Glutamatergic and GABAergic Biomarkers in rTMS for Adolescent Depression

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Study Product:	NeuroStar TMS Therapy® System in XPLOR research configuration
Protocol Number:	May Clinic IRB# 17-004958
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Protocol Date 6 June 2018 Version (3.0)

Version No.	Date	Major Changes
1	16Sept2017	Initial
2	26Sept2017	DSMB recommended changes
3	6June2018	Add MRI/MRS portion and other recommendations from NIMH monitor report
4	10May2019	Complete Baseline assessments at Screening Visit to ease subject burden/ use of i2b2 as a recruitment tool/ IRB approved phone script, email and contact letter used for recruitment
5	20Jan2020	Blurred Vision: FDA requested protocol changes/added side effect "blurred vision" to consent form/changes in AE documentation
6	26 July 2021	The FDA recommended additional screening and monitoring for extrapyramidal twitching/trembling prior to starting the study procedures and throughout the study. The Abnormal Involuntary Movement Scale (AIMS) will be collected at the screening visit and at weekly assessments during Phase I and II investigational treatments and at the 6 month posttreatment visit.

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ATHF	Antidepressant Treatment History Form.
BDI-II	Beck Depression Inventory-II
CDRS-R	Children's Depression Rating Scale, Revised
CFR	Code of Federal Regulations
CGI-S	Clinical Global Impression-Severity Scale
CRF	Case Report Form
CSP	Cortical Silent Period
C-SSRS	Columbia Suicide Severity Rating Scale
CTQ	Childhood Trauma Questionnaire
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GABA	Gamma aminobutyric acid
HIPAA	Health Insurance Portability and Accountability Act
Hz	Hertz
ICF	Intracortical facilitation
IDE	Investigational Device Exemption
IRB	Institutional Review Board
cTBS	Continuous Theta Burst Stimulation
iTBS	Intermittent Theta Burst Stimulation
LDLPFC	Left Dorsolateral Prefrontal Cortex
LICI	Long Interval Intracortical Inhibition
MDD	Major Depressive Disorder
MEP	Motor Evoked Potential
MINI	Mini International Neuropsychiatric Interview
ms	Millisecond
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MT	Motor Threshold
NIH	National Institute of Health
NIMH	National Institute of Mental Health
PDS	Pubertal Development Scale and Tanner Staging
PI	Principal Investigator
QIDS-A17-SR	Quick Inventory of Depressive Symptoms Adolescent Self-Report
RDLPFC	Right Dorsolateral Prefrontal Cortex
rTMS	Repetitive Transcranial Magnetic Stimulation
SAE	Serious Adverse Event/Serious Adverse Experience
SHAPS	Snaith-Hamilton Pleasure Scale
SIT-R3	Slosson Intelligence Test – Revised
SICI	Short Interval Intracortical Inhibition
SOP	Standard Operating Procedure
TASS	Transcranial Magnetic Stimulation Adult Safety Screen

T	Tesla
TBS	Theta Burst Stimulation
TEAE	Treatment Emergent Adverse Event
TEEQ-A and TEEQ-P	Treatment Expectations and Experience Questionnaires Adolescent and Parent Versions
TMS	Transcranial Magnetic Stimulation
TORDIA	Treatment of Resistant Depression in Adolescents
UADE	Unanticipated Adverse Device Effect
UPIRTSO	Unanticipated Problems Involving Risk to Participants or Others
YMRS	Young Mania Rating Scale

Study Summary

Title	Glutamatergic and GABAergic Biomarkers in rTMS for Adolescent Depression
Running Title	Adolescent Biomarker Guided rTMS
IRB Protocol Number	17-004958
Phase	Pivotal
Methodology	Phase I is a double-blind, randomized, biomarker-stratified trial of 1 Hz vs. 10 Hz rTMS. Phase II is for participants who do not respond to Phase I treatment and is a biomarker guided, double-blind trial of continuous vs intermittent theta burst stimulation (TBS). Please note that transcranial magnetic stimulation (TMS) biomarkers will be collected in this protocol during the course of the proposed interventions. Participants in Phase I will also be offered 3 and 7 Tesla Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) scans at the University of Minnesota Center for Magnetic Resonance Research.
Overall Study Duration	5 years
Participant Time Commitment	Phase I – 6 months (1 week screening, 6 weeks of active treatment, 6 month follow up visit) Phase II- 6 months (1 week screening, 2 weeks of active treatment, 6 month follow up visit)
Objectives	To perform an effectiveness and target validation trial of 1 Hz vs. 10 Hz rTMS with a randomized, biomarker-stratified design. The target engagement and stratification biomarker is intracortical facilitation (ICF) a measure of glutamatergic tone in the brain. Cortical GABA, glutamate, and glutamine measured with 7 Tesla MRS will be collected in healthy controls at baseline and depressed participants at baseline and after 6 weeks of TMS treatment in phase I.
Number of Participants	Phase I- 120 participants Phase II- 50 participants (maximum) MRI – 100 Depressed Subjects (Must be Eligible for Phase I) 30 Healthy Controls
Diagnosis and Main Inclusion Criteria	Depressed adolescent (ages 12-18) participants with moderate to severe major depressive disorder (MDD) defined by a score of 40 or greater on the Children's Depression Rating Scale, Revised (CDRS-R) and an interview with the Mini International Neuropsychiatric Interview (MINI).
Study Device	NeuroStar® TMS Therapy System in XPLOR research configuration

Duration of Exposure	Phase I - 6 weeks (30 sessions) and 3 week taper (3 sessions for one week, then 2 sessions for the next week, then 1 session for the last week of the taper). Based on randomization participants will either receive sessions of 1 Hz rTMS at 120 % motor threshold (MT) with 2400 continuous pulses or 10 Hz rTMS at 120 % MT with 4 seconds on and 36 seconds off for 2400 pulses. Both 1 Hz and 10 Hz rTMS sessions will be delivered to the left dorsolateral prefrontal cortex (LDLPFC). Phase II- 2 weeks (10 sessions) of continuous theta burst (cTBS) or intermittent theta burst (iTBS) based on biomarker (ICF) status. Note that TBS sessions involve the delivery of 3 pulses of 50 Hz stimulation given every 200 milliseconds (ms) or 5 Hz. Participants assigned to cTBS will receive 10 daily sessions (5 sessions per week for two weeks) with 120 second trains of uninterrupted TBS for 1800 pulses at 80% MT. Participants assigned to iTBS will receive 10 daily sessions (5 sessions per week for two weeks) with 2 second trains every 10 seconds for a total of 570 seconds for 1800 pulses. Both cTBS and iTBS sessions will be delivered to the LDLPFC. NeuroStar TMS is not an implant. Stimulation is delivered during the daily sessions in the neurostimulation suite.
Reference therapy	Not Applicable
Statistical Methodology	For the primary aim, a linear mixed model analysis of repeated measures will be used to evaluate the repetitive transcranial magnetic stimulation (rTMS) by biomarker status interaction effect on depression severity (CDRS-R score) over 6 weeks of rTMS. For the secondary aim, a within-participant linear mixed model analysis of repeated measures will be used to examine the change in ICF over 6 weeks of rTMS. For the exploratory aims, a within-subjects linear mixed model analysis of repeated measures will be used to examine the change in cortical MRS measures of GABA, glutamate, and glutamine over 6 weeks of rTMS treatment in depressed adolescents. A multiple mediation/moderation path analytic model will be used to estimate the direct effect as well as the total and specific indirect effects of rTMS treatment on depression severity (at the 6-week endpoint) through each potential baseline moderator (anhedonia and adversity).

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the procedures described in this protocol, applicable United States government regulations and Mayo Clinic policies and procedures.

1.1 Background

Adolescent depression is a substantial global public health problem which contributes to academic failure, occupational impairment, deficits in social function, substance use disorders, teen pregnancy, and completed suicides.[1-3] Existing treatment approaches such as psychotherapy, pharmacotherapy, or combinations often have suboptimal results and uncertain safety profiles.[3-6] For example, ongoing controversies regarding the effectiveness and safety of selective serotonin reuptake inhibitors underscore the concern that current treatment modalities do not target relevant, pathophysiology in a neurodevelopmentally informed manner.[5] This is a lost opportunity as intervention early in life could prevent years of disability and suffering on an individual level and a substantial societal economic burden. Unfortunately, interventions for adolescent depressive disorders are often simply adapted from existing adult treatments and studied in heterogenous groups of adolescents[7]. Dimensional and experimental medicine approaches offer the opportunity to optimize existing treatment options and catalyze innovation for adolescent depression.[8,9] For example, anhedonia and historical adversity are key elements of the negative valence systems domain and markers of treatment resistance.[10-12] A refined understanding of anhedonia and adversity would provide the opportunity to develop targeted treatment approaches.

Imbalances in GABAergic and glutamatergic tone play a key role in depression,[13,14] pathophysiologic stress responses,[15] and emotional numbing or anhedonia found in behavioral manifestations of the negative valence system.[16] These GABAergic and glutamatergic imbalances have differential causes, effects, and behavioral manifestations in adolescents as compared to adults.[17-19] For example, recent preclinical work has demonstrated that repeated stress in adolescent rats inhibits GABAergic projections to the amygdala thereby impairing regulatory neurocircuitry.[20] In adult rats, chronic stress facilitates glutamatergic excitatory neurotransmission with ensuing effects on the lateral nucleus of the amygdala, hippocampus, and frontal cortex.[21-23] Developmental differences in frontolimibic GABAergic and glutamatergic tone may underlie variances in adolescent depressive symptom presentations and treatment responsivity.[20,24,25] A deeper understanding of frontolimbic GABAergic and glutamatergic tone in adolescent depression would assist with precision medicine approaches and intervention development. Certain types of transcranial magnetic stimulation (TMS) provide noninvasive measures of cortical GABAergic and glutamategeric tone.[26-28] There are different types of TMS. Repetitive TMS (rTMS) delivers multiple pulses per session over many days or weeks for therapeutic purposes. The effects of rTMS can also be studied as a probe of brain function. Theta burst stimulation (TBS) is a form of rTMS that is thought to have more rapid effects on synaptic plasticity and clinical symptoms than standard rTMS. Single and paired-pulse TMS paradigms are used to study the physiology of the brain. Neurophysiological measures collected with transcranial magnetic stimulation (TMS) such as intracortical facilitation (ICF), short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and the

cortical silent period (CSP) are noninvasive measures of cortical GABAergic and glutamatergic tone.[28-31]

