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PROTOCOL TITLE:

Safety and Efficacy of an Accelerated Protocol of Intermittent Theta Burst Transcranial Magnetic Stimulation (TMS) to Enhance Performance and Promote Resilience in Astronauts (TRISH BRASH)

PRINCIPAL INVESTIGATOR:

Donna Roberts, M.D.

1.0 Objectives / Specific Aims

To address the major challenges in maintaining behavioral health and performance of crews on exploration-class missions, NASA has conducted extensive research in the areas of crew selection, training, and identifying behavioral issues in extreme environments[1,2]. However, evidence from US and Russian spaceflight experience and data from space analog environments indicate that performance and behavioral issues are a significant risk for human space flight, no matter how well selected and trained crew members may be[2]. While these symptoms may result in minor disruptions in Earth-based work environments, their significance becomes greatly magnified by the remoteness of space, potentially jeopardizing mission success and putting astronauts' lives in danger. Future exploration-class missions, such as a mission to Mars, are characterized by long mission durations (2 to 5 years); extreme isolation; hostile environment; extended periods of time within the confinement of the spacecraft; lack of contact with family; and limited social interactions beyond one's team members. There will be extreme performance pressures on crew members who must work optimally together as a team at all times. Therefore NASA must identify and validate countermeasures to promote behavior health and performance (NASA Human Research Roadmap: BMed1, BMed6). NASA has considered the inclusion of psychoactive medications for this purpose, however drugs may have expiration dates shorter than the mission length and the pharmacokinetics of drug administration in microgravity is poorly understood[3] making non-pharmacological approaches desirable.

High frequency repetitive transcranial magnetic stimulation (rTMS) to left prefrontal cortex is a FDA-approved non-invasive brain stimulation for the treatment of major depression. A therapeutic course of rTMS typically consists of approximately 30-40 minutes of high-frequency (i.e., 10 Hz) treatment on each weekday, for 4 to 6 weeks. This protracted schedule often creates logistical hurdles for patients. Recently, a number of investigators have examined accelerated rTMS delivery. Sessions are repeated on the same day to reduce total days of treatment. Accelerated protocols have typically endeavored to fit the conventional dosing scheme (i.e., 54,000-60,000 pulses over 4-6 weeks) into a shorter time period ranging from 3 days to 2 weeks. In the case of either conventional or accelerated rTMS, there has been no systematic examination of dose response.

We propose that similar to novel drug development, advancing accelerated rTMS for enhancing neurocognitive function, stress resilience and to prevent the onset of neuropsychiatric symptoms (e.g., anxiety, depression, anger, irritability) fundamentally necessitates establishing the dose-response function for neurocognitive enhancement. Establishing this dose-response curve would enable the overall goal of study to develop and establish the efficacy of accelerated rTMS to left dlPFC as a non-pharmacological method for enhancing cognitive performance and resilience of high-performing, healthy adults of astronaut age and to extrapolate results to a spaceflight relevant environment (parabolic flight).

Aim 1. Establish the dose-response curve for an accelerated rTMS protocol to left dlPFC for enhancing neurocognitive performance and resilience in a group of adults that mimic the astronaut population. We hypothesize that neurocognitive performance and resilience to stress and psychopathology will show similar dose-response curves and that a dose that maximizes efficacy while minimizing burden will be demonstrated.

Aim 2. Examine the efficacy and safety of accelerated rTMS to left dlPFC for enhancing cognitive performance and resilience. We hypothesize that relative to sham TMS, accelerated rTMS to left dorsolateral prefrontal cortex (dlPFC) will show neurocognitive performance (primary outcome) and resilience enhancements (secondary outcome) in a group of healthy adults that mimic the astronaut population. We additionally hypothesize that accelerated rTMS will be safe as indexed by 1) no clinically significant structural brain changes; 2) no decrements in neurocognitive or affective function; 3) no significant adverse events. We hypothesize that frontal midline theta (and intersite phase synchrony) will be enhanced during successful feedback learning as shown on post- relative to pre- treatment EEG and more so under stress. Under threat, we also expect to see reduced stress as indexed by physiological measures of defensive reactivity (autonomic and startles reflex responding). We additionally hypothesize that active stimulation will be related to lower levels of self-reported stress and neuropsychiatric symptoms and greater resilience.

Aim 3. Assess the biological efficacy of TMS in a microgravity environment. During parabolic flight, resting motor threshold (rMT) will be measured for 10 subjects and compared to rMT obtained before and after flight on the ground. Any change in rMT during flight compared with pre- and post-flight values will be interpreted as an indicator of an altered physiological response to TMS during microgravity and will guide extension of the optimal ground-based accelerated rTMS protocol for spaceflight.

2.0 Background

A1. Transcranial magnetic stimulation. Transcranial magnetic stimulation (TMS) is a medical device that can safely and non-invasively stimulate the brains of awake individuals to induce neuroplasticity[4] and is currently under investigation for numerous applications in behavioral health and performance[5]. Led by the pioneering efforts of our co-Investigator, Dr. Mark George, TMS first received approval from the U.S. FDA in 2008 for the treatment of depression[6,7] and is now used in psychiatric departments throughout the US and Canada[8]. TMS is able to focally stimulate the cortex by creating a dynamic magnetic field generated by a brief but powerful electrical current passed through an electromagnetic coil[9]. The magnetic field induced by TMS diminishes rapidly as distance increases away from the coil. This allows TMS to be used in environments with complex equipment (like a space capsule) without interfering with other devices. For example, we have successfully used TMS to treat pain conditions inside the crowded intensive care unit[10,11].

TMS pulses induce a transient electric field in underlying excitable neuronal tissue. The affected area is approximately 2 to 3 cm below the device in superficial cortex[12], but neural effects are then triggered in interconnected brain areas. TMS pulses cause depolarization of cortical neurons at the stimulation site, which then propagate through connected network nodes[13]. Thus, TMS influences not only regional or local cortex, but also distributed neural networks. Repetitive TMS (rTMS) can further causally influence neural networks with the added power to produce neuroplastic periods of lasting excitation and/or inhibition that reliably persist after the termination of stimulation[14].

The evidence-base for rTMS as a means of prompting therapeutic neuroplasticity has been most persuasive in the case of depression treatment. Proposed as a means of up- regulating prefrontal control over dysregulated limbic activation, high-frequency rTMS is most often applied to left

dorsolateral prefrontal cortex (dlPFC)[15]. Several meta- analyses have now shown that open-label effects sizes are large, while comparisons to sham stimulation are at least moderate to large among even patients highly resistant to pharmacotherapy[16,17].

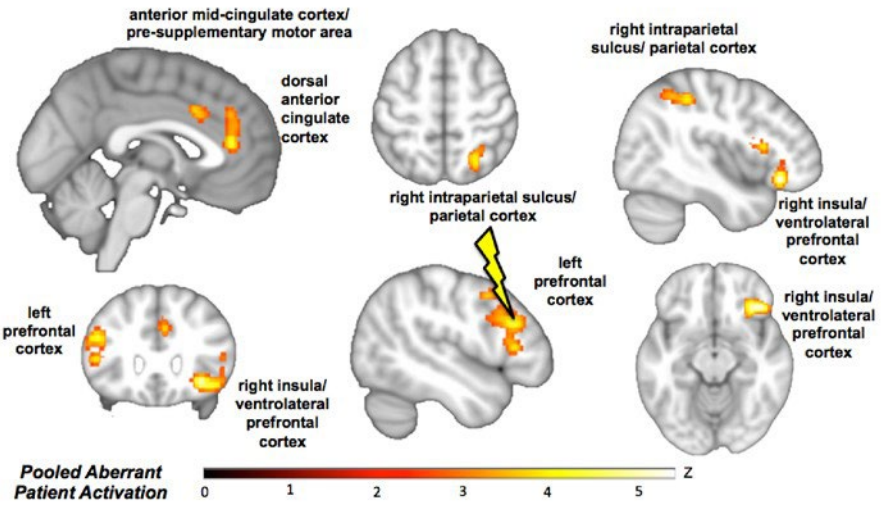


Figure 1. In a transdiagnostic functional neuroimaging meta-analysis of more than 11,000 neuropsychiatric patients and matched control participants (McTeague et al., 2017), the left dorsolateral prefrontal cortex typically targeted in rTMS was a prominent region of hypoactivation during cognitive tasks, in conjunction with other regions that compose the distributed cognitive control or multiple demand network.

A2. Repetitive TMS to target cognitive enhancement and resilience. The proposed mechanism by which rTMS remediates depressive symptoms (i.e., up-regulating prefrontal control over dysregulated limbic activation) implies that rTMS should not only remediate depression, but should be a powerful transdiagnostic intervention for affective and cognitive dysregulation. In fact, high-frequency rTMS to left dlPFC is effective in reducing symptoms as far ranging as pain[18], to craving[19], posttraumatic stress disorder[20], borderline personality disorder[21], and compulsive food restriction[22].

The left dlPFC site typically targeted with rTMS is seated in an area of cortex integral to intact higher order cognition (i.e., executive function)[23]. Co-I McTeague has demonstrated that this left dlPFC region (as well as the rest of the cognitive control or multiple demand network) is commonly hypoactivated during cognitive tasks across neuropsychiatric disorders. This was observed in a transdiagnostic functional neuroimaging meta-analysis of more than 11,000 neuropsychiatric patients and matched control participants (Figure 1)[24]. Furthermore, we have observed during functional magnetic resonance imaging (fMRI) concurrent with TMS, that TMS pulses to the left dlPFC cause BOLD activation in distributed regions of the cognitive control or multiple demand network (Figure 2; Badran et al., in preparation).

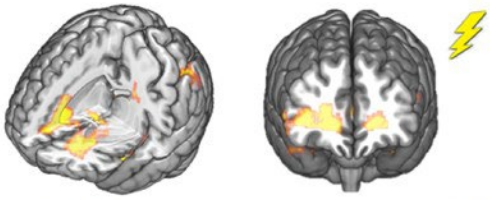


Figure 2. BOLD response during concurrent TMS-fMRI. Stimulation to the left dorsolateral prefrontal cortex affects more distal portions of the cognitive control or multiple demand network (Badran et al., in preparation).

Even among healthy individuals for whom neurocognitive performance has a more restricted range, improved working memory performance has been observed after only a single session of excitatory rTMS[25,26]. Regarding neurocircuit based effects, multiple sessions (i.e., 2 weeks) of excitatory rTMS to left dlPFC among a separate sample of healthy participants have been shown to enhance the efficiency of the cognitive control network during working memory performance[27].

