positive correlations will be used in these calculations. And then the brain network segregation (BNS) will be calculated as: (Rwithin - Rbetween) / Rwithin [216]. BNS will then be compared between baseline and post-intervention to find the intervention related BNS, and then the group differences will also be tested. Linear regression models will be used to test the relationship between BNS and both depression scores and acute mood states.

We also will use TDA-based Mapper analysis (Saggar et al 2018; 2022) to investigate how brain state transitions (at rest) evolve in patients with depression undergoing accelerated iTBS intervention. By constructing high-dimensional manifolds of neuroimaging data and visualizing their topological structures as graphs, we aim to identify patterns of brain state changes associated with treatment. Additionally, we

will characterize the dose-response relationship of iTBS by analyzing how dosage (over the course of treatment) influences these state transitions, providing insights into individualized treatment optimization.

To understand the physiological mechanisms underlying the stimulation effects, we will employ the reduced Wong-Wang (RWW) neural mass model to simulate whole-brain dynamics. This modeling approach will complement our empirical analyses by providing mechanistic insights into how aiTBS influences brain network dynamics. The RWW model will be implemented by modeling each brain region as a neural mass unit described by the RWW equations. Structural connectivity data from diffusion MRI will inform the coupling between regions, and model parameters will be calibrated using resting-state fMRI data from baseline measurements. Stimulation effects will be modeled as external input to the targeted regions, with both direct stimulation effects and network-wide propagation being simulated. statistical analysis of model outputs will be compared with empirical fMRI data using Pearson correlation between simulated and observed functional connectivity patterns, root mean square error between model predictions and actual measurements, and analysis of dynamic functional connectivity states and their temporal evolution. For parameter optimization, we will employ Bayesian optimization to identify model parameters that best reproduce the observed changes in sgACC-DMN connectivity, network-wide reorganization patterns, and individual variation in treatment response. This approach will allow us to systematically explore the parameter space while accounting for the complex interactions between different model components. A crucial component of our analysis will focus on evaluating changes in model parameters, particularly the excitation/inhibition (E/I) balance across brain regions. We will quantify how aiTBS modulates the E/I balance by analyzing changes in local coupling parameters and synaptic gains before and after intervention. Statistical comparisons of these parameters between active and sham conditions will be performed using mixed-effects models with appropriate corrections for multiple comparisons. Additionally, we will examine the relationship between E/I balance changes and clinical outcomes to identify potential biomarkers of treatment response.

This modeling framework will provide insights into the causal chain from stimulation to observed clinical effects, individual variation in treatment response, and optimal stimulation parameters for maximizing therapeutic impact. The analysis will integrate with our primary and secondary endpoints by providing mechanistic explanations for observed changes in functional connectivity and clinical outcomes, while offering a deeper understanding of how aiTBS modulates neural dynamics through changes in local circuit properties and E/I balance.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

Two signed consent forms will be obtained from each participant: (1) Screening consent form and (2) Study consent form. These consent forms will be administered electronically using Adobe Sign.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The study investigator or his designee will explain the research study to the participant and answer any questions that may arise in a private setting. A verbal explanation will be provided in English of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Consenting Processes: There will be two consent forms: one consent for screening procedures, and the other for study and enrollment procedures. For the first consent process, the clinical research coordinator (CRC) will screen the participant's eligibility for enrollment in the study. The study PD or psychiatrist or their designee will consent the participants for the second phase of the consenting process, which involves discussing the risks of the rTMS procedure and MRI scanning. The participant will be consented in the sequence that has been defined by our protocol.

Education and Informed Consent Process: Consent forms will be Institutional Review Board (IRB)-approved, and the participant will be asked to read and review the document. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The signature will be electronically obtained as described above. The consent process will start with an introductory paragraph that describes the study. This statement will be followed by a description of the purpose of the research. The participant will be informed that this is a double blind study and they have a 50% chance of receiving active stimulation and that their participation is voluntary. Next, the duration of study involvement and the procedures involved will be described.