Neurostimulation technologies such as rTMS have great potential as enduring, brain-based interventions for depression in adolescents.[32,33] Treatment with rTMS addresses pathologic imbalances in cortical GABAergic inhibitory and excitatory glutamatergic frontolimbic neurocircuitry.[34-36] At present, adults with broad presentations of depression are typically treated empirically with high frequency (10 Hz) rTMS applied to the LDLPFC as this dosing approach has been widely studied.[37,38] However, prior literature suggests that low frequency (1 Hz) rTMS applied to the right dorsolateral prefrontal cortex (RDLPFC) may be equally effective with superior tolerability.[39-42] Left-sided, 1 Hz rTMS applied to the LDLPFC may have similar antidepressant and neurophysiologic effects as RDLFPC 1 Hz rTMS. For example, early studies with positron emission tomography[43] and near infrared spectroscopy[44] suggest that prefrontal 1 Hz rTMS has a global, bilateral effect on brain activity. Questions of laterality in therapeutic 1 Hz rTMS have been inadequately studied and prior research often utilized right-sided 1 Hz based on dogma more than science.[45-47] Other work posits that patients with depression may have variable responses to these two dosing strategies (1 Hz vs. 10 Hz) necessitating a more sophisticated neurobiological understanding and precision medicine approaches.[34,48] Although rTMS has demonstrated promise for adolescent depression, the mechanisms of 1 Hz vs. 10 Hz rTMS dosing have not been adequately studied in adolescent depression, creating a substantial knowledge gap.[33] Research in this area is critical as the neurobiologic effects of 1 Hz and 10 Hz rTMS in adolescent depression likely diverge from adult depression. [49-51] The study of 1 Hz rTMS in adolescents is particularly critical as it would have advantages in terms of safety and tolerability for widespread dissemination. A basic, neurodevelopmentally informed understanding of standard rTMS dosing approaches (1 Hz and 10 Hz) is an ethical and scientific prerequisite for future large, pivotal studies of patterned dosing such as TBS for adolescent depression.[51,52]

Currently our group is leading a multicenter randomized, sham-controlled trial of rTMS for adolescent depression (A Randomized, Sham-Controlled Trial Evaluation of the Safety and Effectiveness of NeuroStar Transcranial Magnetic Stimulation (TMS) Therapy in Depressed Adolescents). Phase I of the study is a 6 week (30 treatment sessions), randomized, sham-controlled study of 10 Hz rTMS delivered to the left dorsolateral prefrontal cortex (LDLPC). The rTMS is targeted and dosed with the abductor pollicis brevis, "5-cm rule." Phase II is a 6 week open-label treatment course of 10 Hz rTMS for participants who did not respond to treatment in Phase I. Phase III is a follow-up phase for subjects that had clinical benefit in Phase I or II. The target enrollment for this study is 100 participants. Presently, 99 participants have enrolled and it is anticipated that enrollment will close in late 2017 or early 2018. Hence, the proposed dose finding and target validation study would not be expected to run concurrently with the existing industry trial. While the current industry supported effort will provide important systematic data and practical experience, the study design has inherent limitations. As a result, findings will not substantially advance the mechanistic understanding of rTMS in the context of neurodevelopment.[2,5] First, the industry supported study design replicates a longstanding flaw of interventional work in children and adolescents (spanning psychotherapy, pharmacology, and novel therapeutics) in which adult protocols and dosing schedules are simply applied to children or adolescents.[6-8] Second, the current industry supported clinical trial will not inform target engagement efforts nor advance understanding of the neurobiological underpinnings of

adolescent depression and responsivity to 10 Hz rTMS. Third, there is a paucity of data on low frequency (1 Hz) rTMS in adolescents and in general.[9-11] Systematic data demonstrating benefit of 1 Hz rTMS in adolescent depression or subpopulations would have utility as this dosing might be more tolerable, safe, and optimal for widespread delivery.

Hence for the present protocol we have designed an effectiveness and target validation trial of 1 Hz vs. 10 Hz rTMS. While the inclusion of a sham arm would be ideal, there are substantial pragmatic and financial barriers to completing such a study as a single site while concurrently addressing other critical National Institute of Mental Health (NIMH) priorities. Studies of 1 Hz rTMS and mechanistic studies of rTMS in adolescents would address a substantial knowledge gap and could provide further innovations. Unfortunately, there is a low probability that this will be studied systematically outside of a federally funded grant and even less likely that the neurobiologic mechanisms associated with 1 Hz and 10 Hz rTMS in adolescent depression will be adequately studied.

Developmentally informed, targeted interventions with rTMS for adolescent depression could reap important gains on an individual and population level as cortical, GABAergic and glutamatergic tone continues to change throughout development setting the stage for important future functions.[17,53] Prior rTMS research focused on depression often has a fatal flaw as heterogenous groups of patients have been studied with insufficient clinical characterization.[33,52] Anhedonia[54] and adversity[55,56] in depressed adolescents are important dimensional traits to quantify in terms of GABAergic tone, glutamatergic tone, and treatment responsivity to 1 Hz and 10 Hz rTMS. Examining a cohort of depressed adolescents and healthy controls with TMS neurophysiology would deepen our understanding of the role of GABA and glutamate in the negative valence systems domain.[56,57]

Prior work demonstrates that anhedonia is a marker of treatment resistance, poor outcomes, and suicidality. For example, recent multivariate analyses from the landmark Treatment of Resistant Depression in Adolescents (TORDIA) study identified a dimensional measure of anhedonia as the primary predictor of increased time to remission and decreased depression-free days in a large (n=334) cohort of adolescents with treatment resistant depression.[11] Notably patients with historical sexual or physical abuse also had decreased response rates in the TORDIA study.[10] In other recent work, anhedonia severity in depressed adolescents was associated with illness severity, suicidality, duration of illness, and number of depressive episodes.[12] Although incompletely understood, anhedonia and historical adversity appear to have associations with accentuated disruptions in GABAergic[58] and glutamatergic[59,60] tone in frontolimbic circuitry. Recent work demonstrated that adolescents with anhedonia have decreased GABA levels in the anterior cingulate cortex[16] while glutamatergic tone in the basal ganglia was associated with clinical measures of anhedonia.[61] Preclinical work also implicates imbalances of GABAergic and glutamatergic tone in treatment resistance.[23,62] These collective findings suggest that depressed adolescents with anhedonia or historical adversity are a population in great need of effective, brain-based interventions. Dimensional studies of anhedonia and adversity will assist in the development of targeted, neurobiologically informed treatments.[8,9]

MRS Biomarkers of GABAergic and Glutamatergic Tones in rTMS Studies

Prior work demonstrated that the glutamine to glutamate ratio (Gln/Glu) is a sensitive measure of cortical glutamate-glutamine cycle dysfunction in severe psychiatric disorders. In our prior MRS study, 6 weeks of 10 Hz rTMS modulated cortical glutamatergic tone in depressed adolescents. Baseline, posttreatment, and 6 month follow up MRS 3 T scans examined glutamate concentrations in 8 cm voxels from the left dorsolateral prefrontal and anterior cingulate cortices (Figure1). Spectroscopic data were collected with an optimized, intermediate echo time, point resolved spectroscopy (PRESS) sequence and 2-dimensional J-resolved averaged PRESS sequence. After 6 weeks of rTMS, mean Gln/Glu ratios increased from baseline and at 6 month follow-up in both voxels with both sequences. Mixed model repeated measures analysis revealed a significant negative relationship between depression severity as assessed with the CDRS-R and the Gln/Glu ratio in the dorsolateral prefrontal cortex ($\hat{b}$ = –0.00127; 95% CI, –0.00207 to –0.00048; raw P=.003; false discovery rate P=.01). Hence, throughout treatment and follow-up period, decreases in depressive symptom severity had an indirect relationship to increases in the Gln/Glu ratio. This initial work presents many unanswered questions. Resolving GABA, glutamate, and glutamine at 3 T in a single acquisition is challenging to impossible. Special techniques can be used to quantify each of the neurochemicals separately, but that results in three different acquisitions which dramatically increases acquisition time. Our current methodologies do not adequately resolve cortical GABA concentrations and may yield imprecise measures of glutamine concentrations as Cramer-Rao lower bounds (CRLB) criteria are suboptimal. Current work with 7 T MRS demonstrates promise in resolving and quantifying GABA, glutamate, and glutamine in a single acquisition. To our knowledge, 7 T MRS has not been used to study adolescent depression previously.[63,64]

Ultra-High Field MRS for Biomarker Development in Adolescent Depression

Recent work in adult humans with 7 T MRS and stimulated echo acquisition mode (STEAM) sequence has enhanced the detection and resolution of glutamate, glutamine and GABA (Figure2). In young adults (18 to 22 years of age), glutamate and glutamine could be quantified separately in the early visual cortex with very low CRLB of 2.0% ± 0 and 2.7 ± 0.3%, respectively which is not possible at 3 T. Additionally, GABA was quantified with CRLB of 13.5 ± 4.6%. The spectra were acquired in 5 minutes from 2 x 2 x 2 cm3 volume of interest placed in the early visual cortex with STEAM sequence (TR = 5 s, TE = 8 ms, number of averages = 64). Additionally, quantified glutamate, glutamine and GABA concentrations from spectra acquired at TE of 8 ms removes a possible confound of changing T2. Good quality data can be obtained in the frontal lobe using a prototype coil.[63,64]

1.2 Investigational Device

The NeuroStar™ XPLOR® TMS Therapy System will be used to deliver all of the proposed investigational rTMS sessions (1 Hz, 10 Hz, cTBS, and iTBS). The NeuroStar™ XPLOR® TMS Therapy System is a clinical research option for the NeuroStar™ TMS Therapy System that provides features necessary to conduct randomized trials and other TMS research. The NeuroStar™ TMS Therapy System is a computerized electromechanical instrument that produces and delivers brief duration, rapidly alternating (pulsed) magnetic fields to induce electrical currents in localized regions of the cerebral cortex for use in the treatment of MDD in adults who have failed to receive benefit from antidepressant medications. The XPLOR®

research configuration consists of three coils: a known-active coil identical to the NeuroStar™ TMS System treatment coil and two identical, "blinded" coils. One of the blinded coils provides active treatment identical to the known-active NeuroStar™ TMS system treatment coil; the sham coil provides acoustically indistinguishable (from active) pulses and a gentle percussive sensation that simulates scalp sensations produced during active treatment. Please note that the sham and blinded coils will not be used in this protocol.