Taken together, it is not surprising that cognitive improvements have been reported as ancillary benefits to conventional[28-30] as well as accelerated[31] therapeutic rTMS for cognitively intact depressed patients. Accordingly, rTMS has also been shown to improve cognition as the primary outcome in studies of mild cognitive impairment[32].

We propose that because rTMS to dlPFC is targeting cognitive neurocircuitry integral to adaptive cognitive functioning, that promoting neuroplasticity in this network with rTMS could be better optimized to both improve neurocognitive performance as well as enhance resilience to stress and neuropsychiatric dysfunction.

A3. Accelerated theta burst rTMS: Lessening intervention burden toward enhancing adherence amidst the demands of space flight. A therapeutic course of excitatory rTMS typically consists of a single treatment session on each weekday for four to six week. A single session lasts approximately 37.5 minutes with stimulation consisting of 10 Hz trains delivered for 4 seconds on and 26 seconds off. This schedule can be burdensome and reduce adherence. More recently, a number of groups have examined the safety, feasibility, acceptability, and effectiveness of accelerated rTMS delivery[31,33-35] during which sessions are repeated on the same day to reduce total days of treatment, typically spaced by at least 30-60 minutes or more. Safety has also been assessed with both structural and metabolic imaging as well as neurocognitive testing, which has shown no adverse effects on neural integrity and, in fact, gains in cognition[31,33-35]. Furthermore, acceptability results have suggested that accelerated protocols could increase adherence and decrease interruptions to daily obligations[31,33-35].

In a recent further innovation, intermittent theta burst (iTBS) rTMS has been shown to be as efficacious as 10 Hz rTMS in remediating depression[36.] **Notably, a single session of excitatory iTBS rTMS entails 600 pulses in merely 3 minutes.** More specifically, pulses are delivered in triplets at 50 Hz for 2 s (i.e., 5 Hz triplets) and repeated every 10 seconds for a total of 190 seconds (600 pulses). Based on animal work, it has been proposed that theta burst stimulation may be particularly effective in inducing synaptic long-term potentiation due to potential mimicry of endogenous theta rhythms[37]. Cortical theta rhythms as characterized in electroencephalography (EEG) are especially important in the support of human cognitive control[38,39]. Furthermore, among healthy participants theta burst stimulation to left dlPFC has been shown to enhance working and recognition memory performance[40,41].

While the use of iTBS in accelerated protocols is only now emerging for the remediation of neuropsychiatric dysfunction, accumulating safety and efficacy results are promising, even among the most impaired and vulnerable patients[42]. From a practical standpoint, theta burst sessions are typically spaced by approximately 30 minutes to one hour or more with no requirements on the intervening time period. As such, astronauts could undergo multiple 3-minute sessions in a single day with limited interference on space flight demands.

To establish an efficacious intervention, tailored to the logistical demands of spaceflght, we propose two studies (Aims 1 & 2), each of which would implement a cutting-edge high-dose accelerated theta burst protocol for the application of rTMS to enhance neurocognitive performance and resilience to stress and neuropsychiatric dysfunction.

A4. A principled, empirical approach for determining rTMS dose. In the case of either conventional or accelerated rTMS, there has been no systematic examination of dose response. Rather, prior rTMS investigations have relied upon rational decision trees in determining TMS dose. For example, emerging accelerated protocols have typically endeavored to fit the conventional dosing scheme (i.e., 54,000- 60,000 pulses over 4-6 weeks) into a shorter time period ranging from 3 days[33] to 2 weeks[31]. While dose-response curves are typically interrogated as a function of efficacy relative to toxicity, it is also possible to model efficacy in the absence of toxicity, which would be most appropriate in the case of rTMS.

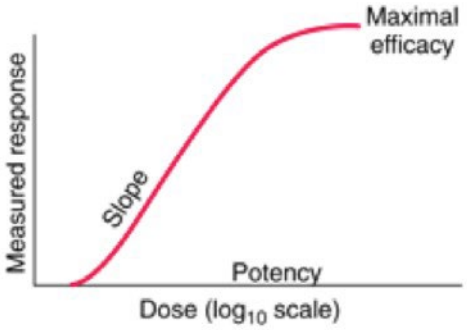


Figure 3. In order to optimize rTMS for neurcognitive enhancement, it is essential to determine the dose-response curve.

We propose that establishing the dose-response curve for enhancing neurocognitive performance among healthy, high functioning individuals, will most efficiently enable us to pinpoint the most efficacious and least burdensome dose to be administered.

A5. TMS and microgravity. Living in a microgravity environment has significant physiological effects on the human body[43-45]. For example, the pharmacokinetics of drug administration is altered in microgravity[46] and psychotropic medications are known to have reduced efficacy during spaceflight[3]. Likewise, there is the potential that TMS may operate differently in a microgravity environment. For example, studying the MRI brain scans of astronaut before and following spaceflight, we have recently shown there is a shift of brain tissue within the skull post-flight[47]. This observed upwards shift of brain tissue could potentially alter the dosing of TMS as the effectiveness of TMS depends on the distance from the TMS coil (which is placed on the scalp) to the underlying cortex[48]. In addition, cephalad fluid shifts which occur in astronauts[49] and during parabolic flight[50-52] may alter the neurophysiological response to TMS. Therefore, as part of this study we will investigate the physiological response to TMS in a microgravity analog environment (parabolic flight).

Several TMS measures of cortical excitability have been described; however the most basic is determination of the TMS resting motor threshold (rMT). The rMT is the minimal magnetic pulse causing muscle contraction as detected by electromyography (EMG) recordings. It is more formally defined as the amount of TMS machine output (intensity) necessary to produce a motor evoked potential (MEP) that exceeds a defined peak-to-peak amplitude (usually 50 μV) 50% of the time in a finite number of trials. The rMT is believed to be an indicator of neuronal membrane excitability[53]. Accurate estimation of rMT is of utmost importance in both research and clinical studies as it is the unit most commonly used for rTMS dosing[54]. Inaccurate estimation of rMT can lead to subject/patient over- or under-dosing.

A6. Conceptual Model and Rationale for Design. We hypothesize that through up regulating cognitive control circuitry with rTMS that an astronaut would have enhanced capacity for successfully contending with the rigors of spaceflight. This would be evidenced foremost in enhanced cognitive control performance. The neurocircuitry undergirding efficient cognitive control overlaps with the neurocircuitry undergirding emotion regulation[55], and as reviewed, rTMS to dlPFC remediates a broad range of affective dysfunction. Thus, we further propose that

accelerated rTMS will promote stress resilience and prevent the onset of neuropsychiatric symptoms (e.g., anxiety, depression, anger, irritability).

Study Arm 1 would establish the dose-response for enhancing cognitive performance with accelerated rTMS to left dlPFC. Stress resilience will be utilized as a secondary outcome. Given the model it would also be reasonable to first establish the objective point at which resilience is up regulated by rTMS. We anticipate that healthy participants analogous to astronauts would likely show little variability in baseline resilience and affective dysfunction. We also anticipate that rTMS as proposed here could prevent the onset of stress and neuropsychiatric symptoms and thus we will longitudinally follow participants for the emergence.

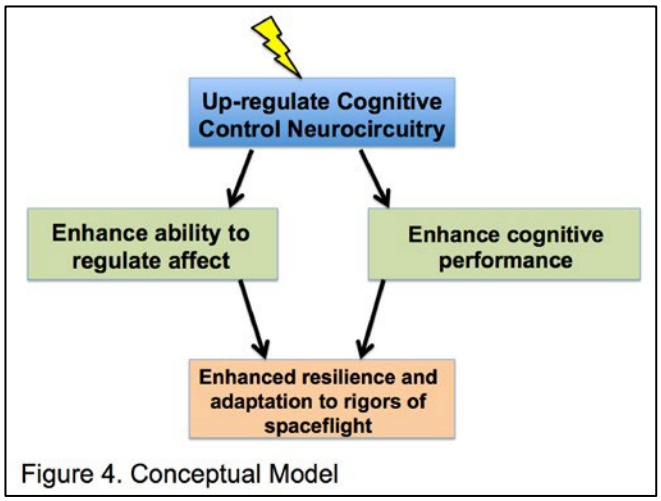


Figure 4. Conceptual Model

Study Arm 2 would utilize the dose established in study one that maximizes cognitive performance gains while minimizing side effects and burden in a double blind, randomized controlled trial. Primary (neurocognitive performance) and secondary (resilience) outcomes would be identical to study 1. In addition, the impact of active versus sham iTBS on cognitive control and resilience under stress will be demonstrated during a challenging learning paradigm under threat of shock. EEG will be used to assess the impact on of iTBS on neurocircuit structure and function. Response stability will be assessed at 6 months and one year. To assess safety, structural MRI adverse event reporting, and cognitive measures will be collected over a long-term 6-month follow up period.

Study Arm 3 study would assess the biological efficacy of TMS in a microgravity environment during parabolic flight.

Study Arm 4 would establish the dose-response for enhancing cognitive performance with accelerated rTMS to left dlPFC, as well as assessing safety. In addition, the impact of each dose of iTBS on cognitive control and resilience under stress will be demonstrated during a challenging learning paradigm under threat of shock. MRI and EEG will be used to assess the impact on of iTBS on neurocircuit structure and function. Response stability will be assessed at 1 month post-treatment. Approval to combine studies 1 and 2 into a 4th arm was approved by our sponsor, due to recruitment delays caused by COVID-19.

B. INNOVATION AND ADVANTAGES AND RELEVANCE TO ASTRONAUTS

B1. Establishing a dose-response curve for rTMS. We propose to innovate in terms of establishing the dose-response curve for rTMS, instead of relying upon rational decision trees typically founded upon the rTMS for depression literature. At present there exists no empirical information as to the dose for maximal benefit in neurocognitive enhancement and stress resilience.

B2. Utilizing an accelerated rTMS delivery schedule. We propose to utilize an accelerated (high-dose) delivery schedule to lessen burden, increase adherence, and promote the utilization of this intervention during space flight with the counter measures NASA already has in place to promote behavior health and performance. Specifically, we propose to examine a range of rTMS doses all within a one-week delivery schedule.

B3. Utilizing an intermittent theta burst stimulation pattern. We propose to innovate by utilizing intermittent theta burst (iTBS), excitatory stimulation, which has been shown to be non-inferior to 10 Hz stimulation in the treatment of depression, but requires only three minutes/session.