The participant will be notified of the discomforts and risks along with the potential benefits. The participant will receive a *Statement of Confidentiality*. The participant will be notified as to the costs for participation (none) as well as the compensation for participation. The participant will be made aware of the research funding source. The participant will be given information regarding the fact that their participation is voluntary. The rights and welfare of the participants will be protected by emphasizing to

them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The participant will be made aware of their alternative options. The participant will then be given the contact information for questions or concerns. The participant will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. All participants will be provided a copy of their signed consent forms.

Finally, after reading the experimental subjects' bill of rights, the participant will be asked for their signature in order to consent and give permission to be in the research study. To ensure the participant has capacity to consent, as part of this process the study psychiatrist or their designee will complete a Capacity to Consent form.

Plan for Review of Consent Document: The signed consent document will be confirmed by the PD, study psychiatrist, or their designee (which includes regulatory staff).

Documentation of Signed Consent: The screening and enrollment logs will be utilized to record the consent and screening of all subjects and the outcome of each screening. These logs will provide a comprehensive list of all subjects who were screened for eligibility and all participants who enrolled into the study. This information will be maintained inREDCap. All subjects who were consented and screened on site will be included in these logs, including screen failures.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, and funding agency. If the study is prematurely terminated or suspended, the Protocol Director (PD) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI and/or sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, as specified and approved by Stanford's IRB.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be securely transmitted to and stored within either Stanford REDCap or Stanford Medicine Box. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study data management systems used by Stanford research staff will be secured and password protected. At the end of the study, all study data will be archived on either Stanford REDCap or Stanford Medicine Box.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

<u>Those regulations require a signed subject authorization informing the subject of the following:</u>

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the Protocol Director, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects who have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period. Subject confidentiality is strictly held in trust by the investigators and the study staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the Protocol Director.

Any data, specimens, forms, reports, and other records that leave the Stanford study site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by the IRB and OHRP.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be stored and analyzed on Flywheel, a fully HIPAA and GDPR compliant platform for medical images. Permission to transmit data to Flywheel will be included in the informed consent, and imaging data will be automatically uploaded to the platform after acquisition. Only lab members with 2-factor authentication will have access privileges. After the study is completed, the de-identified data will continue to be stored on Flywheel in perpetuity for potential use by other researchers, including those outside of the study.

When the study is completed, access to study data will be provided through the Flywheel Medical Imaging Platform and Data Repository. Or alternatively the data can be exported and shared in an encrypted format to be shared.

10.1.5 Key Roles and Study Governance

Principal Investigator	Protocol Director	Study Psychiatrists	Study Psychologist	Neuro-Imaging Lead
Nolan Williams, MD	David Spiegel, MD	Greg Sahlem, MD and Ian Kratter, MD, PhD Thomas Knightly, MD Chris Austelle, M Bora Kim, MD	Flint Espil, PhD	Manish Saggar, PhD
Stanford University	Stanford University	Stanford University	Stanford University	Stanford University
401 Quarry Road Mail Code 5719 Stanford, CA 94305	401 Quarry Road Mail Code 5719 Stanford, CA 94305	401 Quarry Road Mail Code 5723 Stanford, CA 94305	401 Quarry Road Mail Code 5719 Stanford, CA 94305	401 Quarry Road Mail Code 5719 Stanford, CA 94305
nolanw@stanford.edu	dspiegel@stanford.edu	gsahlem@stanford.edu ikratter@stanford.edu thomas.knightly@stanford.edu	espil@stanford.edu	saggar@stanford.edu

		austelle@stanford.edu borakim2@stanford.edu		

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise in study relevant subjects including treatment-resistant depression, TMS, and statistics. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least biannually to assess safety and efficacy data on each arm of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to NIMH.

There are three DSMB members:

1. **Charles Nemeroff:** Dr. Charles Nemeroff is Chair and Professor with the Department of Psychiatry and Behavioral Sciences at Dell Medical School at the University of Texas at Austin. He also directs the Institute for Early Life Adversity Research within the Department of Psychiatry and Behavioral Sciences as part of the Mulva Clinic for the Neurosciences. Prior to joining Dell Med, Dr. Nemeroff was chair of the Department of Psychiatry and Behavioral Sciences and clinical director of the Center on Aging at the University of Miami Miller School of Medicine in Miami, Florida. He received his medical degree and doctorate degrees in neurobiology from the University of North Carolina (UNC) School of Medicine. After psychiatry residency training at UNC and Duke University, he held faculty positions at Duke University Medical Center and at Emory University School of Medicine before relocating to the University of Miami in 2009. He has served as president of the American College of Psychiatrists (ACP) and the American College of Neuropsychopharmacology (ACNP), and he sits on the Scientific Advisory Board and board of directors of the American Foundation for Suicide Prevention (AFSP) and the Anxiety and Depression Association of America (ADAA).