The proposed study also involves the investigational use of the Magstim Model 200 magnetic nerve stimulator to collect cortical neurophysiological biomarkers with single and paired-pulse TMS. Please reference previous I111173-Study Determination. Previous US FDA risk determinations have communicated that single and paired-pulse TMS biomarker studies do not fall into the scope of Investigational Device Exemption (IDE) regulation and do not require an IDE application. Data collected with the Magstim Model 200 magnetic nerve stimulator is for basic physiological research of major depressive disorder. We do not intend to collect data on the safety and effectiveness of the Magstim Model 200 magnetic nerve stimulator.
The Magstim Model 200 will be used to collect the proposed TMS biomarkers: motor threshold (MT), short interval intracortical inhibition (SICI), intracortical facilitation (ICF), long interval intracortical inhibition (LICI), and cortical silent period (CSP). The Magstim Model 200 is a magnetic nerve stimulator FDA approved for the stimulation of peripheral nerves for diagnostic purposes. As with other magnetic stimulators it is made up of two distinct components:

a. A high current pulse generator producing currents of approximately 5,000 amps.
b. A stimulating coil producing magnetic pulses with field strengths of approximately 1 tesla and a pulse duration of 1 ms.

Magnetic stimulation is achieved by discharging the Magstim Model 200 coil near neuromuscular tissues. Current flows from the instrument through the coil in one of two directions (depending on which side of the coil is facing the scalp). If side A is visible and side B is facing the site of stimulation, coil current flows in a counterclockwise direction and within the tissues current flows in a clockwise direction. If side B is visible and side A is facing the site of stimulation, coil current flows in a clockwise direction and within the tissues current flows in the counterclockwise direction. In the coil, current flows from positive to negative. Within tissues, current flow signifies the movement of ions.

The Mayo Clinic Neurostimulation Laboratory is equipped with two single-pulse magnetic stimulators (Magstim 200) and a bi-stim module for the delivery of paired magnetic stimuli at interstimulus intervals as short as 1 ms. A bank of 4 custom-modified electrically isolated bioelectric amplifiers is used to record the motor evoked potential (MEP) for motor threshold determination. These bio-amplifiers are battery powered for AC isolation and customized to reduce artifact induced by magnetic pulses. Features include low distortion and noise. They have especially rapid rate recovery after saturating signals. The amplified signals are digitized with Dell Dimension P200V (200 MHz) personal computers using analogic LS-DAS-12 A/Ds (250 kHz). Computing facilities in the lab include a personal computer for the acquisition of electrophysiological data.

1.3 Preclinical Data

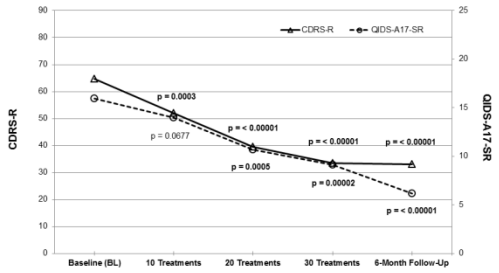
Please see prior IDE applications G060269, G110091, G120121, present IDE application, prior Study Determination (I111173), and all associated references.

1.4 Clinical Data to Date

Please see prior IDE applications G060269, G110091, G120121, present application, prior Study Determination (I111173), and all associated references.

Preliminary Studies: Our prior research with TMS measures of ICF[53,65] and investigation studies of 10 Hz rTMS[66-70] form the basis of this application. Collectively, this body of work suggests that ICF has utility as a biomarker in depressed adolescents[53,65], rTMS may modulate cortical measures of glutamatergic tone,[70] and that investigational, therapeutic rTMS protocols have feasibility for adolescents with moderate to severe depressive symptoms.[64,69] Our prior work informed a multicenter, industry sponsored clinical trials of rTMS. To our knowledge, clinical trial efforts have only examined standard adult dosing of 10 Hz rTMS in adolescents with no consideration or examination of developmental differences, mechanistic effects, and biomarkers. To our knowledge, no prior studies of adolescent depression have examined rTMS dose comparisons (1 Hz vs. 10 Hz) or explanatory biomarkers in the context of variable dosing.[33,52] Developmentally informed studies of 1 Hz and 10 Hz rTMS for adolescent depression are essential as adolescents may have differential treatment and neurophysiological responses. An enhanced understanding of the neurophysiology of rTMS in adolescents would inform future work with intermittent theta burst stimulation and could catalyze the development of new experimental dosing strategies for rTMS in adolescent depression.

Figure 1. Feasibility of 10 Hz rTMS for Adolescent Depression.



Our lab has now treated more than 30 depressed adolescents in the course of three distinct studies of 10 Hz rTMS, at 120% motor threshold, applied to the left dorsolateral prefrontal cortex for 30 sessions over 6 weeks.[66,69] Initial results are encouraging in terms of feasibility and patient retention. Primary clinical outcome measures with the clinician rated CDRS-R and secondary self-report measures with the 17 item Quick Inventory of Depressive Symptomatology Adolescent Version Self-Report (QIDS-A17-SR) demonstrated significant improvements throughout the 6 week treatments, posttreatment, and at a 6 month follow up visit (Figure 1). The durability of clinical effect at 6 months is noteworthy and seldom seen in interventional studies of adolescent depression. However, these initial efforts have simply examined adult dosing schedules of 10 Hz rTMS and are not informed by target engagement strategies.

1.5 Study Rationale and Risk Analysis (Risks to Benefits Ratio)

1.5.1 Study Rationale

Repetitive transcranial magnetic stimulation (rTMS) is a promising brain-based treatment for adolescent depression, but target engagement dosing strategies are not adequately understood.[33] A leading theory suggests that rTMS addresses pathological imbalances in cortical gamma-aminobutyric acid (GABA) and glutamatergic tone. There is a substantial knowledge gap regarding precision medicine approaches for dosing rTMS and the mechanisms associated with 1 Hz and 10 Hz rTMS in adolescent depression. Disruptions in excitatory and inhibitory neurotransmission are also implicated in negative valence and depression. Anhedonia and early adversity are associated with poor outcomes in adolescent psychiatric disorders and may be linked to accentuated imbalances of cortical glutamatergic and GABAergic tone[10,12,15,16,71]. Clarifying the mechanisms of rTMS for adolescent depression could catalyze intervention discovery for treatment resistant populations.[47,52]

To solve these problems we propose a novel dose finding and target validation study of rTMS for adolescent depression with a biomarker-stratified, randomized design. [72] Intracortical facilitation (ICF), [63] a transcranial magnetic stimulation (TMS) measure of cortical glutamatergic tone is the target engagement biomarker for the proposed trial. A cohort of adolescents (ages 12-18) with clinically significant depression defined as 40 or greater on the CDRS-R will be stratified according to baseline ICF (high defined as > 1.5 or low defined ≤ 1.5)[26,28,65] and then randomized to 6 weeks of either left prefrontal 1 Hz or 10 Hz rTMS. The research team will collect weekly ICF and CDRS-R measures during treatment. We will assess baseline anhedonia with a composite measure from relevant CDRS-R and Beck Depression Inventory-II (BDI-II) items. We will assess lifetime adversity with the Childhood Trauma Questionnaire (CTQ). Depressed adolescent participants who do not respond to 1 Hz or 10 Hz rTMS will be offered the opportunity to participant in an exploratory, 2 week trial of iTBS or cTBS (novel dosing strategies of TMS therapy). An ideal study would include a sham arm. As noted there are substantial financial and pragmatic barriers to this in the proposed study. Further, the proposed study is focused on developing a mechanistic understanding of different doses of rTMS and the effectiveness of 1 Hz rTMS compared to 10 Hz as this has not been adequately addressed in previous work.

Aim 1 examines the relative efficacy of 6 weeks low frequency (1 Hz) or high frequency (10 Hz) left prefrontal rTMS according to a baseline ICF (ICF-15 % MEP amp) measure of glutamatergic tone in depressed adolescents. We will employ a biomarker stratified design. Depressed adolescents will be stratified by baseline ICF low (≤ 1.5) or ICF high (>1.5) groups prior to randomization.

- We expect that 6 weeks of 1 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) in depressed adolescents with high baseline ICF compared to adolescents with low baseline ICF.
- We expect that 6 weeks of 10 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) in depressed adolescents with low baseline ICF compared to adolescents with high baseline ICF.

- 6 weeks of 1 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) than 6 weeks of 10 Hz rTMS in depressed adolescents with high baseline ICF.
- 6 weeks of 10 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) than 6 weeks of 1 Hz rTMS in adolescents with low baseline ICF.

Aim 2 examines changes in weekly ICF and CDRS-R across 6 weeks of either 1 Hz or 10 Hz rTMS.

- We anticipate that ICF measures will decrease in depressed adolescents receiving 6 weeks of 1 Hz rTMS.
- We anticipate that ICF measures will increase in depressed adolescents receiving 6 weeks of 10 Hz rTMS.
- Depressive symptom improvement will have a direct relationship with ICF decrease in depressed adolescents receiving 6 weeks of 1 Hz rTMS and an indirect relationship with ICF increase in depressed adolescents receiving 10 Hz rTMS.

Exploratory Aim 3

Aim 3a: To compare 7 T MRS cortical measures of GABAergic tone and glutamatergic neurochemistry in depressed adolescents and healthy control adolescents.

Aim3b: To examine potential changes in cortical GABAergic and glutamatergic neurochemistry after a six week course of either 1 Hz or 10 Hz rTMS in depressed adolescents.

Hypothesis:

1. Depressed adolescents will have decreased cortical levels of GABA as assessed with 7 T MRS compared to healthy control adolescents.

2. Depressed adolescents will have increased cortical levels of Glutamate as assessed with 7 T MRS compared to healthy control adolescents.

3. Bilateral cortical 7 T MRS measures of GABA will increase after 6 weeks of 1 Hz rTMS in depressed adolescents.

4. Bilateral cortical 7 T MRS measures of Gln/Glu will decrease after 6 weeks of 1 Hz rTMS in depressed adolescents.

5. Bilateral cortical 7 T MRS measures of GABA will decrease after 6 weeks of 10 Hz rTMS in depressed adolescents.

6. Bilateral cortical 7 T MRS measures of Gln/Glu will increase after 6 weeks of 10 Hz rTMS in depressed adolescents.

Exploratory Aim 4 examines clinical moderators of treatment (anhedonia and adversity) in this sample of adolescents undergoing treatment with 1 Hz and 10 Hz rTMS.