3.0 Study Endpoints

Study Arms 1 2 & 4 neurocognitive performance is the primary treatment outcome measure. Stress resilience and acceptability/tolerability are secondary treatment outcome measures. For Study Arm 3, biological efficacy of TMS in microgravity is the primary treatment outcome measure. Recruitment for Study Arm 1 will cease once 40 participants have completed all procedures and fulfilled randomization to all doses. Recruitment for Study Arm 2 will cease once 60 participants have completed all procedures and fulfilled randomization to all doses. Recruitment for Study Arm 3 will cease once 10 participants have completed all procedures. Recruitment for Study Arm 4 will cease once 50 participants have completed all procedures and fulfilled randomization to all doses.

4.0 Inclusion and Exclusion Criteria/ Study Population

Participants who are aged 22-55 (inclusive), who mimic the demographics of the astronaut population. We conservatively expect an 80-90% retention rate, as dropout is typically fewer than 10% in double-blind trials of conventional daily (87) as well as accelerated rTMS (86, 89). Study Arms 1 2 & 4 will all have the same inclusion/exclusion criteria. Study Arm 3 will have similar inclusion/exclusion criteria with additional criteria involving exposure to parabolic flight.å

Study Arms 1 2 & 4 Inclusion Criteria. Participants must be 22-55 years of age: endorse good health with no history of mental or physical illness or implanted metal; college graduates (associates degree or higher); negative urine pregnancy test if female of childbearing potential; English is primary language; capacity to consent; willingness to adhere to rTMS schedule and assessments.

Study Arms 1 2 & 4 Exclusion Criteria. Participants will be excluded for the following: any current psychiatric diagnosis or current Clinical Global Impression ratings of psychiatric illness > 1; neurodevelopmental disorders; current physical illness; history of CNS disease, concussion, overnight hospitalization, or other neurologic sequelae), tumors, seizures, meningitis, encephalitis, or abnormal CT or MRI of the brain; frequent or severe headaches; history of a continuing significant laboratory abnormality; any psychotropic medication taken within 5 half-lives of procedure time; pregnancy or intention to become pregnant during rTMS or follow-up period; any head trauma resulting in loss of consciousness; visual impairment (except glasses); inability to complete cognitive testing; currently breast feeding; active participation or plan for enrollment in another evidence-based clinical trial affecting psychosocial function; repeated abuse or dependence upon drugs (excluding nicotine and caffeine) currently taking medications that lower the seizure threshold; taking either of the medications including stimulants, modafinil, thyroid

medication, or steroids; implanted devices/ferrous metal of any kind; history of seizure or seizure disorder; inability to determine motor threshold; claustrophobia or other condition that would prevent the structural MRI assessment of pre and post-treatment, and 6 months later.

Study Arm 3 Inclusion/Exclusion Criteria. Study Arm 3 will have modified inclusion criteria regarding age, as the participants must be 23-61 years of age, as well as identical exclusion criteria with the following additional exclusion criteria related to the experience of parabolic flight:

Participants will be excluded for the following: History of motion sickness and/or motion discomfort; fear of flying or previous inability to tolerate flying; visual impairment (except glasses), ear disease, hearing loss or balance disorders; neck, back or other spinal problems; diabetes; history of GERD or other blood disorders; high or low blood pressure; heart or vascular trouble, stroke, history of angina or chest pain; stomach, liver, esophageal or intestinal trouble; weakened limbs or joints or broken bones within the past year; subthreshold behavioral health issues, such as panic attacks, fear of heights, fear of flying or fear of closed spaces; currently pregnant; dizziness, blackouts, fainting spells, or loss of consciousness for any reason; recent severe illness, surgery, or admission to hospital; medical rejection, medical discharge from military or other disabilities; lung disease, breathing problems, asthma or others.

5.0 Number of Subjects

Study Arm 1: Dose-finding (enrollment *n = 50 to complete assessments and treatment for n=40*): To contend with dropout we propose to enroll 50, with the aim of completing assessments and treatment for 40 participants. We conservatively expect an 80-90% retention rate, as dropout is typically fewer than 10% in double-blind trials of conventional daily[87] as well as accelerated rTMS[86,89]. Recruitment would cease once 40 participants have completed all procedures and fulfilled randomization to all doses.

Study Arm 2: Efficacy & Safety (enrollment *n = 70 to complete assessments and treatment for n=60*): To contend with dropout we propose to enroll 70, with the aim of completing assessments and treatment for 60 participants. We conservatively expect an 80-90% retention rate, as dropout is typically fewer than 10% in double-blind trials of conventional daily[87] as well as accelerated rTMS[86,89]. Recruitment would cease once 60 participants have completed all procedures and fulfilled randomization to the most efficacious dose determined by Study Arm 1.

Study Arm 3: Biological Efficacy of TMS in Microgravity *(n = 10)*: To contend with dropout we propose to enroll 10 participants.

Study Arm 4: Dose-finding & Safety (enrollment *n = 60 to complete assessments and treatment for n=50*): Due to recruitment delays due to COVID-19, we have proposed to our sponsor (and have received approval) to combine studies 1 and 2. To contend with dropout we propose to enroll 60, with the aim of completing assessments and treatment for 50 participants. We conservatively expect an 80-90% retention rate, as dropout is typically fewer than 10% in double-blind trials of conventional daily[87] as well as accelerated rTMS[86,89]. Recruitment would cease once 50 participants have completed all procedures and fulfilled randomization to the most efficacious dose determined by Study Arm 1.

6.0 Setting

Study Arms 1 2 & 4: All procedures will take place in private assessment and rTMS treatment rooms at the MUSC Brain Stimulation Laboratory (BSL) in the Institute of Psychiatry. The MRI scanning for Study Arm 2 and 4 will occur at the Center of Biomedical Imaging at 30 Bee Street on the Medical University of South Carolina campus.

The option to remotely complete the consenting process and SCID interview will be given to participants in order to limit in-person visits for a multitude of reasons, including COVID19 precautions. Phone calls or video conferencing through Webex will be utilized in order to properly administer and oversee the completion of these assessments.

Study Arm 3: Screening, interview and assessments will take place in private assessment rooms at the MUSC Brain Stimulation Laboratory (BSL) in the Institute of Psychiatry. The study treatment procedures will take place at Sanford, Florida - The ZERO-G Experience®

7.0 Recruitment Methods

Participant recruitment for Study Arms 1, 2, 3 & 4 will include flyers, handouts, electronic and physical bulletin board postings, social media/message boards (i.e., Craigslist, MUSC Broadcast Research studies section, Yammer, Facebook, Twitter, Instagram), newsletter/media advertisements, and recruitment talks at local community events/organizations and surrounding community as well as through the MUSC Psychiatry. The flyers will be used as the advertisement for all the different recruitment platforms. Participants who make contract through the QR code will be directed to a secure REDCap survey asking for their preferred contact date and time to discuss the study. Participants who make contact based on recruitment efforts will be given a description of the study purpose, procedures, and potential risks and benefits of the study by phone. The potential participant will be invited to ask questions until they are satisfied and can make a decision to proceed or not with the phone screen. If the potential participant agrees to continue, a phone screen will be conducted to determine eligibility for the next phase (in-person clinical interview) of the study.

8.0 Consent Process

Informed Consent. If the potential participant passes the telephone screen and decides to come in for the interview, a signed informed consent will be necessary at the outset before beginning the interview. The PI, Dr. Roberts or trained study staff will administer the consent process in an office at the MUSC Department of Radiology or the Institute of Psychiatry. The consent form describes the study procedures and ensures participants of the confidentiality of their responses. The consent form further reminds participants that they have the option to withdraw from the study at any time and will receive proportional payment or they can refuse to answer certain questions and continue in the study with full compensation. The consent form contains thorough descriptions of the research protocol including the procedures, benefits and risks, compensation, right to non-participation, review processes, emergency medical treatment, financial responsibility, and privacy issues. Prior to beginning the clinical assessment session or any scan, participants must demonstrate ability to read, verbalize understanding of, and sign informed consent documentation. Two fields within the consent form enables participants to consent to whether study personnel may keep their contact information and contact them in the future regarding other research studies. Enrollment is not contingent upon responses to this query. Participants will be

allowed to take the consent form home for further review. Each day participants will be queried about their comfort with continuing the study.

9.0 Study Design / Methods

This study involves three aims divided into four separate study arms. Study Arm 1 Dose Finding; Study Arm 2 Efficacy & Safety; Study Arm 3 Biological Efficacy of TMS in Microgravity; Study Arm 4 Dose Finding & Safety.

Recruitment, Screening & Intake Assessment. For Study Arms 1, 2, 3 & 4, healthy participants would be recruited from the local and surrounding community. Eligibility would be screened in-person or via phone. Participants who meet probable inclusion criteria endorse good health, demographics of astronauts with no history of mental illness, neurological disorders, substance abuse or implanted metal as determined Telephone Screening-Control would be invited for an intake assessment. The Telephone Screening-Control questionnaire would include a TMS safety screener, the 4-item Patient Health Questionnaire (PHQ-4 item), PTSD Checklist Screener (PCL-6), and 7-item Generalized Anxiety Disorder (GAD-7). E-consent will be an option if the coordinator does not feel the participant is able to come into the laboratory for consenting for any reason, but primarily due to safety precautions surrounding the COVID-19 pandemic. The e-consent will be emailed through REDCap and approved research personnel will go through the e-consent with the participant over the phone or over video conferencing. The participant will receive a copy of the signed e-consent by email from the research personnel. The coordinator will offer to send the consent form to the participant in advance to provide ample time for review. Following the consent, the remainder of the intake assessment can also be performed remotely. This includes the Structured Clinical Interview for DSM-5. Study Arm 3 participants will have additional assessments to assess capability to be in parabolic flight.

Study Arm 1: Dose Finding

rTMS dosing parameters, targeting, and rationale for proposed design. We chose to alter dose as a function of sessions, with a single session defined as 600 pulses at 120% motor threshold, 50 Hz triplets for 2 s, and repeated every 10 s for a total of 190 s to left dlPFC (Table 1). Ten doses would be block randomized to participants (block size to be determined by Dr. Ramakrishnan) to ensure that the upper and lower doses are acquired in tandem in approximately equal rates.