2. **Ian Cook:** Dr. Ian Cook holds the Joanne and George Miller and Family Endowed Chair in Depression Research and is a Professor of Psychiatry and Biobehavioral Sciences at the David Geffen School of Medicine and a Research Scientist at the Semel Institute for Neuroscience and Human Behavior. Dr. Cook received his Bachelor's degree with high honors from Princeton University and his medical degree from the Yale University School of Medicine. He completed his psychiatry residency training at UCLA's Neuropsychiatric Institute, where he also was an NIMH-funded research fellow. Dr. Cook served on the Executive Committee on Practice Guidelines of the American Psychiatric Association, and he guided the electronic dissemination of their evidence-based guidelines in psychiatry.

3. **Rita Popat:** Dr. Popat is a Clinical Associate Professor in the Department of Epidemiology and Population Health at Stanford University. Her research interest focuses on the epidemiology of

Parkinson's disease and amyotrophic lateral sclerosis, specifically evaluating the genetic and environmental contributions to these neurodegenerative disorders. She is also interested in studying the relation of cognition, estradiol exposure (endogenous and exogenous), and genetic factors. At Stanford she additionally co-directs the MS program in Clinical Research & Epidemiology and is a member of the steering committee for the Stanford Medicine Teaching and Mentoring Academy.

10.1.7 Clinical Monitoring

Safety oversight of this study will be under the direction of a Data and Safety Monitoring Board (DSMB), as detailed in section 10.1.6. Dr. David Spiegel will serve as the study medical monitor. We will hold internal weekly meetings to review significant changes study recruitment, enrollment numbers, AE/SAEs, and overall study progress. All participant safety data will be captured in eCRFs. Study psychiatrists will review participant eCRFs periodically to ensure adequate clinical monitoring.

10.1.8 Quality Assurance and Quality Control

We will perform internal quality management of study conduct, data collection, documentation and completion, per Quality Management SOP..

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical study is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

Staff Training:

- rTMS Training: Every TMS operator on this study will be trained in basic knowledge of brain physiology, basic mechanisms of TMS, the potential risks of the rTMS procedure, and the physiological changes induced by rTMS[182]. All trainings will be led by the PD, study psychiatrists, or trained TMS operator. This training will be stored in REDCap. In order to operate the rTMS device, all operators must have approval from a trained TMS operator.

- MT Training: The PD, study psychiatrists, or trained TMS operator will train all TMS operators and relevant study personnel to acquire proper motor thresholds for TMS intervention via visual observation and neuronavigated mapping combined with an automated threshold-quantification technique named PEST (Parameter Estimation by Sequential Testing). Training will include determination of MT via visual observation and neuronavigated mapping combined with the PEST technique. In order to acquire MT using the TMS coil, all operators must have approval from a trained and certified TMS operator.

- MRI Scanning: Study personnel who perform MRI scanning will have to receive appropriate MRI training from the Stanford Center for Neurobiological Imaging (CNI) in accordance with their protocols (https://cni.stanford.edu/wiki/MR_Protocols), as all MRI scans will be conducted in their facility.

Ongoing quality control will include regular data verification and protocol compliance checks to be performed by the senior research staff and the research team (designated study staff). An ongoing review of study procedures will be done to ensure that the privacy of participants and confidentiality of data are not violated. There will also be adequate provisions for monitoring the collected data to ensure the safety of participants and to maintain the confidentiality of the research data. The CRC will be responsible for addressing quality assurance issues (correcting procedures that are not in compliance with protocol) and quality control issues (correcting errors in data entry).

Source data comprise all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. When applicable, information recorded on the CRF shall match the source data recorded on the source documents.