Hypothesis:

6 weeks of low frequency (1 Hz) rTMS vs. high frequency (10 Hz) rTMS will have greater efficacy (as assessed by CDRS-R) in depressed adolescents with anhedonia and adversity compared to depressed adolescents without anhedonia and adversity

The proposed project will examine an rTMS dosing, target engagement strategy for adolescent depression and refine understanding of GABAergic and glutamatergic contributions to negative valence. Findings will inform the clinical dissemination of rTMS for adolescent depression and provide insights to further treatment innovation for disorders of the negative valence systems domain early in life.[9,52]

To our knowledge the NeuroStar® TMS Therapy System in XPLOR research configuration has been used most often in prior investigational studies of adolescent depression.[33,69] Our group has existing and ongoing collaborations with Neuronetics as documented in referenced IDE applications: G060269, G110091, G120121. The present, proposed IDE application would deliver investigational, therapeutic TMS therapy (1 Hz, 10 Hz, cTBS, and iTBS) with the NeuroStar® TMS Therapy System in XPLOR research configuration.

Although the interventional component of the study (randomized biomarker stratified trial of 1 Hz or 10 Hz rTMS and extension trial of cTBS or iTBS) is considered more than minimal risk to adolescents, TMS treatments offer a potential direct benefit for the individual participants.[33,47] Notably all depressed, adolescent participants will received an active form of TMS. There is no placebo or sham condition. In addition, the schedule of mood assessments and daily assessments during the interventional portion of the study for any adverse events has been developed for the purpose of monitoring the participants' well-being.

All families and participants for this study will be provided with a consent/assent form describing this study and providing sufficient information for Participants to make an informed decision about their participation in this study. This consent/assent form will be submitted with the protocol for review and approval by the Mayo Clinic IRB. The formal consent/assent of a participant, using the approved IRB consent/assent form, must be obtained before that participant undergoes any study procedure. This form must be signed and dated by the participant the participant's legally-authorized representative, and the individual obtaining informed consent/assent.

1.5.2 Anticipated Risks and Protections

TMS and TBS Safety Concerns in Humans

This study will utilize different types of TMS. Single and paired-pulse TMS is used to collect neurophysiologic data. Therapeutic rTMS (1 Hz, 10 Hz, cTBS, and iTBS) applies multiple magnetic pulses to the brain over many (30-40 in this protocol) sessions for treatment purposes.[32,73]

Potential risk of seizure: There is a small risk of seizure associated with the use of TMS. This is regarded as the most serious adverse event ever reported in TMS studies to date. Seizures have

been reported to result from single-pulse TMS but have occurred most frequently with repetitive rTMS.[74,75] The risk for seizure with iTBS may be higher than standard rTMS (1 Hz or 10 Hz).[74] Participants who have a past history of neurological disorders such as strokes or epilepsy have the greatest risk. This incidence is still low (0.6% or less) which is comparable to the reported incidence of seizures with antidepressant medications..[74,75] The role of TMS in some case reports of seizures is equivocal[76,77] but the possibility of TMS inducing seizures in participants without prior neurological pathology cannot be entirely discounted. There have been 17 case reports of seizures during TMS in healthy participants, depressed participants, or participants with other disorders not thought to confer a seizure risk (such as pain).[72] It is also noteworthy, that in a recent, multicenter trial of rTMS involving 301 adult patients receiving rTMS at 120% motor threshold, 3,000 pulses per session, five days a week for 4-6 weeks (this is rTMS delivered at the upper limits of recommended safety guidelines) there were no seizures nor elevated rates of serious adverse events.[38] Reported seizures associated with rTMS have also been self-limited and beyond careful monitoring and neurological assessment have not required acute treatment. It is also important to note that there is no evidence that a single seizure, or even a series of induced seizures, makes a subsequent seizure more likely in an otherwise healthy individual. However, it is also true that there are potential psychosocial consequences of a seizure, including the potential psychosocial impact. There are 3 case reports of seizures in adolescents with depression undergoing rTMS treatments. In two of these instances the adolescents were taking psychotropic medications.[78,79] In one instance the adolescent used alcohol the night before rTMS treatment.[79] One case report described a seizure in an adolescent undergoing deep TMS.[80] In our experience thus far and to our knowledge there have been no seizures in depressed adolescents receiving NeuroStar rTMS treatments.

With regard to the potential for seizures, all patients will be carefully screened for current or historical factors that may increase the risk of seizures (see Exclusion criteria). The Transcranial Magnetic Stimulation Adult Safety Screen (TASS) will be used to specifically query for any potential seizure risks. Neurologic disease which may increase the risk for seizures is part of the exclusion criteria.[74,75] Patients undergoing TMS in the proposed study will not be taking medications which could potentially lower their seizure threshold. All TMS sessions will be supervised by a board-certified child and adolescent psychiatrist and all personnel involved will be provided with training in the identification of potential prodromal signs or symptoms of a seizure, particularly the identification of more subtle behavioral events which may herald the occurrence of a frontal lobe seizure, such as changes in level of consciousness or inattention. All TMS sessions will be conducted in the neurostimulation suite with access to medical facilities and equipment in the event a seizure should occur. Seizure monitoring will be by visual observation. Investigators have the capability to manage a seizure if it were to occur. The Mayo Clinic Neurostimulation Lab has direct access (within minutes) to emergency medicine services.

In the event of a seizure, the supervising physician will initiate appropriate clinical care as defined within the clinical rTMS practice. In the management of seizures, attention must be taken to minimize the risk of aspiration, and when possible guiding the patient into the left lateral decubitus position is desirable. Because seizures in the context of TMS are brief (typically <60 seconds) and without serious physical sequelae, efforts will be focused on preventing complications of the seizure rather than initiating any specific medication that is not required unless a seizure is prolonged. If a prolonged seizure (>60 seconds) is identified, appropriate

response measures will be initiated which may include notifying a hospital-based pediatric rapid response team and/or escorting participant to the emergency medicine department upon stabilization (located directly across from TMS building). The patient may be provided lorazepam (or equivalent benzodiazepine available in the resuscitation cart) to abort the seizure. To date, status epilepticus has never been described following rTMS.[47,74,75]

If a seizure occurs during the study, TMS treatment as part of the study will be discontinued. Ongoing monitoring of mood and neurocognitive symptoms per the study protocol will be offered. Any seizure will be reported to the Mayo Clinic IRB, Data and Safety Monitoring Board (DSMB), Neuronetics, and the FDA as required per agreements, regulations, and policies.

Neuropsychological function after TMS: Several studies have examined the short-term effects of rTMS administration across a range of stimulation parameters, and found little or no evidence to suggest a change in cognitive function as a result of rTMS administration.[66,74,75] Although there is no evidence to suggest an untoward effect on cognitive function as a result of rTMS administration the study team will collect neurocognitive data at baseline, posttreatment (Phase I and Phase II), and at a 6 month follow-up visit. A Mayo Clinic Consultant, Pediatric Neuropsychologist, [redacted] [redacted] PhD will oversee the implementation, training, administration, quality assurance, and data analyses of neurocognitive testing (NIH Toolbox)

Potential risk of alteration of auditory threshold: During TMS, the coil produces an audible, high energy click. Existing evidence suggests that TMS can result in transient changes in auditory threshold if no precautions are taken (such as wearing earplugs during TMS). Ear plugs are required for all participants and study personnel during any TMS procedure. These are worn routinely during TMS protocols to mitigate this risk.[38,74] Participants will undergo audiometry testing at baseline and upon completion of 6 week rTMS (1 Hz or 10 Hz) and once again (if applicable) after the two week trial (of either cTBS or iTBS).

Potential for unintended behavioral change: (e.g., worsening of depression or induction of mania): Crying has been reported in some patients receiving left prefrontal rTMS. There is no evidence to suggest that a frank worsening of depression may be seen in patients with major depression receiving rTMS. On the other hand, mania has been observed in bipolar disorder patients who have received rTMS.[38,74,75] Dr. Croarkin and the study team will be available 24 hours a day to address any concerns participants or families have regarding changes in behavior. Adverse events are monitored at every treatment visit. Dr. Croarkin or a board-certified child and adolescent psychiatrist will remain in the neurostimulation suite (in a room adjacent to the rTMS treatment room) during all rTMS treatment sessions.

Suspected treatment-induced mania will be evaluated using the Young Mania Rating Scale (YMRS). A YMRS score of ≥ 20 will prompt administration of the M.I.N.I.(age 18) or M.I.N.I. KID to determine whether or not the Participant meets the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for mania. A YMRS score of 20 or greater will be considered an adverse event (AE) and will be monitored using the YMRS; the emergence of DSM-5 verified mania will be treated as a serious adverse event (SAE) and will also be monitored using the YMRS.

Please note that Dr. Croarkin works with a team of Board Certified Child and Adolescent Psychiatrists with expertise in adolescent depression, suicidality, severe psychiatric disorders in childhood, rTMS protocols, and clinical research. These psychiatrists are co-investigators and colleagues. One of these physicians (in the event Dr. Croarkin is away) or Dr. Croarkin is present in the treatment suite during rTMS delivery and available 24 hours a day in the event of emergencies. While our group has never had a participant with emergent mania in an rTMS protocol, we have experience and comfort in addressing psychiatric emergencies in general. We have standard operating procedures that the team of psychiatrists are comfortable with and have experience implementing. For example, this team collectively has had experience in hospitalizing research participants. The overriding guiding principal for our research team is that participant safety is our central priority. Any decisions regarding clinical monitoring, referral for higher levels of treatment, and ongoing study participation are informed by what will optimize each participant's safety and clinical status. Beyond this, the Mayo Clinic Division of Child and Adolescent Psychiatry (11 board certified child and adolescent psychiatrists) always has an individual on call for psychiatric emergencies. While these colleagues are not co-investigators, they receive training on a regular basis regarding ongoing adolescent research efforts. All members of the Child and Adolescent Psychiatry Division have proficiency and comfort in dealing with psychiatric emergencies. They contact the PI (Dr. Croarkin) or the covering research child and adolescent psychiatrist with any emergency involving a research participant. Research participants are ultimately offered standard of care interventions based on clinical presentation and need regardless of stage of participation (to include emergent evaluation in outpatient clinic, emergency medicine visits, or psychiatric hospitalization in Mayo Clinic, Generose 1-West, an 18 bed inpatient unit which is located directly below our neurostimulation treatment suite).