Table 1. Dosing Parameters by Weekday and Total for Determining Dose-Response Curve

	# of Active Sessions by day*					Total		
Dose Step	Monday	Tuesday	Wednesday	Thursday	Friday	# Active Sessions	# Active Pulses	# Sham Sessions
1	1	1	1	1	1	5	3000	45
2	2	2	2	2	2	10	6000	40
3	3	3	3	3	3	15	9000	35
4	4	4	4	4	4	20	12000	30
5	5	5	5	5	5	25	15000	25
6	6	6	6	6	6	30	18000	20
7	7	7	7	7	7	35	21000	15
8	8	8	8	8	8	40	24000	10
9	9	9	9	9	9	45	27000	5
10	10	10	10	10	10	50	30000	0

*A single session=600 pulses at 120% rMT, iTBS triplets at 50 Hz for 2 s and repeated every 10 s for a total of 190 s to left dlPFC.

A MagVenture MagPro TMS System would be utilized and is already in place at the Brain Stimulation Laboratory. To control for differences in treatment time for the different doses, all participants would be in the TMS chair for the same duration. That is, all participants would perceive receiving treatment for ten, 3-minute sessions on each weekday for one-week. Each session would be separated by at least 10 minutes or more depending on stimulator availability and participant schedule. In addition to block randomization to dosing parameters, Dr. Ramakrishnan would randomize each participant's daily active/sham sessions. For example, if a participant were assigned to receive two active sessions in a given day, five sessions must be sham. This would further maintain the integrity of the blind. Additionally, a focal electrical sham developed by Co-I Dr. George and colleagues[77] would be used which simulates the sound and sensation of active TMS. This entails a pre-treatment individualized titration of electrical stimulation until sham and active blocks of rTMS are indistinguishable to participants. Participants then receive an individualized level of sham stimulation throughout treatment. This has been shown in a series of trials to bolster the integrity of the blind[78-80]. Guided by existing studies, the dose range is 3,000-30,000 pulses for a total of 5 to 50 active rTMS sessions. Each participant would perceive ten 3-minute sessions (each separated by 10 minutes) over each of five contiguous days. A single session=600 pulses of iTBS at 120% rMT, triplets at 50 Hz for 2 s and repeated every 10 s for a total of 190 s (600 pulses) to left dlPFC targeted via F3 of the 10-20 system. Probabilistic coil placement to left dlPFC via F3 was chosen as opposed to individualized, neuronavigation-based targeting to the participant's structural scan to maximize the potential to readily implement the approach by crewmembers in during spaceflight. In order to further prove the precision of using neuronavigation equipment compared to using cap measurements alone, coordinators will concurrently run an observational study during the treatment visits which will not require additional invested time by the participant.

To assess intervention acceptability, participants would be asked to rate pain and discomfort on a visual analog scale after each session[86]. At the end of treatment participants would also complete a 5-item questionnaire about confidence in treatment procedures and mechanisms. To assess intervention safety, adverse events would be examined. To assess intervention feasibility, recruitment and retention rates would be examined. To assess intervention efficacy, neurocognitive, resilience, and symptom measures would be repeated after the last treatment session, and at 1 month post- treatment.

To assess neurocognitive performance, participants would complete the WinSCAT battery[60] (a computerized battery which takes about 10 minutes to complete and includes tests of reaction time, short term memory, and visual memory and is used by NASA to assess cognitive function in astronauts during International Space Station flights), the NIH Toolbox[62] (a comprehensive set of neuro-behavioral measure that assess cognitive, emotional, sensory, and motor functions), as well as subtests from the Penn Computerized Neurocognitive Battery[61]. The battery includes well-validated computer- adaptations of neuropsychological tests (approximately 60 - 90 minutes total). Tasks assess domains including: information-processing speed, sustained attention/vigilance, verbal memory, working-memory capacity, cognitive flexibility, episodic memory, performance monitoring, resistance to interference, spatial memory, set shifting, inhibition/impulsivity, verbal learning, episodic memory, sensorimotor function and processing speed.

To assess resilience, stress, and affective function, participants would complete the Connor-Davidson Resilience Scale[63], a 25-item scale assessing hardiness, sense of control, adaptability,

stress endurance and self-confidence. The 17-item Hamilton Scale for Depression (HAM-D)The 10-item Perceived Stress Scale[64] would be completed to assess subjective stressors and their impact. The 99-item self-report Inventory of Depression and Anxiety Symptoms (IDAS-II)[65] would be completed as a broad measure of psychopathology and related functional impairment. The measure includes 18 non-overlapping factor-analytically-derived symptom subscales covering a wide range of psychopathology dimensions such as anxiety, depression, mania, insomnia, appetite).

To further characterize the sample, participants would complete Structured Clinical Interview for DSM-5[66] and two sections of the Wechsler Abbreviated Scale of Intelligence-II (WASI-II)[67] Matrix Reasoning and Vocabulary. To assess substance use and abuse, the Fagerstrom Test for nicotine dependence, Drug Abuse Screening Test (DAST-10) Questionnaire, and Alcohol Use Disorders Identification Test (AUDIT).

To assess trajectory of changes in symptoms and psychosocial function, the abbreviated 7-item IPF (Brief Inventory of Psychosocial Functioning; B-IPF) would be administered at intake, after the last treatment session, and then via telephone or RedCap survey, each week for four weeks following treatment completion. The 6-item Patient Reported Outcome Measurement Information System (PROMIS), 4-item Patient Health Questionnaire (PHQ-4), Generalized Anxiety Disorder-7 (GAD-7), and 6-item PTSD Checklist (PCL-6) would also be administered. These measures would be revised to query the last 7 days. Weekly symptom changes and demographic data would be assessed via RedCap.

Table 2. Visit Summary Study Arm 1: Dose Finding

Session Number	Task Description	Location at MUSC	Visit Duration
0	**Eligibility Screening**	Telephone	30 minutes
1	**Consent*** **Interview*** **Neurocognitive Battery** **Questionnaire Battery** **Motor Threshold Testing**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	20-30 minutes 30 minutes 90 minutes 1 hour 10-15 minutes
2	**rTMS day 1**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Ten 3-minute treatment sessions separated with10 minutes or more between each session.

			Total Time: –2 - 4 hours
3	**rTMS day 2**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Ten 3-minute treatment sessions separated with 10 minutes or more between each session. Total Time: –2 - 4 hours
4	**rTMS day 3**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Ten 3-minute treatment sessions separated with 10 minutes or more between each session. Total Time: –2 - 4 hours
5	**rTMS day 4**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Ten 3-minute treatment sessions separated with 10 minutes or more between each session. Total Time: –2 - 4 hours
6	**rTMS day 5**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Ten 3-minute treatment sessions separated 10 minutes or more between each session. Total Time: –2 - 4 hours
7	**Neurocognitive Battery** **Questionnaire Battery**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	90 minutes 1 hour
8	**1 month post-treatment Neurocognitive Battery**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	90 minutes 1 hour

	1 month post-treatment Questionnaire Battery		

*****Option to complete remotely due to COVID-19 precautions.

Study Arm 2: Efficacy & Safety

Assessment & rTMS. The assessment and TMS methods from study 1 will be identical, except that 60 participants (enrollment n=70 to complete assessments and treatment for n=60 to contend with drop out) will be randomized to one dose of active or sham iTBS rTMS. **The dose will be determined as the most efficacious dose from Study 1, minimizing side effects and burden.** In addition, to determine longer-term safety of the intervention, participants will complete neurocognitive, resilience and symptom measures pre- and immediately post-treatment as well as 1, 2, and 6 months later.

MRI assessment. To more specifically examine safety, participants who received active stimulation will undergo MRI sessions (FLAIR, diffusion, T2* and volumetric sequences) for assessment of structural changes at pre- and post-treatment, and 6 months later.

Physiological assessment. The impact of active versus sham iTBS on function will be further assessed with electrocortical and physiological activity during a challenging feedback-based response-learning paradigm[39,70]. This learning task is essentially a more difficult version of the Wisconsin Card Sorting task. More specifically, participants must dynamically learn different categories of stimuli as well as the exceptions. Participants receive feedback (correct/incorrect) on each trial to shape learning. Intermittently, and disproportionately on incorrect trials to increase stress, participants are instructed to increase performance speed. Additionally, trials are embedded in counterbalanced blocks of threat of unpredictable shock. Half of the blocks are "No Shock" probability blocks, whereas half of the blocks are "Unpredictable Threat of Shock." Thus, in addition to performance demands participants must contend with a psychologically threatening and unpredictable context during performance. Typically, more efficient learning and performance is predicted by increased midline theta oscillations (4-8 Hz) and increased intersite phase synchrony during feedback, and further enhanced under threat or stress among those with successful performance[39]. Responses to blocks of unpredictable threat and no threat will be compared in terms of autonomic measures (heart rate and skin conductance) as well as the responses to 50 ms, 98 dB white noise probes, which elicit a blink response (i.e., startle reflex) measured with electromyography (EMG). The reactions captured in these measures reliably increase as a function of stress and aversion as well as lack of resilience[71-74]. In summary, we predict that EEG metrics of cognitive control will be enhanced pre- to post-treatment in the active relative to sham group, and more pronounced under threat, indicative of enhanced cognitive control and resilience. We predict that concurrent physiological metrics of stress reactivity during unpredictable relative to no threat of shock will be reduced pre- to post-treatment in the active relative to sham group, indicative of improved resilience. Shock level will be individually titrated prior to the learning procedure. EEG will be assessed pre- and immediately post-treatment as well as 1 and 6 months later (64-channel Brain Products ActiChamp System). Startle, heart rate and skin conductance will be assessed with Biopac EMG, EKG, and EDA modules.

Table 3. Visit Summary Study Arms 2 and 4: Dose-finding/Efficacy & Safety

Session Number	Task Description	Location at MUSC	Visit Duration
0	**Eligibility Screening**	Telephone	30 minutes
1	**Consent** **Interview** **Neurocognitive Battery** **Questionnaire Battery** **Motor Threshold Testing**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	20 – 30 minutes 30 minutes 90 minutes 1 hour 10 – 15 minutes
2	**MRI Safety Screen** **Pre-treatment Structural MRI and functional MRI** **EEG assessment**	MUSC Center for Biomedical Imaging	5 minutes 1 – 2 hours 1-2 hours
3	**rTMS**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Up to ten 3-minute treatment sessions separated with 20-30 minutes or more between each session. Total Time: 3 - 5 hours
4	**rTMS**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Up to ten 3-minute treatment sessions separated with 20-30 minutes or more between each session. Total Time: 3 - 5 hours
5	**rTMS**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Up to ten 3-minute treatment sessions separated with 20-30 minutes or more between each session.