Following written standard operating procedures, this monitor will verify that this clinical study is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical study staff at the site under the supervision of the site investigator. A dedicated data manager will oversee the collection and organization of study data, and the investigator will ensure the accuracy, completeness, legibility, and timeliness of the data reported.

All study data, except for medical images, blood specimens, and select consent forms, will be stored in an electronic data capture (EDC) system called REDCap. Study data stored on REDCap will include CRFs as well additional clinical data such as adverse events (AEs), current medications, TMS session parameters. Medical images will be stored on Flywheel (see section 10.1.4). Documentation of the blood draws will be extracted from EPIC and will be filed accordingly in REDCap.

To ensure the protection and confidentiality of patient information, we will have two REDCap databases. One database will contain any PHI collected during the study and will be accessible to only the PD, the lab data manager, and the clinical coordinators collecting and entering this data. A separate database will contain all non-PHI data collected during the study. Subjects in this database will be identified using only their study identifier. Lab members collecting and entering this data will have access to this REDCap.

Edit checks, electronic queries, and audit trails are built into REDCap to ensure accurate and complete data collection and security. In addition, pdf copies of the case files will be created for each subject where completed CRFs will be securely stored..

The REDCap database will be centralized on a dedicated file server located on a secure rack in the Stanford University Forsythe Hall data center. The data center is maintained by Stanford University IT Services and includes secured entry, 24/7 monitoring, environmental control systems, fire detection and suppression, and an Uninterruptible Power Supply (UPS) for protection against power anomalies. A Backup and Recovery Service (BaRS) is available. The file server is only accessible through the Stanford University network (SUNET), however off campus access to the file server through SUNET is sometimes necessary and a secure connection is available through the Stanford Virtual Private Network (VPN) service. This file server has been utilized in the past to store databases for previous studies and also used the VPN service without incident. All raw data forms received will be promptly stamped, entered or scanned, and filed in a locked filing cabinet. No information will be released to persons other than the patient without permission. Confidentiality is assured. Results of all studies are published in a manner that does not reveal the identity of individual subjects.

The database and web servers will be secured through controlled physical access. For security reasons, and in compliance with regulatory guidelines, remote access to the EDC system is only granted to users who complete 2-factor authentication by providing a correct username and password as well as confirm their identity using a physical device. Access codes are non-transferrable. Personnel who have not undergone training will not access the study eCRF until appropriate training is completed and documented. The eCRF data elements do not reside on the user's workstation; they are securely transmitted to a database as forms are completed or updated. Protocol-specified source documents (e.g. hospital discharge summaries, operative/procedural reports) will be retrieved as necessary. Copies of all study-related documentation will be retained within the central laboratory. Case files will be located in a secured area at the study site. All completed CRFs will be de-identified and subjects will be referred to using only their assigned study subject identifier and initials. Information stored in the source documents will be safeguarded according to institutional guidelines.

Access to the database will be restricted and only the database manager can view and update the complete database. Research assistants will be able to modify parts of the database to enter data or make necessary corrections to the data. Access to specific parts of the database can be granted to other project members with permission obtained through the Protocol Director. All personnel will be required to successfully complete HIPAA and CITI group 7 training. Access to the database on the file server is monitored and controlled through SUNET and separate SUNET IDs and passwords are required. Secure email alternatives will be used to safely communicate information or send data between project members. Stanford IT Services provides a Secure Email service, which is very easy and seamless to use. To achieve email encryption, only the word "SECURE:'' needs to be added to the subject line in an email message. Stanford School of Medicine Information Resources and Technology (IRT) group also provides MedSecureSend (MSS) service for large files up to 20GB. Secure Email is only available to project members and collaborators with SUNET IDs. For collaborators outside of campus, a MedSecureSend email message can include an option to invite a recipient to create a MedSecure account before the content of the email can be opened or read.

Participants who agree to have their blood drawn will have their blood spun down, and the plasma will be aliquoted and stored in a -80 degree Celsius freezer. Labels will have no personal identifiers. A portion of these plasma samples may be shipped on dry ice to our collaborators at New York University and/or University of Antwerp. The remaining portion will be stored at Stanford. Buffy coat will also be

collected and stored at Stanford. Some or all of these samples may be sent outside of Stanford for analysis.