Potential risk of movement of metallic objects in the patient's body: Because of the brief, but intense local magnetic field induced by the TMS coil, any metallic object in the vicinity of the coil, namely within the head region, may slightly move as a result. Patients with metallic objects in or near their head are excluded from the study. Patients will be specifically instructed to remove any magnetically sensitive jewelry or other objects near their head. Patients with metallic objects in or near their head are excluded from the study. Patients will be specifically instructed to remove any magnetically sensitive jewelry or other objects near their head.[38,74,75]

Potential for development of headache or local pain at the site of stimulation: TMS administration may result in the development of a transient headache during or after the TMS session, and the development of pain localized near the site of the stimulation coil. These effects are thought to be due to the direct activation of muscles and nerves near the stimulating coil. This is more common with rTMS and TBS than with single or paired-pulse TMS. TMS sessions or measurements will be discontinued immediately in the event of any significant scalp discomfort or pain. Participants will be counseled on the use of ibuprofen to relieve pain or discomfort as necessary.[38,74,75]

Unimproved depression or Clinical Deterioration: Continued depression or worsening of depressive symptomatology is always a risk of depression, even for those treated with medication and/or psychotherapy. Clinical deterioration due to the underlying psychiatric conditions is also a risk. The PI (Dr. Croarkin) is a board-certified child and adolescent

psychiatrist, and the research team at Mayo Clinic has prior research experience with child and adolescent depressed populations. The research team includes 3 other board certified child and adolescent psychiatrists with experience delivering investigational rTMS to adolescents, extensive experience with suicidal adolescents, and clinical research experience. Should worsening of depression occur, Dr. Croarkin (or a child and adolescent psychiatrist research team member) will follow standard policies and procedures developed by the Mayo Clinic Child and Adolescent Psychiatry Division in the Department of Psychiatry and Psychology. The study psychiatrist will implement strategies to ensure each partcipant's safety and optimal clinical outcome (e.g., comprehensive assessment of depressive symptomatology including possible lethality, immediate notification to the adolescent participant's legal guardian, and referral to community treatment providers). Implementation of strategies to mitigate worsening of depression will be tailored to the specific needs of the individual.

During the consent process, participants and families will be educated about the possibility of significant changes in mood, suicidal thinking, and/or behaviors during the treatment. These changes could include emergence of mania, worsening of depression, and/or suicidal thinking and behaviors. Both the participants and parent(s) will be told to initiate contact with their study doctor, if the participant experiences any significant mood or behavior changes including suicidal ideation. Principal Investigator contact information is included in the consent/assent document. Furthermore, during the treatment phases of the study, participants will have frequent contact with the study team (5 visits per week) and assessments weekly where changes of psychological symptoms will be queried. Ongoing monitoring for worsening of depression and emergence of suicidal ideation and/or behaviors will be evaluated using the CDRS-R, C-SSRS, and clinical global impression-severity scale (CGI-S).

Suicidal behavior: Patients with current suicidal ideation or past suicide attempts are eligible for the study (unless outpatient treatment is not clinically indicated), as ongoing suicidal ideation and behaviors are frequently seen in depressed youth. Therefore, ongoing suicidal ideation or worsening of suicidal ideation and behavior is a risk. The PI (Dr. Croarkin) is a board-certified child and adolescent psychiatrist with extensive experience treating adolescents with suicidal behavior. The research team includes three other board certified child and adolescent psychiatrists. Dr. Croarkin or the research child and adolescent psychiatrist will follow standard policies and procedures developed by the Mayo Clinic Child and Adolescent Division and implement strategies (e.g., comprehensive assessment of depressive symptomatology, assessment of suicidal ideation [inclusive of intent, means, and plan], immediate notification of the adolescent participants' legal guardian, referral for admission to Generose, 1-West, the Mayo Clinic Child and Adolescent Inpatient Psychiatry unit [in cases of immediate lethality]). Implementation of strategies to mitigate suicidal behavior will be tailored to the specific presenting needs of the individual.

Please note that Dr. Croarkin works with a team of Board Certified Child and Adolescent Psychiatrists with expertise in adolescent depression, suicidality, severe psychiatric disorders in childhood, rTMS protocols, and clinical research. These psychiatrists are co-investigators and colleagues. One of these physicians (in the event Dr. Croarkin is away) or Dr. Croarkin is present in the treatment suite during rTMS delivery and available 24 hours a day in the event of emergencies. We have standard operating procedures that the team of psychiatrists are

comfortable with and have experience implementing. For example, this team collectively has had experience in hospitalizing suicidal research participants. The overriding guiding principal for our research team is that participant safety is our central priority. Any decisions regarding clinical monitoring, referral for higher levels of treatment, and ongoing study participation are informed by what will optimize each participant's safety and clinical status. Beyond this, the Mayo Clinic Division of Child and Adolescent Psychiatry (11 board certified child and adolescent psychiatrists) always has an individual on call for psychiatric emergencies. While these colleagues are not co-investigators, they receive training on a regular basis regarding ongoing adolescent research efforts. All members of the Child and Adolescent Psychiatry Division have proficiency and comfort in dealing with psychiatric emergencies. They contact the PI (Dr. Croarkin) or the covering research child and adolescent psychiatrist with any emergency involving a research participant. Research participants are ultimately offered standard of care interventions based on clinical presentation and need regardless of stage of participation (to include emergent evaluation in outpatient clinic, emergency medicine visits, or psychiatric hospitalization in Mayo Clinic, Generose 1-West, an 18 bed inpatient unit which is located directly below our neurostimulation treatment suite).

MRI/MRS Safety Concerns in Humans

Potential Risks

The effects of magnetic fields in an MRI scanner have been extensively studied, and there are no known significant risks with an MRI exam. Some subjects may be bothered by feelings of confinement (claustrophobia), and by the noise made by the magnet during the procedure. Subjects will wear earplugs while in the magnet. Subjects with pacemakers, implanted defibrillators or other implanted electronic or metallic devices may not participate. Subjects with braces may not participate in the MRI/MRS scan portion of the study.

Clinical Assessments and Neurocognitive Testing with NIH Toolbox

The psychiatric assessment, interview, and rating scales in this study are time consuming and may bring up upsetting issues. If child abuse is detected, the research team is obligated to follow mandatory, federal, state, and institutional reporting laws. On occasion participants clinically deteriorate during a research assessment. Dr. Croarkin or a board-certified child and adolescent psychiatrist from the research team will be available 24 hours a day for these situations. A Mayo Clinic Consultant, Pediatric Neuropsychologist, [redacted], PhD will oversee the implementation, training, administration, quality assurance, and data analyses of neurocognitive testing (NIH Toolbox)

Confidentiality

The potential for a breach in confidentiality always exists, specifically with the written research data and study databases. However, information that is obtained will be stored in locked file drawers in locked offices; data will have identifying information sheered from it to prevent loss of confidentiality, all computers and databases will be password protected with passwords available to limited study personnel, and all staff must sign confidentiality certificates.

Loss of confidentiality is a potential risk because participants will be asked to disclose their recent medications history, information pertaining to demographics, family history, and psychiatric history. Since code numbers will be given to each participant and data will be stored by code number, the risk of loss of confidentiality will be minimal. Only personnel working on this project at Mayo Clinic and the University of Minnesota Center for Magnetic Resonance Research will have access to the data. Only data that are pertinent to the study will be collected so that seriousness of loss of confidentiality will be minimized. The alternative to participating in this project is to decline. Participants will be at no risk if they decline to participate.

1.5.3 Potential Benefits

Although the interventional component of the study (randomized biomarker stratified trial of 1 Hz or 10 Hz rTMS and extension trial of cTBS or iTBS for nonresponders) is considered more than minimal risk to adolescents, TMS treatments offer a potential direct benefit for the individual participant.[33] [81,82] Notably all depressed, adolescent participants will received an active form of TMS. There is no placebo or sham condition. In addition, the schedule of mood assessments and daily assessments during the interventional portion of the study for any adverse events has been developed to optimize monitoring the participant's well-being.

Standard TMS dosing (1 Hz and 10 Hz) has been applied more broadly than TBS to children and adolescents in the course of investigational, therapeutic studies.[83] The intent for the proposed intermittent theta-burst stimulation (iTBS) and continuous theta-burst (cTBS) exploratory extension trial (Phase II of the proposed study) is to maximize the infrastructure of the proposal for both research participants and our field. Theta burst stimulation (TBS) is an alternative dosing approach in which 3-pulse 50 Hz bursts are delivered at 5 Hz. With iTBS, 2 second trains are delivered to the LDLPFC every 10 seconds for 1800 pulses putatively inducing plasticity changes that resemble long-term potentiation. Continuous theta-burst stimulation (cTBS) delivers 2 second trains of 5 Hz stimulation continuously for 1800 pulses In general it has been speculated that iTBS sessions increase cortical excitability and that cTBS sessions decrease cortical excitability.[83-85]

In the course of a NIMH career development award focused on neurostimulation the PI/sponsor has taken every opportunity to interact with national and international clinical communities that focus on the delivery of TMS therapies. This has included volunteering for the Clinical TMS Society Board of Directors, attending International Brain Stimulation conferences, and visiting multiple national and international TMS clinics and laboratories. From these experiences it is clear that TBS protocols are increasingly delivered in clinical settings to adults and adolescents despite a relatively underdeveloped understanding of the in vivo neurophysiological effects in humans. This is particularly concerning in the context of developing children. Systematic study and data collection (efficacy measures, safety, adverse events, and neurophysiological data) focused on TBS is critical in this context. Based on prior experience of the PI, research participants (and their parents) who do not receive benefit from the proposed, primary, experimental therapeutic protocol (Phase I: 6 weeks of either 1 Hz or 10 Hz rTMS) would appreciate the availability of an additional treatment protocol with an experienced center in which safety is maximized. Practically, an extension trial of TBS would also present an

opportunity to maximize resources and collect novel pilot data for future grant submissions (Exploratory Clinical Trials of Novel Interventions for Mental Disorders R61/R33).[8]

1.6 Anticipated Duration of the Clinical Investigation

It is anticipated that the proposed study will be five years in duration. For individual participants, Phase I (biomarker stratified, randomized trial of 1 Hz vs. 10 Hz rTMS) is 10 weeks in duration. Phase II (trial of cTBS vs iTBS based on ICF biomarker status) is 3 weeks in duration.