			Total Time: 3 - 5 hours
6	**rTMS**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Up to ten 3-minute treatment sessions separated with 20-30 minutes or more between each session. Total Time: 3 - 5 hours
7	**rTMS**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	Up to ten 3-minute treatment sessions separated with 20-30 minutes or more between each session. Total Time: 3 - 5 hours
8	**Post-treatment Neurocognitive Battery** **Post-treatment Questionnaire Battery**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	90 minutes 1 hour
9	**MRI Safety Screen** **Post-treatment Structural MRI and functional MRI** **EEG assessment**	MUSC Center for Biomedical Imaging	5 minutes 1 – 2 hours 1 – 2 hours
10	**1 month post-treatment Neurocognitive Battery** **1 months post-treatment Questionnaire Battery** **EEG assessment**	MUSC Brain Stimulation Laboratory in the Institute of Psychiatry	90 minutes 1 hour 1 – 2 hours

<u>Study Arm 3: Biological Efficacy of TMS in Microgravity</u>

To assess the biological efficacy of TMS during microgravity, we will determine resting motor threshold (rMT) in 10 subjects before, during and following parabolic flight (sessions 1-3). To determine rMT, we will briefly apply single pulse TMS. We will use a standard clinical algorithm (adaptive parameter estimation by sequential testing) to determine rMT of the abductor pollicis brevis muscle in an automated fashion. The measurement takes 20 seconds to perform (20 stimuli using single pulse TMS)[75,76].

Pre-flight data collection: First, on the ground pre-flight, the optimal location for stimulus induction will be identified. This location will be marked on the scalp in order to facilitate quick and reproducible identification of this site during the in-flight and post-flight sessions. At this location the TMS coil will be fixed firmly in place and the rMT will be determined. The subjects will also undergo a 10 minute standard computerized cognitive battery (WinSCAT) used by NASA to assess cognitive function in astronauts during International Space Station flights[60].

In-flight data collection: The in-flight rMT measurement will be performed by placing the coil in the same location identified pre-flight. The coil will be secured in place using stabilization hardware developed with Zero-G similar to the fixation hardware in use in our lab. The flight profile includes 30 parabolas each with approximately 25 seconds of microgravity. This will allow up to 2-3 in-flight measurements per subject providing a margin for human error.

Post-flight data collection: The same procedure will also be utilized to obtain the post-flight measurement. The subjects will also undergo a computerized cognitive battery (WinSCAT) post-flight.

Any significant change (based on established values in normal controls[54]) in rMT during flight compared with pre- and post-flight values will be interpreted as an indicator of an altered physiological response to TMS during microgravity.

The research team (Drs. George and Roberts and trained research assistants) will be aboard the flight and perform all research procedures. Drs. George and Dr. Roberts are licensed physicians who have extensive experience safely performing TMS. Dr. Roberts has previous parabolic flight experience. The research assistants will come from the brain stimulation laboratory and will have been trained in all aspects of TMS application and safety prior to the flight. In addition, they will have been responsible for the application of TMS in the brain stimulation laboratory gaining expertise in applying TMS during ongoing TMS trials prior to the parabolic flight.

Additional data collection: The same procedures will be utilized to obtain the rMT in three different body positons (upright, supine, Trendelenburg) and WinSCAT cognitive battery.

Experimental set up

See the figure which shows our set up on the ground. To record motor evoked potentials, signals will be amplified using a 1902 amplifier, digitized with a 1401 ADC and displayed using Signal Software (Cambridge Electronic Design, Cambridge, England) which will be used on loan from our laboratory. For cortical stimulation, a portable TMS system will be used. A laptop computer will be used to control the automated software. Zero G Corporation will work with the research team to install this hardware in the aircraft and to perform safety checks.

Zero-G has extensive experience with the aircraft and implementing hardware setups. NASA has used Zero-G parabolic flights to train astronauts and certify spaceflight hardware for more than 15 years. Zero-G Corporation has performed a feasibility analysis and has given preliminary pre-

approval for this study. Approximately 2 months prior to the flight, a detailed structural review of the experimental set up will be performed. As a commercial airline, Zero-G is regulated by the FAA. Any structural change to the aircraft such as bringing experimental equipment on board must be approved by the FAA. Therefore our experimental set up will undergo structural analysis by the FAA as part of a designated engineering review. Upon receiving approval from the FAA, we will be confirmed for a flight date.

On the day prior to the flight, the study team members will work with Zero G to load the study equipment into the aircraft and to perform safety checks assuring the test equipment is ready for flight (See study schedule below). During this time, the participants will have free time.

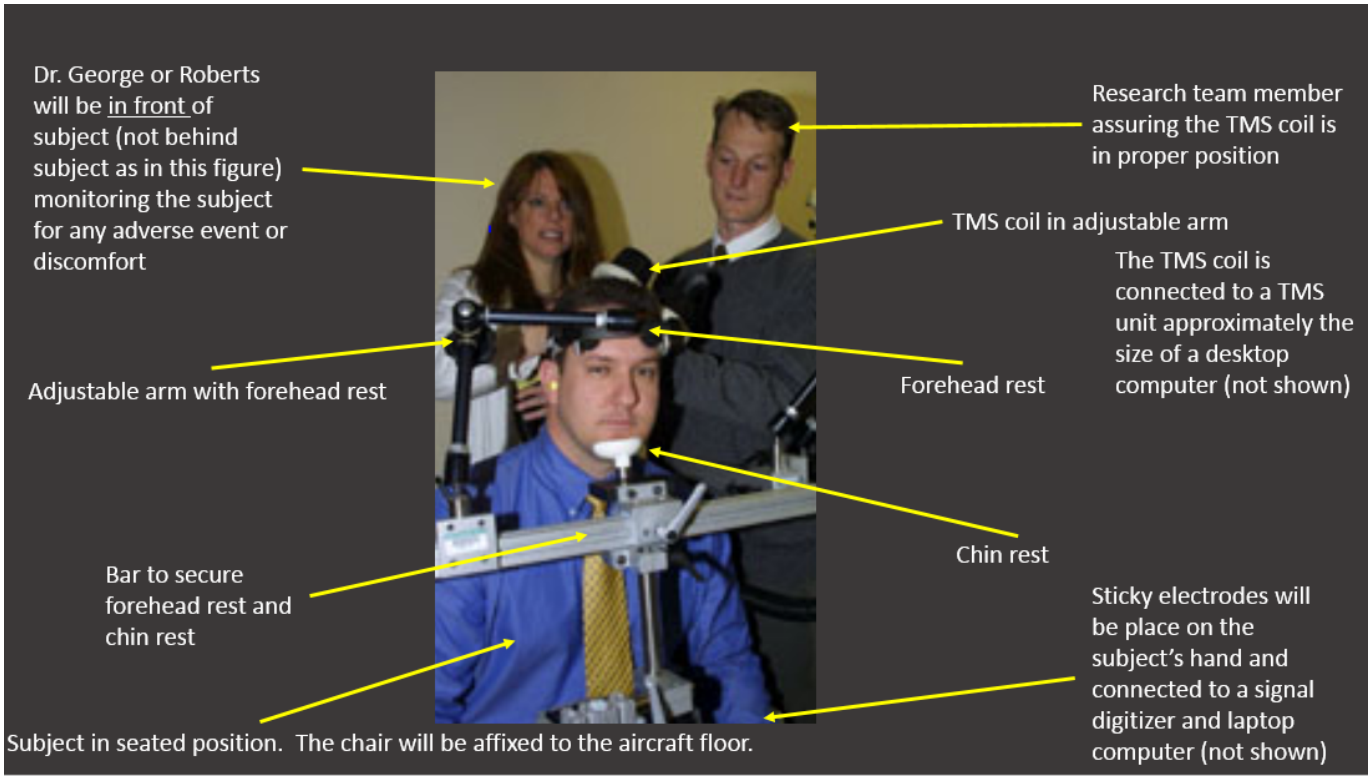
Research activities during parabolic flight

Upon entering the aircraft, subjects and the research team will be directed to an area in the back of the plane which contains standard aircraft seating. Subjects and the research team will remain in this area during take-off and flight until the aircraft reaches an appropriate altitude. The subjects and research team will stay seated until signaled by Zero G personnel that it is time to move to the section of the plane where all experimental procedures will take place. At that time, the parabolic portion of the flight will begin.

For each parabola, Zero-G personnel provide the study team with adequate notice that the microgravity portion of the flight is approaching. This gives the research team time to get the subject in place in preparation for the upcoming TMS application. During TMS, the subject will be seated in a chair fixed to the floor of the aircraft and wear a seatbelt. (See the figure). The TMS coil will be securely fixed in place using an adjustable arm attached to the chair. The subject will place his/her head in a chin rest and forehead rest attached to the chair. The subject will hold their head against the TMS coil for the 20 seconds of TMS. One member of the research team will start the software which will control application of the TMS pulses and ensure the subject's head is in the appropriate position against the TMS coil. Another member of the research team (Dr. George or Dr. Roberts) will closely monitor the subject for any discomfort or adverse event. After the 20 seconds of TMS, the subject will move from the experimental set up to the nearby designated location where subjects remain when not actively involved in the study as the research team prepares the next subject for the next parabola.

During parabolic flight, go-pro cameras will be used to video record the study.

All emergencies are handled in accordance with FAA regulations. Throughout the flight Drs. George and Roberts will observe the subjects for any sign of an adverse event requiring medical attention. If such an event occurred, Dr. George or Roberts will notify the Zero-G flight director who will inform the pilot to end the parabolic flight maneuvers and to go immediately to the closest airport to get medical treatment for the subject. Drs. George and Roberts will ensure the subject is in a safe position and monitor the subject until emergency personnel arrive. All Zero-G personnel are trained in basic life support and cardiopulmonary resuscitative equipment is available on the plane.



Travel/transportation

The parabolic flight will take place in Sanford, Florida. Participants will be required to make their own transportation arrangements to Sanford, Florida and for the return trip to the Charleston area. The study will reimburse subjects for gas mileage from the Charleston area to Sanford, Florida and for the return. Participants should plan on travel to Sanford, Florida the day before the planned study date. A hotel reservation will be made for the participants by the study team and the cost of the hotel stay for two nights will covered by the study. Participants will also be reimbursed a per diem for meals. Reimbursement rates will be per MUSC policy (currently 58 center per mile and $32 per day per diem pro-rated). This has been reviewed and approved by MUSC Risk Management and the approval email from Risk Management is attached.

Study schedule

Listed are the activities of each day and who will be involved (study team and/or participants).