10.1.9.2 Study Records Retention

In accordance with NIH grant policy, all electronic and paper records for this study will be maintained for at least three years from when the annual Federal Financial Report (FFR) is submitted to the NIH. Permission to destroy any records after this time is at the discretion of the principal investigator and the department or laboratory.

10.1.10 Protocol Deviations

A protocol deviation is defined as any noncompliance with the clinical study protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. In general protocol deviations are not allowed, except as stipulated below. We will continually monitor for instances of protocol deviation. Should a deviation occur we will record the deviation in a pre-designated subject-specific Protocol Deviation CRF and report the reason and nature of the deviation to both the Stanford IRB and the Data Safety Monitoring Board (DSMB) responsible for this study. All documentation will be reported depending on the type of deviation (see below for time-frames of reporting). Protocol deviations that constitute unanticipated problems involving risks will be promptly reported to the Stanford IRB.

A protocol deviation that constitutes an *unanticipated problem involving risks to subjects or to others* will be reported promptly to the IRB, as follows:

1. Emergency deviations: When a deviation occurs in an emergency situation, such as when a deviation from the protocol is required to protect the life or physical well-being of a participant. The Stanford IRB will be notified as soon as possible and no later than 10 days after the PD has been informed of the emergency situation. The PD or his designee will submit a report to the Stanford IRB.

2. Major, non-emergent deviations without prior approval: A planned deviation that is non-emergent and represents a major change in the protocol as approved by the Stanford IRB. The Stanford IRB must approve the request before the proposed change is implemented. The PD or co-investigators must submit non-emergent deviations to the IRB for review. If a major, non-emergent deviation occurs without prior IRB approval the event is considered non-compliance. Non-compliance will be reported to the IRB promptly. The PD or co-investigator's failure to report promptly any major, non-emergent deviation for which the PD did not obtain prior approval is itself an incident of non-compliance.

3. Protocol deviations that are only minor or administrative: At Stanford, minor or administrative protocol deviations are defined as those, which do not *affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects*. If a protocol deviation occurs which meets this definition, the deviation will be reported to the Stanford IRB at the time the continuing review application is submitted. Examples of minor or administrative deviations could include: follow up visits that occurred outside the protocol required time frame because of the participant's schedule, or blood samples obtained at times close to but not precisely at the time points specified in the protocol.

The above practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this study will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting the PI.

We will adhere to the NIH Grants Policy on Availability of Research Results: Publications, Intellectual Property Rights, and Sharing Biomedical Research Resources. Specifically, transfers of research materials out of the laboratory to other researchers will be made under terms no more restrictive than in the Simple Letter Agreement for the Transfer of Materials or the Uniform Biological Materials Transfer Agreement (UBMTA) and made without reach-through requirements. Should any intellectual property arise that may be patentable, Stanford will ensure that the related technology (materials and final research data) remains widely available in a timely fashion to the research community in compliance with policies and regulations governing research awards from the NIH.

After the outcome data have been published, the data will be made available to individuals or institutions who have received IRB approval to conduct secondary data analyses on the dataset. The final dataset will include data on mood, effects of psychological and neuromodulatory intervention on mood and fMRI neuroimaging of regional brain activity, functional connectivity, and structure.

Thus, we will make the data and associated documentation available to users only under a data sharing agreement that provides for: (1) a commitment to using the data only for research purposes and not to identify any individual participant; (2) a commitment to securing the data using appropriate computer protection technology; and (3) a commitment to destroying or returning the data after analyses are completed. The datasets will be put on a computer disk (CD) that is encrypted. Once verification of IRB approval is obtained, we will send the encryption code and CD in separate mailings to the investigator(s). All storage and sharing of data will be in accordance with Stanford IRB policy and approval, and HIPAA guidelines. By requesting IRB approval, we will be able to track recipients of the data, the type of data analyses proposed, and subsequent research findings. This data will be compiled in our database and may be of interest to NIH as well as lend itself to subsequent secondary data analyses that are important to the fields of emotion regulation, CNS neurophysiology, fMRI, and depression treatment.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this study will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this study. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AE	Adverse Event
aiTBS	Accelerated Intermittent Theta-Burst Stimulation
ATHF	Antidepressant Treatment History Form
BaRS	Backup Recovery Service
BNS	Brain Network Segregation
BPD	Borderline Personality Disorder
BOLD	Blood-oxygen-level-dependent
CAC	Clinical assessment committee
CEN	Central Executive Network
CFR	Code of Federal Regulations
CITI	Collaborative Institutional Training Initiative
CNI	Stanford Center for Cognitive and Neurobiological Imaging
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019
CRC	Clinical Research Coordinator
CRF	Case Report Form
cTBS	Continuous Theta Burst Stimulation