2 Study Objectives

The proposed study is a dose finding, effectiveness, and target validation study of rTMS for adolescent depression with a biomarker stratified design. [72] Intracortical facilitation (ICF), [65] a transcranial magnetic stimulation (TMS) measure of cortical glutamatergic tone is the target engagement biomarker for the proposed trial. In phase I, a cohort of adolescents (ages 12-18) with clinically significant depression defined as 40 or greater on the Children's Depression Rating Scale-Revised (CDRS-R) will be stratified according to baseline ICF (high defined as > 1.5 or low defined ≤ 1.5) and then randomized to 6 weeks of either left prefrontal 1 Hz or 10 Hz rTMS. The research team will collect weekly ICF and CDRS-R measures during treatment. We will assess baseline anhedonia with a composite measure from relevant CDRS-R and Beck Depression Inventory-II (BDI-II) items. We will assess lifetime adversity with the Childhood Trauma Questionnaire (CTQ).

Participants in Phase I will be offered the opportunity to have 3 and 7 T MRI and MRS looking for biomarkers at baseline and after 6 weeks of treatment. A subgroup of depressed adolescent participants (up to 50) who do not respond to 1 Hz or 10 Hz rTMS will be offered the opportunity to participant in an exploratory, 2 week, trial of iTBS or cTBS based on ICF biomarker status (Phase II of the study). Participants with high baseline ICF (defined as > 1.5) will be assigned to cTBS treatment. Participants with low ICF (defined ≤ 1.5) will be assigned to iTBS.

7 T MRS measures Glutamate and GABA biomarkers in healthy adolescents and depressed adolescents. The overall goals are to examine cortical Glutamate and GABA neurochemical markers in adolescent depression and the impact of 1 Hz and 10 Hz rTMS on cortical glutamate and GABA neurochemical markers when compared to the healthy adolescent cohort.

2.1 Primary Objective

Aim 1 examines the relative efficacy of 6 weeks low frequency (1 Hz) or high frequency (10 Hz) left prefrontal rTMS according to a baseline ICF **(ICF-15 % MEP amp)** measure of glutamatergic tone in depressed adolescents. We will employ a biomarker stratified design. Depressed adolescents will be stratified by baseline ICF low (≤ 1.5) or ICF high (>1.5) groups prior to randomization.

- We expect that 6 weeks of 1 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) in depressed adolescents with high baseline ICF compared to adolescents with low baseline ICF.
- We expect that 6 weeks of 10 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) in depressed adolescents with low baseline ICF compared to adolescents with high baseline ICF.
- 6 weeks of 1 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) than 6 weeks of 10 Hz rTMS in depressed adolescents with high baseline ICF.
- 6 weeks of 10 Hz rTMS will have greater efficacy (assessed by improvement in CDRS-R) than 6 weeks of 1 Hz rTMS in adolescents with low baseline ICF.

2.2 Secondary Objective

Aim 2 examines changes in weekly ICF and CDRS-R across 6 weeks of either 1 Hz or 10 Hz rTMS.

- We anticipate that ICF measures will decrease in depressed adolescents receiving 6 weeks of 1 Hz rTMS.
- We anticipate that ICF measures will increase in depressed adolescents receiving 6 weeks of 10 Hz rTMS.

Depressive symptom improvement will have a direct relationship with ICF decrease in depressed adolescents receiving 6 weeks of 1 Hz rTMS and an indirect relationship with ICF increase in depressed adolescents receiving 10 Hz rTMS.

Exploratory Aim 3

Aim 3a: To compare 7 T MRS cortical measures of GABAergic tone and glutamatergic neurochemistry in depressed adolescents and healthy control adolescents.

Aim3b: To examine potential changes in cortical GABAergic and glutamatergic neurochemistry after a six week course of either 1 Hz or 10 Hz rTMS in depressed adolescents.

Hypothesis:

1. Depressed adolescents will have decreased cortical levels of GABA as assessed with 7 T MRS compared to healthy control adolescents.

2. Depressed adolescents will have increased cortical levels of Glutamate as assessed with 7 T MRS compared to healthy control adolescents.

3. Bilateral cortical 7 T MRS measures of GABA will increase after 6 weeks of 1 Hz rTMS in depressed adolescents.

4. Bilateral cortical 7 T MRS measures of Gln/Glu will decrease after 6 weeks of 1 Hz rTMS in depressed adolescents.

5. Bilateral cortical 7 T MRS measures of GABA will decrease after 6 weeks of 10 Hz rTMS in depressed adolescents.

6. Bilateral cortical 7 T MRS measures of Gln/Glu will increase after 6 weeks of 10 Hz rTMS in depressed adolescents.

Exploratory Aim 4 examines clinical moderators of treatment (anhedonia and adversity) in this sample of adolescents undergoing treatment with 1 Hz and 10 Hz rTMS.

Hypothesis:
6 weeks of low frequency (1 Hz) rTMS vs. high frequency (10 Hz) rTMS will have greater efficacy (as assessed by CDRS-R) in depressed adolescents with anhedonia and adversity compared to depressed adolescents without anhedonia and adversity

The proposed project will examine an rTMS dosing, target engagement strategy for adolescent depression and refine understanding of GABAergic and glutamatergic contributions to negative valence. Findings will inform the clinical dissemination of rTMS for adolescent depression and provide insights to further treatment innovation for disorders of the negative valence systems domain early in life.[9,52]

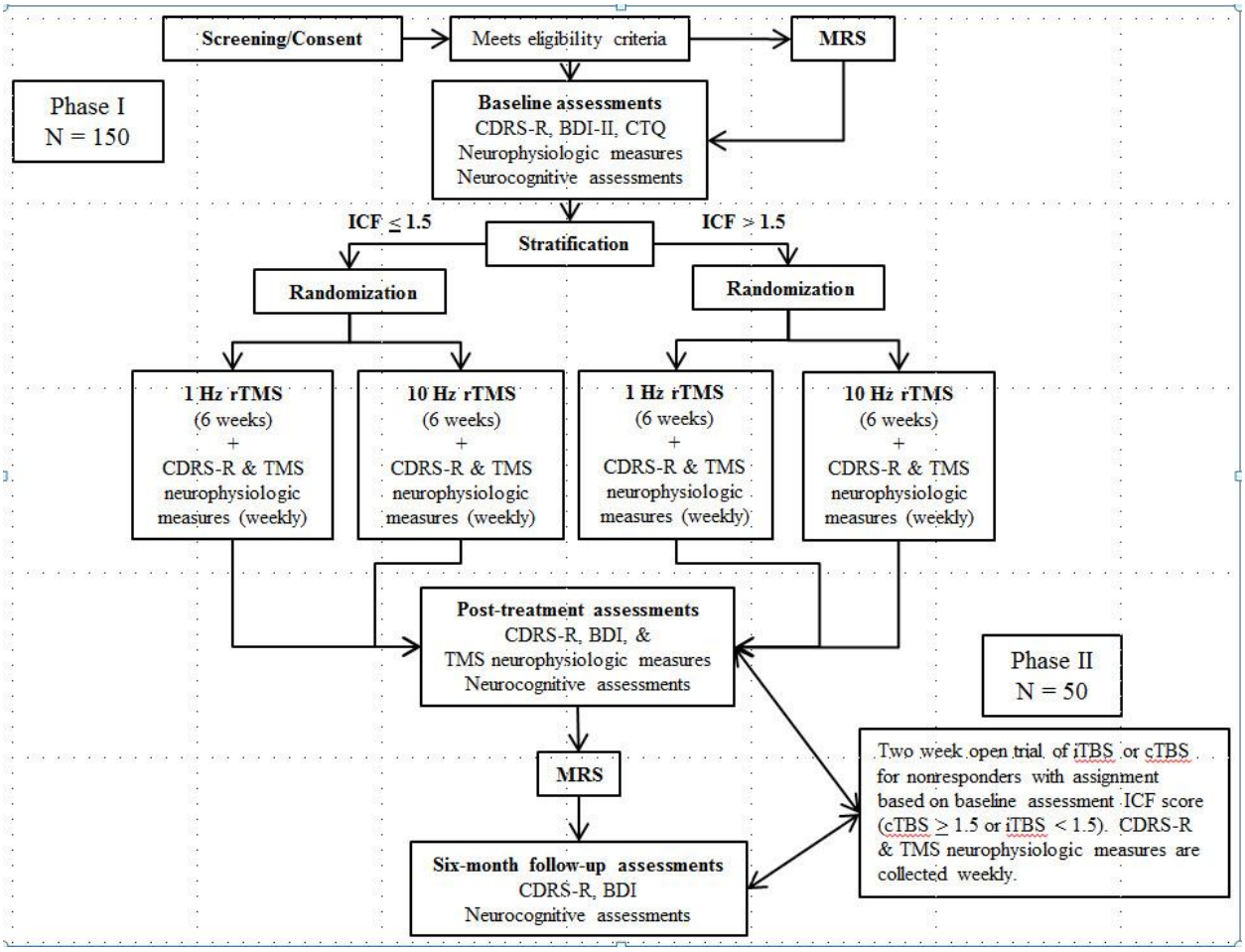
To our knowledge the NeuroStar® TMS Therapy System in XPLOR research configuration has been used most often in prior investigational studies of adolescent depression.[33,69] Our group has existing and ongoing collaborations with Neuronetics as documented in referenced IDE applications: G060269, G110091, G120121. The present, proposed IDE application would deliver investigational, therapeutic TMS therapy (1 Hz, 10 Hz, cTBS, and iTBS) with the NeuroStar® TMS Therapy System in XPLOR research configuration.

Although the interventional component of the study (randomized biomarker stratified trial of 1 Hz or 10 Hz rTMS and extension trial of cTBS or iTBS) is considered more than minimal risk to adolescents, TMS treatments offer a potential direct benefit for the individual participants.[33,47] Notably all depressed, adolescent participants will received an active form of TMS. There is no placebo or sham condition. In addition, the schedule of mood assessments and daily assessments during the interventional portion of the study for any adverse events has been developed for the purpose of monitoring the participants' well-being.