Day prior to study	Travel to Sanford, Florida	Study team	Participants

Day 1: Test Readiness Review			
8:00am:	Arrive	Study team	Participants
8:30am:	Mission Briefing	Study team	Participants
9:15am:	Experiment set-up	Study Team	Participant - Free Time
9:30am:	Test Readiness Review (TRR)	Study Team	Participant - Free Time
12:00pm:	Finalize TRR	Study Team	Participant - Free Time
2:00pm:	Load experiments on G-FORCE ONE	Study Team	Participant - Free Time
5:00pm:	Complete experiment loading	Study Team	Participant - Free Time

Day 2: Flight Day			
8:00am:	Check-in	Study team	Participants
8:30am:	Pre-flight data collection	Study team	Participants
9:00am:	Security Check & Boarding	Study Team	Participants
10:00am:	ZERO-G Weightless Lab Flight	Study Team	Participants
12:30pm:	Post-flight data collection	Study Team	Participants
1:00pm:	Return to Charleston area	Study Team	Participants

Study Arm 4: Dose Finding

Assessment & rTMS. The assessment and TMS methods from study 1 will be identical, except that 50 participants (enrollment n=60 to complete assessments and treatment for n=50 to contend with drop out) will be randomized to one of ten doses of active iTBS rTMS. In addition, to determine longer-term safety of the intervention, participants will complete neurocognitive, resilience and symptom measures pre- and immediately post-treatment as well as 1 month later.

MRI assessment. To more specifically examine safety, all participants will undergo MRI sessions (FLAIR, diffusion, T2* and volumetric sequences) for assessment of structural changes at pre- and post-treatment,.

Physiological assessment. The impact of each dose of iTBS on function will be further assessed with electrocortical and physiological activity during a challenging feedback-based response-

learning paradigm[39,70]. This learning task is essentially a more difficult version of the Wisconsin Card Sorting task. More specifically, participants must dynamically learn different categories of stimuli as well as the exceptions. Participants receive feedback (correct/incorrect) on each trial to shape learning. Intermittently, and disproportionately on incorrect trials to increase stress, participants are instructed to increase performance speed. Additionally, trials are embedded in counterbalanced blocks of threat of unpredictable shock. Half of the blocks are "No Shock" probability blocks, whereas half of the blocks are "Unpredictable Threat of Shock." Thus, in addition to performance demands participants must contend with a psychologically threatening and unpredictable context during performance. Typically, more efficient learning and performance is predicted by increased midline theta oscillations (4-8 Hz) and increased intersite phase synchrony during feedback, and further enhanced under threat or stress among those with successful performance[39]. Responses to blocks of unpredictable threat and no threat will be compared in terms of autonomic measures (heart rate and skin conductance) as well as the responses to 50 ms, 98 dB white noise probes, which elicit a blink response (i.e., startle reflex) measured with electromyography (EMG). The reactions captured in these measures reliably increase as a function of stress and aversion as well as lack of resilience[71-74]. In summary, we predict that EEG metrics of cognitive control will be enhanced pre- to post-treatment as a function of dose, and more pronounced under threat, indicative of enhanced cognitive control and resilience. We predict that concurrent physiological metrics of stress reactivity during unpredictable relative to no threat of shock will be reduced pre- to post-treatment, more so at higher doses, indicative of improved resilience. Shock level will be individually titrated prior to the learning procedure. EEG will be assessed pre- and immediately post-treatment as well as 1 month later (64-channel Brain Products ActiChamp System). Startle, heart rate and skin conductance will be assessed with Biopac EMG, EKG, and EDA modules.

10.0 Data Management

Confidentiality. Any discussion of identifying sensitive information will occur in private rooms at the Brain Stimulation Laboratory, 30 Bee Street Center for Biomedical Imaging, or the Department of Radiology. Regarding documentation, participants' names will appear only on the IRB-approved Consent, HIPAA, and payment forms, initial screening form, and in a separate key file that links individual participant names and contact information to a random participant identification code. The participant identification code will be assigned to the individual during Visit 1 and all subsequent data collection will reference this code. The key linking individual identifying information to the participation code will be maintained in an electronic database accessible only to the PI and her designees in a password-protected file on an encrypted and password protected network (MUSC LAN). The questionnaire data collected through RedCap will be referenced by participant ID only and collected via a HIPAA compliant interface and downloaded to the MUSC server once a participant has completed participantion. If a participant consents to participate at the interview, the initial screening form will be entered into a secure electronic database according to the assigned participation code and then locked in the PI's office, separate from payment and consent forms including heath information. If a participant declines consent or is lost to follow-up (i.e., defined as not appearing for intake within 1 month of screen), the screening form will be securely shredded. The consent, HIPAA, and payment forms will be kept in a locked cabinet in the PI's locked office. All other collected paper (e.g., interview responses) and electronic (e.g., questionnaire, neurocognitive, MRI data) files will be identifiable only by participant code and stored in locked file cabinets or on the secure MUSC LAN at the Institute of Psychiatry (non-MRI

data) and Center for Biomedical Imaging (MRI data). The key file linking names to IDs will be deleted after data collection is complete.

Consent and HIPAA forms will be kept on file for 6 years. Contact information is kept on file for 6 years if the participant consents to allowing their information to remain active in our files, or the contact information is destroyed immediately after the study is completed if participants chose that option on the consent form. Although individual-subject analyses may be written up in publications, the individual subjects producing that data will never identified by name or initials, or any other identifying information.

All study personnel will complete all annual MUSC CITI training concerning confidentiality and research ethics.

The PI will monitor data quality on a weekly basis to ensure integrity, completeness, and fidelity to the IRB protocol.

Statistical Plan.

Arms 1 and 4. Dose-response relationships for the primary and secondary efficacy endpoints of change in neurocognitive performance (composite measure), EEG, and resilience, stress and symptoms would be assessed using the multiple comparisons procedure with modeling (MCP-Mod) approach for 1 and 2 month outcomes. To determine the reliability of a dose response, MCP-Mod fits a set of pre-specified candidate dose-response curves to the data using a multiple-comparisons technique. The pre-specified dose-response curves to be tested for resilience and neurocognitive performance would be Emax, logistic, and linear. Significant curves would then be used to develop inferences on optimal doses for the randomized controlled trial in aim 2. Descriptive statistics would be performed on acceptability ratings, adverse events, and dropout rates. Linear mixed- effects models would assess the trajectory of pain/discomfort ratings.

The rationale for participant/dose ratio. Dose-response studies are not powered in the traditional manner. In order to resolve the shape of the continuous dose response function, it is statistically more efficient to select more doses as opposed to more participants[57,58]. The number of observations within each dose is primarily to account for sampling variability, which could be achieved with a minimum of 2 to 3 observations. Capturing variability is essential to the planned analyses, which underweight the points of greatest variability in estimating the response curve. To contend with dropout we propose to enroll 60, with the aim of completing assessments and treatment for 50 participants. We conservatively expect an 80-90% retention rate, as dropout is typically fewer than 10% in double-blind trials of conventional daily[59] as well as accelerated rTMS[31].

Arm 2. Descriptive statistics would be performed on acceptability ratings, adverse events, and dropout rates. Linear mixed-effects models would assess group x pre/post rTMS change in neurocognitive performance, resilience and stress self-report measures, and EEG and ERP metrics. Regarding statistical power, we based predicted effect sizes on findings of neurocognitive enhancement by Holtzheimer et al.[31] in an accelerated rTMS protocol, specifically, reliable change of 5 standard units on the RBANS (SD=8.9). Specifically, group sample sizes of 27 achieve 80% power to detect a difference of 5.000 (SD=8.9) in a design with 3 repeated measurements having a Compound Symmetry covariance structure, the correlation between observations on the same subject is 0.300, and alpha level is 0.050. We propose to enroll 70 people to contend with dropout with target completion reached with 30 participants each completing active and sham conditions (60 participants total).

Arm 3. A power analysis was performed to determine the number of subjects needed to study physiological effects of simulated microgravity on motor threshold determination. With our study design, each subject will be his/her own control. For calculation of the study's power, we used data of Wassermann[54] who examined session-to session variability of motor threshold in 19 subjects on three separate occasions and computed the average coefficient of variation in motor threshold across sessions as 0.058±0.036. Based on this data, a total of 10 subjects will have a 99% chance (at a two sided 5% significance level) to detect a change in motor threshold due to an experimental intervention (parabolic flight) with an effect size of 1.61.

11.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The PI's plan for ensuring safety and data integrity follows.

Quality Control. QC will include regular data verification at weekly meetings with the PI, Co-Is and study personnel. This will include verification of the documentation (Integrity of the Consent and HIPPA, scores on the Assessments, MRI scanning information), study progress and participant status, any adverse events, and any protocol deviations. Events determined by the PI to be unanticipated problems involving risks to subjects or others (UPIRTSOs) will be reported by the PI to the IRB as soon as possible and no more than 10 working days per policy.

Safety Training. Before any investigator or assistant is allowed to enter the scanner room, they are required to take an extensive MRI safety course (with annual refresher courses) that cover powering down (or quenching) the magnet for patient safety and with established procedures for expediting participant contact with emergency medical personnel, should the need arise. These courses are run by the MUSC Center for Biomedical Imaging and are a prerequisite for obtaining privileges to book and use scanner time. Prior to administering TMS all personnel must be trained and certified by Dr. George/Dr. Roberts.

Seizure Risk. Participants will already be lying down on the scanner bed during scans or reclined in a chair during rTMS. In the event of a seizure in the scanner, participants will be taken out of the magnet bore immediately and the barriers on both sides of the detachable bed will be raised to prevent injuries from falls. This event will qualify as a medical emergency and procedures outlined below will be followed.

Medical Emergencies:

1. Emergency responding in the scanner is facilitated by having two research staff running a scan.

In the event of an emergency, one of these individuals remains with the participant and undocks the scanner bed from the magnet bore. This bed can easily be wheeled out of the scan room to facilitate speedy access to arriving emergency medical personnel. The second researcher calls 9-1-1 from the scanner suite and gives details of the participant's level of medical distress and location. Next, this person goes out to the front of the scanner building to flag down arriving emergency personnel and to direct them to the participant.

2. Dr. George and Dr. Roberts are licensed physicians and will be on call during all sessions and rTMS sessions (or a similarly trained physician) to respond to any more subtle potential medical situations arising from doing TMS/fMRI. Dr. George will be responsible for training research personnel in detecting the onset of seizures.

3. These guidelines are in full agreement with the Center for Biomedical Imaging safety protocols and with published guidelines by a panel of experts in conducting TMS/rTMS and TMS/neuroimaging work for both research and clinical purposes[95].