CTQ	Childhood Trauma Questionnaire
DMN	Default Mode Network
DSM-5	Diagnostic and Statistical Manual of Mental Disorders- version 5
EC	Ethics Committee *or* Effective Connectivity
ECG	Electrocardiography
ER	Emotion Regulation
eCRF	Electronic Case Report Form
ECT	Electroconvulsive Therapy
EDC	Electronic Data Capture
EDTA	ethylenediaminetetraacetic acid
eIRB	Electronic IRB
FC	Functional Connectivity
fcMRI	Functional Connectivity Magnetic Resonance Imaging
FDA	Food and Drug Administration
FIGS	Family Interview for Genetic Studies
fMRI	Functional Magnetic Resonance Imaging
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IC	Institute or Centre
ID	Identification
IDE	Investigational Device Exemption
IEAE	Intervention-emergent AE
IMC	Independent Monitoring Committee
IMS	Immediate Mood Scaler
IND	Investigational New Drug
IQ	Intelligence Quotient
IRB	Institutional Review Board

IRT	Resources and Technology group
ISI	Intersession Interval
ISTS	International Society for Transcranial Stimulation
iTBS	Intermittent Theta-Burst Stimulation
ITT	Intent to Treat
LAC	Acetyl-L-carnitine
L-DLPFC	Left Dorsolateral Prefrontal Cortex
LTD	Long-term Depression
LTP	Long-term Potentiation
MADRS-CR	Montgomery-Asberg Depression Rating Scale-clinician rated
MADRS-S	Montgomery-Asberg Depression Rating Scale- Self Report
MC	Motor Cortex
MDD	Major Depressive Disorder
MI	Myocardial Infarction
MINI	MINI International Neuropsychiatric Interview
MRI	Magnetic Resonance Imaging
MSM	Maudsley Staging Method
MSSI	Modified Scale for Suicidal Ideation
MSS	MedSendSecure
MT	Motor Threshold
NCT	National Clinical Trial
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NHC	Normal Healthy Control
NSR	Nonsignificant Risk
OHRP	Office for Human Research Protections
PD	Protocol Director
PEST	Parameter Estimation by Sequential Testing

PHI	Protected Health INformation
PI	Principal Investigator
PID	Participant identification number
QC	Quality Control
REDCap	Research Electronic Data Capture
rMT	Resting Motor Threshold
RS	Resting-State
RS FC	Resting-State Functional Connectivity
rTMS	Repetitive Transcranial Magnetic Stimulation
SAE	Serious Adverse Event
SCC	Subcallosal Cingulate
SCID	Structured Clinical Interview for DSM-5
sgACC	Subgenual Anterior Cingulate Cortex
SoA	Schedule of Activities
SN	Salience Network
SUNet	Stanford University Network
TASS	TMS Safety form
TBS	Theta-Burst Stimulation
TMS	Transcranial Magnetic Stimulation
TRD	Treatment Resistant Depression
UPS	Uninterruptible Power Supply
UP	Unanticipated Problem
US	United States
vlPFC	Ventrolateral Prefrontal Cortex
VPN	Virtual Private Network
YMRS	Young Mania Rating Scale