All families and participants for this study will be provided with a consent/assent form describing this study and providing sufficient information for Participants to make an informed decision about their participation in this study. This consent/assent form will be submitted with the protocol for review and approval by the Mayo Clinic IRB. The formal consent/assent of a participant, using the approved IRB consent/assent form, must be obtained before that participant undergoes any study procedure. This form must be signed and dated by the participant the participant's legally-authorized representative, and the individual obtaining informed consent/assent.

3 Study Design

3.1 General Design



Phase I will enroll a cohort (N=120) of adolescents (ages 12-18) with clinically significant depression defined as 40 or greater on the Children's Depression Rating Scale-Revised (CDRS-R). Participants will be stratified according to baseline ICF (high defined as > 1.5 or low defined ≤ 1.5) and then randomized to 6 weeks of either left prefrontal 1 Hz or 10 Hz rTMS. The research team will collect weekly ICF and CDRS-R measures during treatment. We will assess baseline anhedonia with a composite measure from relevant CDRS-R and Beck Depression Inventory-II (BDI-II) items. We will assess lifetime adversity with the Childhood Trauma Questionnaire (CTQ). Participants will have baseline neurocognitive testing. Participants will have a six month follow-up visit with depression severity measures (CDRS-R and BDI-II) and neurocognitive assessments.

Up to 100 Depressed adolescents will be offered the opportunity to undergo magnetic resonance spectroscopy (MRS) measure at baseline (prior to any Phase I TMS treatment) and posttreatment visits (within 2 months after 6 weeks of TMS treatment in Phase I). A comparison group of 30 healthy control adolescents will be offered the opportunity to undergo MRS at one time point. A subset of up to 50 depressed adolescents who do not respond to Phase I TMS treatment will be offered the opportunity to continue in Phase II (within 2 months of completion of Phase I treatment) for two weeks of either iTBS if (ICF ≤ 1.5) or cTBS if (ICF is >1.5).

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Magnetic Resonance Imaging: Depressed and control participants will undergo 3 T structural MRI and ultra-high field (7 T) MRS (7-T, 90–cm horizontal bore magnet interfaced with a Siemens console) to examine GABA, glutamate, and glutamine concentrations in the occipital cortex (OCC) Dr. Malgorzata Marjanska is a co-investigator on this grant and will supervise the collection and analyses of all MRI/MRS data at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota. Please note that travel time from Mayo Clinic Rochester to the CMRR is approximately 1 hour. Participants will be remunerated for travel and all study visits.

Recent work in adult humans with 7 T MRS and short echo time sequences has enhanced the detection and resolution of GABA, glutamate and glutamine. Spectra will be acquired from a voxel placed in the OCC using the STEAM sequence. Voxels will be prescribed using anatomical landmarks from T1-weighted images. Spectra will be stored separately for subsequent automatic frequency and phase correction (in-house written Matlab routines) using the NAA signal at 2.01 ppm. Summed spectra will be analyzed using LCModel 6.1-4A (Stephen Provencher Inc., Oakville, ON, Canada) and simulated basis sets with a metabolite-nulled macromolecule spectrum measured in vivo.. No baseline correction, zero-filling or apodization functions will be applied to the in vivo data prior to the analysis. LCModel fitting will be performed over the spectral range from 0.5 to 4.2 ppm. The quantification will be performed using the unsuppressed water signal obtained from the same voxel. Concentrations will be corrected for cerebrospinal fluid content (the tissue composition will be obtained vial voxel segmentation using T1-weighted images acquired at 3 T).

The first MRS will take place following the screening visit but before the baseline/treatment visit occurs. Therefore, a 7 day window is the allowed timeframe for the first MRI and MRS scan. The second MRS will take place within 2 months of completing all rTMS phase I treatments and assessments and before beginning Phase 2 treatments. Screening will include University of Minnesota Center for Magnetic Resonance Research systematic safety assessments for safety to undergo both rTMS and ultra-high field (7 T) MRI/MRS.

Please note that travel time from Mayo Clinic Rochester to the CMRR is approximately 1 hour. Participants will be remunerated $100.00 per scan for their time and travel expenses.

Research participants: We will enroll participants ages 12 to 18 with the capacity to provide informed assent and parents (for participants younger than age 18) with the capacity to provide informed consent. Capacity to consent will be assessed with comprehension assessments. We are budgeted to recruit 120 depressed adolescents over five years for a target of 100 evaluable subjects (120 accounts for attrition based on our prior experience). We are budgeted to recruit 30 healthy adolescent participants for MRS comparison and test-retest studies. These goals are realistic given our group's experience with therapeutic rTMS and biomarker studies. Screening will include systematic safety assessments for safety to undergo both rTMS and ultra-high field (7 T) MRI/MRS.

Participants who fail to receive clinical benefit from Phase I (defined as a CDRS-R score of 40 or greater at post-treatment) will be offered enrollment in Phase II (N=50 max). The decision to enroll in Phase II can be made by the subject no more than 2 months after the last day of Taper Week 3. During phase II, participants will be assigned to 2 weeks of cTBS if their intracortical facilitation measure (baseline assessment for TBS extension trial) is >1.5 (building on the current speculation that cTBS would be expected to decrease cortical excitability and ICF measures). Participants will be assigned to 2 weeks of iTBS if their ICF (baseline assessment for TBS extension trial) is $\leq$ 1.5 (building on current speculation that iTBS would increase cortical excitability and ICF measures. All participants in Phase II will have a six month follow-up visit with depression severity measures (CDRS-R and BDI-II) and neurocognitive assessments.

3.2 Primary Study Endpoints

The primary study endpoint is depression severity (measured by CDRS-R total) over the 6-week study period.

3.3 Secondary Study Endpoints.

The secondary study endpoint is intracortical facilitation (ICF) over the 6-week study period.

3.4 Table 1 Primary Safety Endpoints

Assessment	Description
Adverse events	Participants will be evaluated for adverse events at each visit. Serious adverse events (SAEs) will be reported as they occur to the Mayo Clinic IRB and USA FDA (as per regulations). Systematic data collection focused on adverse events will include the Pediatric Adverse Event Rating Scale and Physical Symptom Checklist. These rating scales will be administered at baseline, weekly (during all rTMS, cTBS, and iTBS treatment weeks), at the 6 month follow up visit, and at study exit.
Auditory thresholds	Participants will undergo audiometry testing at baseline and upon completion of 6 week rTMS (1 Hz or 10 Hz) and once again (if applicable) after the two week open extension trial (of either cTBS or iTBS).
C-SSRS – Columbia Suicide Severity Rating Scale	This is collected to assess lifetime suicidal ideation and behavior prior to participation, reassessed weekly (during 6 week trial of 1 Hz vs 10 Hz rTMS and if applicable 2 week extension trial of cTBS or iTBS) during treatments, posttreatment, and 6 month follow up visit.
NIH Toolbox Cognition Battery	http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox Cognition measures: http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/cognition The NIH Toolbox Cognition Battery, recommended for ages 7+, consists of tests of multiple constructs. It yields individual measure scores and the following summary scores: Education, Handedness, Attention (Flanker Inhibitory Control and Attention Test), Episodic memory (Picture Sequence Memory Test, and Auditory Verbal Learning Test), Executive Function (Dimensional Change Card Sort Test, Flanker Inhibitory Control and Attention Test), Language (Picture Vocabulary Test, Oral Reading Recognition Test), Processing Speed (Pattern Comparison Processing Speed Test), Working Memory (List Sorting Working Memory Test). Collected at baseline, posttreatment, and 6 month follow up.
Pediatric Adverse Event Rating Scale	This is a 51 item; self-report measure collected at baseline, weekly during rTMS treatments, posttreatment, and at 6 month follows up visit for adverse event monitoring.
Physical Symptoms Checklist	This is a 46 item measure to be collected by the study team (under the supervision of the PI) at baseline, weekly during rTMS treatments, posttreatment, and at 6 month follow up visit for adverse event monitoring.

Assessment	Description
Vital Signs	(Pulse, blood pressure, height, and weight) will be collected at baseline, upon completion of 6 week rTMS (1 Hz or 10 Hz) once again (if applicable) after two week open extension trial (of either cTBS or iTBS), and at 6 month follow up visit.
YMRS-Young Mania Rating Scale	This is an 11 item measure used to assess for manic symptoms. The YMRS scale will be completed at baseline and repeated during the course of study participation if symptoms of mania occur in a study participant. If the YMRS is positive, A MINI/MINI-Kid will be repeated to evaluate for mania. If the study participant meets full DSM-5 criteria for mania or hypomania he or she will be discontinued from the study and followed clinically.

4 Participant Selection, Enrollment and Withdrawal

4.1 Inclusion Criteria

Inclusion Criteria:
- ages 12-18, male or female
-Depressed adolescent participants will have a primary diagnosis of MDD based on a clinical and structured interview with the MINI
-Depression symptoms severity of a 40 or greater based on evaluation with the Children's Depression Rating Scale Revised (CDRS-R) at screening and baseline visits. Further, the total score of the baseline CDRS-R score must not have had a 25% or greater decrease from the screening CDRS-R score
-The duration of the current episode of depression must be 4 weeks or more but 3 years or less.
-For any participant currently receiving antidepressant medication, the referring clinician must determine that insufficient benefit is being received from this treatment and it is clinically appropriate to discontinue the existing antidepressant. Participants will need to have stopped taking antidepressants at least 1 week prior (4 weeks for fluoxetine) to the baseline visit.
-Participants in psychotherapy are eligible provided that this was initiated 4 weeks prior to enrollment and that the frequency of visits will be maintained during study participation.

4.1.1 Inclusion Criteria Healthy Controls

-ages 12-18, male or female

4.2 Exclusion Criteria

Exclusion Criteria:
-The following psychiatric comorbidities are exclusionary: psychotic disorders, bipolar disorders, anorexia nervosa, bulimia nervosa, and substance use disorders within the past year (with the exception of caffeine and tobacco)
-A positive urine drug screen at baseline
-Seizure history
-Family history of epilepsy in a first degree relative

-Head trauma with loss of consciousness for greater than 5 minutes
-Any true positive findings on the rTMS safety screening form.
- Contraindications to MRI/MRS (CMRR Subject Safety Screening Form)
-Any concurrent psychotropic medications (for potential participants receiving antidepressants or psychotropic medications, the referring clinician must determine that insufficient benefit is being received from the treatment and if clinically appropriate, discontinue existing antidepressants and other psychotropic medications).
-Prohibited concomitant medications (See Appendix A)
-Pregnancy or suspected pregnancy in female Participants (assessed with urine pregnancy test)
- Conductive, ferromagnetic, or other magnetic-sensitive metals implanted in the subject's head within 30 cm of the treatment coil excluding the mouth that cannot safely be removed. Examples include cochlear implants, implanted electrodes/stimulators, aneurysm clips or coils, stents, bullet fragments, jewelry and hair barrettes.
-Prior brain surgery
-Risk for increased intracranial pressure such as a brain tumor
-Any unstable medical condition
-History of treatment with ECT or TMS Therapy for any disorder
-Use of any investigational drug within 4 weeks of the baseline visit
-Initiation of a new psychotherapeutic treatment within the past 4 weeks
-Suicide attempt within the previous 6 months that required medical treatment or ≥ 2 attempts in the past 12 months, or has a clear cut plan for suicide and states that he/she cannot guarantee that he/she will inform a family member or call his/her psychiatrist or the investigator if the impulse to implement the plan becomes substantial during the study; or, in the investigator's opinion, is likely to attempt suicide within the next 6 months.