Ethical Research Practices. Ethical guidelines for clinical research will be followed strictly and all information obtained in the study will be kept strictly confidential. Data will be assigned coded identifiers and all names will be removed from study assessment and outcome data. Files linking participant names or identifying information to the coded identifier will be stored in a password-protected file, on a password protected desktop computer in a locked laboratory. Demographic and other identifying information will be stored separately from consent forms to eliminate the possibility of participant identification; signed consent forms will be locked in secure cabinets separate from data files. The files linking names to IDs will be deleted at the conclusion of the research project. De-identified data will be stored indefinitely following the conclusion of the project. Only the PI and active research staff will have access to the de-identified data. The PI and all research staff and mentors will be responsible for and will comply with mandated reporting rules. All researchers will be obligated to demonstrate that they have remained abreast of all guidelines and rules related to the Health Insurance Portability and Accountability Act (HIPAA). Each member of the research staff will complete focused training on each task for which they are responsible and will perform ongoing quality control for others performing similar work. The PI and/or study coordinator will produce quarterly administrative reports describing study progress including accrual, demographics, and participants' status. Reports will describe adherence to inclusion/exclusion criteria and the study protocol in addition to any unanticipated problems in the category of risks to participants or others as well as any adverse events. All collected data will be obtained for research (and participant safety) purposes only.

Other protections against risk. In designing this trial, the research team constantly struggled with the tension between adding more tests or interventions and participant burden. As was discovered in the pilot study by George and colleagues[86], this design is feasible, does not impose unreasonable expectations of time or effort, or expose patients to risks or limit them from the best available care.

Adverse Events & Trial Safety. Potential conflicts of interest will be reported using the NIH rules for disclosure. Adverse Events (AEs)/Serious Adverse Events (SAEs) occurring during the course of the project will be collected, documented, and reported in accordance with protocol and IRB reporting requirements. All research staff involved with adverse event reporting will receive general and protocol specific AE/SAE training including identification, assessment and evaluation, and documentation and reporting. A research specialist will identify any potential adverse events during the course of the study from participant self-report and administration of the visit assessments and procedures. The research assistant will provide information to the PI, who will be responsible for AE/SAE assessment and evaluation including a determination of seriousness and study relatedness.

An Adverse Event (AE) is defined as any unwanted change, physically, psychologically or behaviorally, that occurs in a study participant during the course of the trial is an adverse event. A Serious Adverse Event (SAE) is defined as an adverse event that has one of the following outcomes:

- Results in death,
- Is life-threatening,

- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, OR
- Requires intervention to prevent one of the above outcomes.

AEs/SAEs are documented and reported as per protocol and IRB requirements. Research staff will identify adverse events and obtain all available information to assess severity, seriousness, study relatedness, expectedness, outcome and the need for change or discontinuation in the study intervention. Adverse events are generally documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information if available should be documented in a progress note in the research record as appropriate to allow monitoring and evaluating of the AE. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms are completed and disseminated to the appropriate persons and within the designated timeframes as indicated above. For each AE/SAE recorded, the research staff will follow the AE/SAE until resolution, stabilization or until the participant is no longer in the study as stated in the protocol. When a reportable SAE is identified, the research staff will notify the MUSC Institutional Review Board (IRB) within 24 hours and complete the AE report form in conjunction with the PI. The MUSC IRB meets monthly and is located at 165 Cannon Street, Rm. 501, Charleston, SC 29425. Communication with the IRB is through email, memos, official IRB forms, and online reporting.

If complete information is not available when the initial 24-hour SAE report is disseminated, follow-up information will be gathered to enable a complete assessment and outcome of the event. This information may include hospital discharge records, autopsy reports, clinic records, etc. The research staff will attach copies of source documents to the SAE report for review by the PI.

We will report adverse events to the Medical University of South Carolina (MUSC) Institutional Review Board (IRB), but no later than 10 working days after the investigator first learns of the event. The MUSC IRB AE reporting requirements are as follows: All deaths that occur during the study or 30 days post termination from the study are required to be reported as adverse events even if they are expected or unrelated. Other adverse events are reportable to the MUSC IRB if the AE is unexpected AND related or possibly related AND serious or more prevalent than expected. All three criteria must be met for an AE to be reported to the MUSC IRB. The IRB definition of unexpected is that the AE is not identified in nature, severity or frequency in the current protocol, informed consent, investigator brochure or with other current risk information. The definition of related is that there is a reasonable possibility that the adverse event may have been caused by the drug, device or intervention. Reportable AEs are reviewed by the IRB Chair and reported to the IRB Board at the next meeting.

The potential risks and benefits and methods to minimize these risks are outlined above. The research staff will report any unexpected AEs or any scores of “severe” on the side-effect symptom rating form or any FDA-defined serious AEs to the PI within 24 hours so that the PI can decide on the appropriate action. All unexpected AEs will be monitored while they are active to determine if treatment is needed. Study procedures will follow the FDA’s Good Clinical Practice Guidelines (www.fda.gov/oc/gcp).

Data and Safety Monitoring Plan (DSMP). The PI, Dr. Roberts will be in charge of 1) providing scientific oversight; 2) reviewing all adverse effects or complications related to the study; 3)

monitoring enrollment; 4) reviewing summary reports relating to compliance with protocol requirements; and 5) providing advice on resource allocation. Dr. Roberts will meet quarterly in-person and as necessary with the CoI-s as an internal Data Safety Monitoring Committee (DSMC) to review progress. The recommendations of the DSMC will be reviewed and the PI will take appropriate corrective actions as needed. At each meeting the DSMC will:

- Review the research protocol and plans for data and safety monitoring.
- Evaluate the progress of the three studies, including periodic assessments of data quality and timeliness, participant recruitment, enrollment, and retention, participant risk versus benefit, integrity of the intervention, and other factors that can affect study outcome.
- Consider factors external to the study when interpreting the data, such as scientific developments that may impact the safety of study participants or the ethics of the study.
- Make recommendations to the MUSC IRB for continuation or termination of the studies.
- Protect the confidentiality of study data and monitoring.

Institutional Review Board (IRB). The MUSC IRB will review and approve the funded protocol; review patient and provider consent forms, ensure protection of patient privacy and safety, and monitor the study on an ongoing basis. Adverse events will be reported to MUSC IRB as they occur. Annual reports to MUSC IRB will indicate enrollment rates, adverse events, new findings that may influence continuation of the study, and reports of the DSMB.

12.0 Withdrawal of Subjects

Early withdrawal of study subjects. As stated on the Informed Consents, all subjects reserve the right to withdraw from the clinical investigation at any time. The Investigator for any of the following reasons may discontinue subjects from the study:

- Subject is found to have entered the study in violation of the protocol.
- Subject withdraws consent to participate in the study.
- Subject is noncompliant with procedures set forth in the protocol.
- Subject experiences an Adverse Event that warrants withdrawal from the study.
- It is in the Investigator's opinion that it is not in the subject's best interest to continue.
- Subject displays other abnormal laboratory, medical or clinical findings for which clinical intervention should take precedence over study participation including:
    a) Development of significant neuropsychiatric symptoms
    b) Generalized seizure
    c) Inpatient hospitalization
    d) Unable to complete treatment in the designated time frame

Any participant who informs research staff of an intention to withdraw from the study will be asked to return for a final safety visit, whereby the complete range of post-treatment assessments will be performed. If a participant is lost to follow up, three documented attempts will be made to contact the participant.

13.0 Risks to Subjects

Risks associated with TMS and MRI.

Challenges will include patient tolerability to TMS, the MRI scanner, and the study clinical and neurocognitive assessments. The primary safety concern, the same as that for conventional once-daily rTMS, is risk of seizure. However, all reported TMS-induced seizures during conventional or accelerated protocols have been self-limiting, and NONE required further intervention to stop the seizure; no post-seizure sequelae or recurrent seizures developed. Extensive precautions with regard to suicidal ideation/homicidal ideation risk will be followed.

Due to the novel nature of the proposed study, Table 4 is included to provide a representative range of the stimulation parameters and populations examined with accelerated rTMS. The dosing range of these studies covers the range proposed in this study. In these as well as other accelerated rTMS studies, the authors have demonstrated the safety, feasibility and acceptability of accelerated, high-dose studies of rTMS in neurologically intact samples. Of pertinence to the issue of safety as well as the conceptual model proposed here, Holtzheimer et al.[81] showed reliably enhanced neuropsychological performance on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) from baseline to six weeks after an accelerated, high-dose rTMS protocol in patients with major depression. Furthermore, Baeken et al.[82,83] as well as Herremans et al.[84] and Williams et al.[85] additionally collected structural and functional imaging and demonstrated no adverse changes pre- to post-accelerated rTMS. Taken together, these studies demonstrate that the potential risks of accelerated rTMS are likely similar to conventional once-daily rTMS.

Table 4 . Representative accelerated rTMS studies for neuropsychiatric conditions.

Study	Total Pulses	Days	Total # Sessions	Stimulation Intensity	Disorder	Frequency	N
Holtzheimer et al.[81]	15,000	2	15	100%	Major Depression	10 Hz; 5s train, 25s ITI	14
Baeken et al.[82,83]	31,200	4	20	110%	Major Depression	20 Hz; 1.9s train; 12s ITI	15
Herremans et al.[84]	31,200	3	15	110%	Alcohol Use Disorder	20 Hz; 1.9s train; 12s ITI	19
Desmyter et al. [92]	32,400	4	20	110%	Major Depression	54 bursts of three; 2s train; 8s ITI s	50
Duprat et al. [93]	32,400	4	20	110%	Major Depression	54 bursts of three; 2s train; 8s ITI s	50
George et al. [86]	54,000	3	9	120%	Suicidal ideation	10 Hz;	41
McGirr et al. [34]	60,000	10	20	120%	Major Depression	10 Hz; 5s train, 25s ITI	27

Modirrousta et al. [94]	90,000	3	30	110%	Major Depression	10 Hz; 5s train, 25s ITI	18
Williams et al. [85]	90,000	5	10	90%	Major Depression	54 bursts of three; 2s train; 10s ITI	7
Schulze et al. [91]	120,000-180,000	10-15	20-30	120%	Major Depression	20 Hz; 2.5s train; 10s ITI	65

Weighing potential risks related to TMS. Based on previous clinical applications of left prefrontal rTMS in depression and in various other psychiatric disorders, as well as risks published by researchers and communicated experiences, it is hypothesized that left prefrontal rTMS (as proposed) is likely to be effective in enhancing cognition. This study is an important necessary step to characterize the safety, feasibility, efficacy and also durability of the proposed quality of life enhancing effect.