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.1	11JAN2021	- changed 'SAINT' to 'aiTBS' - addition of severe BPD as an exclusion criterion - addition of BPD diagnostic module (if indicated) to screening visit - update to statistical design to include random blocks of 2, 4, 6 - update of personnel - addition of data quality check log - MRI training certification process updated	Changes made as per DSMB suggestions and clarify study workflow.
2.0	05OCT2021	- Soterix Neuronavigation System replaced with Localite TMS Navigator - MagPro XP 120V Edition replaced with MagPro X100 - Standard MADRS-S replaces 24-hour version of MADRS-S for visit 3, baseline, and 1-month assessments - Clearly state that MSSI will be performed at screening and then will be included as part of a clinical consult with PI or study psychiatrist if triggered by a score of 3 on item 9 of the MADRS-S ('Zest for life' item) - David Spiegel, MD, added as protocol director under 'Key Roles and Study Governance' section. - Two new exclusion criteria added related to maximum treatment dose and stability of symptoms - Justification for newly added exclusion criterion related to stimulation dose added	Changes made as per DSMB suggestions, study updates, and to clarify study workflow

		- DSMB to meet at least triannually - Motor threshold may be checked as part of screening visit. - SoA updated to include mSSI at screening, baseline, and as part of a clinical consult if triggered by a score of 3 on item 9 of the MADRS-S ('Zest for life' item) - SoA updated to include PSQI at screening and baseline. - Updated language regarding use of OTC medications, timing of dosing of any hypnotic medications, and use of any anxiolytic medications. - Updated language on definition of a screen failure. - Clarified informed consent process - Study responsibilities supervised by protocol director (PD) - Use of EMG for measurement of MT removed - Lidocaine application schedule modified	
3.0	04FEB2022	-'Urine Analysis' broken into 'Urine Drug Screen' and 'Urine Pregnancy Test - Urine Drug Screen added to Visits 3-8 -Addition of C-SSRS to screening visit - Update of SOE to include FIGS, CTQ, and blood draw if participant opts into optional substudy - Removed minimum HAM-D score as an inclusion criterion - Changed recent suicide attempt exclusion criterion to past one year (instead of past 90 days) - Modified definition of 'recent use' of a rapid-acting antidepressant in an exclusion criterion - Update of lifestyle considerations	General update

		to increase accuracy - Added clarifying details related to the definition of screen failure and the process of rescreening. - Study responsibilities supervised by protocol director (PD) - Removed line related to AE follow up that is redundant to section 8.3.1. - Addition of a weekly phone call by CRC for check in between Visits 8 and 9 - Addition of optional blood draw and questionnaires added - Addition of C-SSRS to screening instruments - Addition of Adobe Sign for remote consent electronic signatures - Clarified time period for AE assessment. - Updated definition of SAE to enhance clarity. - Removed lines related to SAE reporting timeline that are redundant to section 8.3.6 - Removed lines related to expected AEs that are redundant to section 8.3.3.3 - Added table (previously in section 8.3.4) and expanded discussion of expected - AEs Removed table (moved to section 8.3.3.3) - Clarified time period of AE assessment, time period for following of an AE, and AE screening process - Removed line related to period of AE assessment that is redundant to section 8.3.4 - Storage of blood specimens specified	
3.0	25FEB2022	- Optional blood draws - Adobe Sign Consenting - Removed HAMD and update to eligibility criteria and lifestyle considerations	General update

3.1	20APR2022	- Clarified language and timepoints for optional blood draws - Clarified blood specimens are not saved in REDCap - Added specimen processing, storage, shipping, and labeling language - Added terms to abbreviations list	
3.2	09SEP2022	- Allow screening data in REDCap to be shared between BSL studies	General update
3.2	31MAR2023	- Added Spanish recruitment material	General update
3.3	19May2023	- Clarified side effects associated with Lidocaine and MRIs	Clarification
3.3	19May2023	- Listing of study psychiatrists	Update
3.4	30Nov2023	Removed Box eCRF backup language	Update
3.4	30Nov2023	Added University of Antwerp as a collaborator who may receive samples.	Update
3.4	30Nov2023	Updated total blood draw volume to 30 mL.	Update
3.4	30Nov2023	Updates to amendment history	Update
3.4	30Nov2023	Added a timetable for eligibility criteria	Clarification
3.4	30Nov2023	Removed outdated video language	Clarification

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12 APPENDICES