Potential worsening of neuropsychiatric symptoms with TMS. Several studies have thus far demonstrated the feasibility of using rTMS in depression without any indicators of exacerbation of symptoms. The research team will work closely with participants to familiarize them with the nature of this experimental setup. All staff will also be trained to be alert to any emergence of neuropsychiatric symptoms and/or psychosocial impairment. An ethical issue concerning the use of variable doses was considered at length; however, this concern is made easier by understanding that all participants will be given an active course of rTMS. Thus, even the lowest doses have previously shown efficacy in treating depression and may improve cognition among healthy participants in the study 1. Additionally, all participants will be assessed throughout each day for emerging side effects.

Potential risk of a seizure with TMS. There is a risk that TMS can cause a seizure; but it is rare. The risk of seizure induction is related to the intensity, duration, frequency and rest interval of stimulation. Following the adoption and widespread use of the safety guidelines from a National Institute of Neurological Disorders and Stroke (NINDS) workshop on TMS, only 20 seizures have been reported since 1997, and they usually involved parameters of "higher settings" then the "safe range". To our knowledge, stimulation with the parameters and settings proposed in this study should not cause seizures. Each subject's stimulus intensity is determined by his or her motor threshold and will be carefully calculated before beginning treatment. Our study patients will be free from using known stimulants and medications that are known to increase the risk of seizure (e.g., theophylline).

Other potential effects of TMS on brain tissue. TMS is thought to be safe, with no brain damage, despite extensive use in humans and other animals. Dr. George and colleagues[90] have recently completed a case report of a maintenance treatment of rTMS for depression over a year, where a depressed patient received a total of [(16,000 x 2 trials) + (8,000 x 12 trials)] = 32,000 + 96,000 = 128,000 stimuli over a year period. The patient's MRI showed no structural changes at the end from baseline. The patient experienced no seizures and had tolerated the procedure equally throughout the successive trials. Dr. George and colleagues have also reported a safety study[88]

looking at the MRI scans before and after 2 weeks of daily left prefrontal rTMS for depression. Specifically, no structural changes were found in the left prefrontal lobe of patients who received active rTMS compared to placebo. More specific to the current study, Baeken et al.[82,83] as well as Herremans et al.[84] and Williams et al.[85] additionally collected structural and functional imaging and demonstrated no adverse changes pre- to post-accelerated rTMS.

Potential changes in cognitive function. There have been no reports of deleterious changes (more than a minute) in cognitive function (memory, attention, etc.) in rTMS studies. Safety studies specifically looking for these changes did not find any effects of rTMS. Holtzheimer et al.[81] showed reliably enhanced neuropsychological performance on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) from baseline to six weeks after an accelerated, high-dose rTMS protocol in patients with major depression. This study will assess for potential changes in cognitive function with pre- and post-treatment cognitive batteries designed to look for potential TMS effects, if they exist.

Potential hearing loss. The discharge of the rTMS coil generates a high-energy click that may cause cochlear damage. Humans exposed to rTMS have shown temporary increases in auditory threshold (especially at high frequencies) lasting at least 5 minutes and less than 4 hours[89]. Foam earplugs can protect against these changes and will be worn by the patients and researchers present during TMS sessions. Due to high dosage delivery in this proposal, participants will be instructed to ensure proper fit of earplugs prior to each session.

Safety in case of pregnancy. This protocol will exclude pregnant and breastfeeding women (women of child bearing age will undergo a pregnancy test prior to enrollment).

Risk of facial twitching and skin irritation. The TMS coil can cause facial twitching, skin irritation, or both, which can be acutely unpleasant. This typically often reduces over the course of treatment. Additionally, all patients will have a foam insert placed between the coil and their scalp for comfort and this typically reduces this discomfort. Furthermore, facial twitching and skin irritation are typically only acute and subside with the end of stimulation.

Risk of a first-degree burn. The TMS coil can heat up during use. The machine used in this study has two major protective engineering features: (1) an external heat monitor that will shut down the system if the coil gets too warm; and (2) a liquid-coiled coil design that keeps the coil much cooler than previous models. Additionally, all patients will have a foam insert placed between the coil and their scalp for comfort and also to act as additional thermal protection. The TMS treater will periodically monitor coil temperature during each treatment.

MRI risks. Exposure to magnetic field strengths used in the present study is not shown to be a significant health risk. Risks to an unborn fetus from exposure to the MRI field strength used in the proposed research (3 Tesla) are unknown. Therefore, pregnant females and those who may become pregnant (unwilling to follow study restrictions limiting chances of conception) will not be allowed to participate. Participants will be asked to lie still and awake for 30 minutes in the scanner and this can occasionally result in soreness, stiff back, etc. Participants will be queried approximately every 10 minutes about their comfort. The main risk associated with MR imaging is the possibility of introducing metal to the magnet or its close proximity. Participants are thoroughly screened to prevent metal being brought into the MR environment. Other potential hazards of MRI scanning include: collision hazards, noise, neurostimulation at rapid sampling

rates (i.e., short TRs), body temperature changes, helium, and nitrogen hazards. The MRI facility is tested regularly by internal and external safety monitoring teams. These risks are minimal, and the facility is run within FDA guidelines. All investigators and research assistants running participants in the Center for Biomedical Imaging are thoroughly trained in MR safety as a requirement to run scans.

Risks associated with the parabolic flight (ZERO-G Experience®).

Injury or Illness. There is risk of injury or illness from sudden changes in gravity, altitude and/or turbulence, and could include physical contact of one's body with the interior of the aircraft and other participants during weightlessness, as well as objects and liquids floating in the aircraft. There is the danger of property damage, personal injury or illness (minor or serious) and/or death resulting from weightlessness, the conduct of the ZERO-G Experience®, flight malfunction or mechanical failure and/or anything else related to the ZERO-G Experience®. The risks of such Injuries & Damages are involved in such high adventure programs as the ZERO-G Experience® and subjects may have to exercise extra care for their own person and for others around them in the face of such hazards and that despite exercising such care Injuries & Damages may occur.

Hazards and Risks Unforeseen and Foreseen. This experience involves exposure to a variety of hazards and risks foreseen and unforeseen, which are inherent in or may result from each ZERO-G Experience® and some of which cannot be eliminated without destroying the unique character of the ZERO-G Experience®.

Lack of Medical Personnel. There may not be rescue or medical personnel on board the aircraft to address and treat the Injuries & Damages to which the participants (subjects and study team personnel) may be exposed to as a result of the ZERO-G Experience®. However, Drs. George and Roberts will be aboard the flight and all Zero-G personnel are trained in basic life support and cardiopulmonary resuscitative equipment is available on the plane.

Nausea or Vomiting due to Motion Discomfort. Motion discomfort can lead to nausea or vomiting. During the preflight training, the ZERO-G Experience® staff will provide some useful tips on in-flight movements that will help participants to reduce their chance of motion discomfort. Unlike the astronauts in training, the flyers will experience 30 parabolas, which is enough to provide a pleasurable experience but not enough to cause motion discomfort. An often-made mistake by flyers is not eating prior to the flight. ZERO-G provides a flight- friendly meal and encourages all participants to eat before and during the preflight training. Flying on an empty stomach is not recommended and can aid in creating motion discomfort.

The Zero-G Experience Risks of Other Persons. Injuries & Damages can occur by natural causes or activities of other persons, other participants, the Mission Director and Coaches, assistants, the study team personnel, or other third parties, as a result of negligence, the conduct of the ZERO-G Experience® or because of other reasons.

Decompression and Loss of Consciousness. While the probability of an explosive decompression on the ZERO-G aircraft is similar to any other Part-121 operation, commercial airplane operation, the implications of not getting oxygen into the body immediately could be significant including permanent brain damage or death.

Unknown Risk of Performing single pulse TMS during Parabolic Flight

Subjects will be exposed to TMS during parabolic flight for at most 40 seconds. However, as TMS has previously not been performed in a microgravity environment, all risks associated with TMS during parabolic flight cannot be known. Microgravity may result in unknown altered cerebral physiology which may alter the response to TMS and it is for this reason that study arm 3 is being performed.

Nevertheless, the investigators have taken several precautions to reduce the chance that a subject would be exposed to any serious risks from combined TMS and parabolic flight:

- First and foremost, only single pulse TMS will be performed. As discussed above, the risk of seizure induction with TMS is related to the intensity, duration, frequency and rest interval of stimulation. For study arm 3, only single pulse TMS will be performed such that TMS pulses will be spaced 1 second apart.

- The intensity of TMS will be very low. The intensity will be set to be at most just above the amount needed to cause a brief thumb twitch.

- The subjects will be exposed to a small number of TMS pulses (at most 40 TMS pulses total during parabolic flight)

- The duration of exposure to TMS during parabolic flight will be very brief. During parabolic flight, resting motor threshold will be determined. This will take at most only approximately 40 seconds total per subject.

- Drs. George and Roberts will be present on the flight and in constant observation of the subjects. At any sign of an adverse event requiring medical attention, they will notify Zero-G flight personnel who will inform the pilot to end the parabolic flight maneuvers and return to the airport to get medical treatment for the subject. 911 will be called and emergency personnel will meet the team at the airport.

14.0 Potential Benefits to Subjects or Others

Establishing the dose-response curve for enhancing neurocognitive performance with accelerated rTMS could efficiently identify the most efficacious range of doses and thus significantly advance the capacity for successfully contending with the rigors of spaceflight for astronauts. Participants in this study may or may not individually experience neurocognitive enhancement. While all doses are in the demonstrated therapeutically effective range, some doses may be more effective than others. Some people might consider parabolic flight (approximately $5000 value) as a potential benefit.

15.0 Sharing of Results with Subjects

We will inform participants of any new or relevant information that might influence their desire to continue participating in the study. We will also provide participants with a summary of their clinical outcomes.

16.0 Drugs or Devices (if applicable)

We will use a MagVenture MagProsystem with a Cool-B65 coil to deliver TBS to subjects. Access to the device will be limited to those who are trained to deliver the treatment and have been certified by Mark George, M.D.

Prefrontal rTMS at this intensity, frequency and number of stimuli has been considered "non-significant risk" by the FDA and the MUSC IRB for well screened depressed patients or healthy adults. (See the uploaded letter from the FDA; FDA_George.pdf). It is also FDA approved (Oct 2008) for the treatment of depression.

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