4.2.2 Exclusion Criteria Healthy Controls

-History of depression or any psychiatric treatment
-Subjects found to be pregnant at time of screening will be ineligible
-Positive urine drug screening
-Contraindications to MRI/MRS (CMRR Subject Safety Screening Form)

4.3 Participant Recruitment, Enrollment and Screening

Participants will be recruited from the general community and referrals from local providers. Currently Dr. Croarkin directs a research program and outpatient clinic for adolescent mood disorders. Our group historically has been successful in the recruitment of similar samples.

Additional resources will be utilized for recruitment of potential participants including:

- from within the clinical and referral practices of the clinical study site
- referrals from other care providers within the treatment communities
- radio advertisements
- invitation letters sent to parents of potentially eligible participants
- print advertisements, including but not limited to brochures, flyers, and listings in research classifieds
- trial listing with ClinicalTrials.gov
- trial listing on university and clinical study site website

- social media

All advertising materials will be reviewed and approved by the Mayo Clinic IRB prior to use.

Potentially interested adolescents and their families may be provided with printed participant educational materials to aid them in making an informed decision regarding their willingness to participate in this study. In addition, parents of potentially eligible patients identified via i2b2 search will be contacted via IRB-approved email and follow-up telephone calls if necessary.

Institutional Review Board (IRB) approval will be obtained for the consent and assent form. The investigators will describe the study in full and complete a series of written comprehension questions to verify an understanding of the discomforts and potential benefits to the study. Participants and parents will then be asked to sign the consent and assent forms prior to participation. Dr. Croarkin or a research team member that Dr. Croarkin has trained and supervises will explain the details of the study, answer any study related questions, and obtain informed assent and consent from the participants and their parents or legal guardians. Dr. Croarkin or a board-certified child and adolescent psychiatrist will be available 24 hours a day to address any questions participants or parents have in signing informed consent or in the course of study participation. Dr. Croarkin and the study team will reinforce that declining study participation does not impact accessibility to standard psychiatric treatment within the Mayo Clinic system or surrounding community. Dr. Croarkin will offer ongoing standard clinical care or appropriate referrals (based on patient preference) to patients who exit the study or complete all study procedures.

After providing informed consent and assent, participants will undergo an initial clinical assessment with a structured diagnostic interview (MINI), urine drug screen, safety screens, symptom rating scales, and a neurocognitive battery with the NIH tool box.[86] Participants will then undergo TMS neurophysiology testing to generate a baseline ICF (interstimulus interval of 15 milliseconds) measure for the purpose of stratification. Baseline visits study procedures can be performed on the same date at Screening visit procedures or over 2-3 separate visits based on Participant preference and comfort. Upon completion of baseline measurements and procedures, participants will be randomized to 1 Hz or 10 Hz rTMS.

Preventing participant attrition during investigational, therapeutic TMS: Treatment sessions are delivered 5 days per week consistent with prior systematic research.[33,47,83, 38] We will offer treatment sessions on the weekend (for a total of 5 weekly sessions) when necessary to accommodate the participant scheduling constraints over a period of 6 weeks. Hence participants will be given 42 opportunities to complete 30 sessions of rTMS. During the first week of rTMS treatment intensity may be adjusted for participant comfort to a minimum of 80% motor threshold and titrated by 10% daily to 120% of resting motor threshold. We have found that these scheduling and titration procedures are highly effective for retaining adolescent participants for investigational therapeutic rTMS

It is anticipated that 150 participants will be screened to enroll 120 participants for Phase I. A maximum of 50 participants will be enrolled in Phase II. Prior clinical experience and research with 10 Hz and 1 Hz rTMS suggests that approximately 50% of Phase I participants (N=60) will

not respond to treatment and thus will be eligible for Phase II. Please note that Phase II is an exploratory study and also intended to provide an addition resource for participants who do not respond to standard rTMS approaches. Based on prior work it is unlikely that no more than 50 participants will be eligible and interested in enrollment in Phase II.

4.4 Early Withdrawal of Participants

4.4.1 Participant Withdrawal, Data Collection, and Follow-Up

Participants may withdraw voluntarily from the study at any time. Participants will be withdrawn from the study by the PI/Sponsor if a participant:

- experiences a seizure,
- experiences a DSM-5-confirmed treatment-induced mania,
- is non-compliant with study procedures; or
- endorsed illicit drug use or has a positive urine drug screen during the study

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

Discontinuation information [e.g., date and the reason(s) for discontinuation] will be recorded in the Participant's CRF (Case Report Form). Participants who discontinue prematurely should complete the Week 6 assessment procedures within 2 days following their last TMS treatment session. See the Schedule of Events for the specific procedures to be performed at this discontinuation visit.

Participants withdrawn from the study due to an AE will be followed up for 30 days or until resolution. Participants withdrawn from the study will not be replaced, regardless of the reason for withdrawal. An effort will be made to determine why a participant does not return for the required visits or is dropped from the study. This information will subsequently be recorded on the participant's CRF.Participants will be encouraged to remain compliant with all expected study visits. Non-adherence to expected study visits will be documented and may result in removal from the study. This will be clearly discussed during the consent/assent process and reinforced throughout the study through regular screening for issues with compliance.

Dr. Croarkin will assist with clinical referral for all participants who voluntarily withdraw from the study or are withdrawn by the research team and PI. Specifically, Dr. Croarkin will offer ongoing clinical care or community referrals based on the preference of the participant.

5 Study Device

5.1 *Description*

The NeuroStar TMS Therapy® System is a computerized electromechanical instrument that produces and delivers brief duration, rapidly alternating (pulsed) magnetic fields to induce electrical currents in localized regions of the cerebral cortex. It is a non-invasive tool used for the treatment of Participants with MDD who have not achieved sufficient clinical benefit from antidepressant pharmacotherapy. The NeuroStar TMS Therapy System has been FDA-cleared for use in adults. NeuroStar XPLOR® is a clinical research option for the NeuroStar TMS Therapy System that provides features necessary to conduct randomized controlled trials and other TMS research. The active treatment modality is essentially the same for both the NeuroStar TMS Therapy System and NeuroStar XPLOR.

The NeuroStar XPLOR TMS System consists of the following equipment and software:

- Mobile Console (includes processor module, power module, mast, gantry, halo, and display arm)
- System Software; the XPLOR option also includes a dedicated system software application (software version 1.7.5 or greater) with its own dedicated database of participant treatment history, TMS TrakStar® application, and TrakStar database that operates only with the corresponding XPLOR Software, maintaining isolation, security, and data integrity of the research.
- TMS TrakStar® Data Management System software
- Therapy Coils included with the XPLOR option is a set of three TMS coils:
    - a blinded sham or placebo coil; the sham coil is acoustically matched to protect the integrity of the blind,
    - a blinded active coil, and
    - a standard treatment coil that can be used for performing motor threshold level determination or open-label treatment.
- Head Support System (includes laser positioning aid and coil positioning guide)
- Treatment Chair
- Positioning Cushions (to enhance comfort and positioning of the participant in the required posture for the duration of the treatment session)

The NeuroStar TMS Therapy System also requires supplies and disposables for each treatment session:

- Head Cushion Liner
- Head Side Pad Liner
- Head Positioning Straps
- Earplugs
- SenStar® Treatment Link (a single-use medical device)
- Used SenStar Treatment Link Return box

5.2 Method for Assigning Participants to Treatment Groups

Each participant will be assigned a unique identification upon signing the informed consent and assent form. This number will consist of an assigned 3-digit identifier. Participants will be identified by their assigned number and initials on the CRFs and source documents. The PI/Study Sponsor will maintain a master list linking Participant names and the identifying information indicated above

A Phase I randomization schedule will be generated by Dr. Nakonezny (Study biostatistician). Participants will not be randomized until all entry (inclusion and exclusion) criteria have been met and all pre-study procedures have been satisfactorily completed. After stratification based on ICF biomarker status, eligible participants will be randomly assigned to receive one of the two treatment regimens (1 Hz or 10 Hz rTMS), and the next available randomization number for the participant's stratum will be recorded in the screening log and on the participant's CRF. Randomization numbers may not be reassigned. A permuted-block procedure will be used to randomly assign the treatment conditions, with a fixed block size for each stratum. Phase II is a trial of cTBS or iTBS for Phase I participants who did not have an adequate clinical response in Phase I. Participants will be assigned to 2 weeks of cTBS if their intracortical facilitation measure is >1.5 (building on the current speculation that cTBS would be expected to decrease cortical excitability and ICF measures). Participants will be assigned to 2 weeks of iTBS if their ICF (baseline assessment for TBS extension trial) is $\leq$ 1.5.

Collection of ICF and TMS neurophysiology measures: TMS data will be collected with standard procedures published previously.[65] Participants are seated in a comfortable chair during the procedure. All participants and research team members wear ear plugs during testing sessions. Surface electromyography (EMG) readings are recorded from the abductor pollicis brevis (APB) muscle. The research team will ensure participants maintain relaxation during the procedures with audio feedback tests. TMS stimulation will be applied to the hand area of the contralateral cortex with a figure-of-eight magnetic coil (with a coil diameter of 70 mm on each loop) using the Magstim 200 magnetic stimulator device. The TMS coil is held tangentially on the head with the handle backward at 45 degrees laterally from the midline to determine resting motor threshold. The optimal coil position is located by moving the coil in 1-cm increments over the motor cortex. The resting motor threshold (RMT) is defined as the stimulation intensity which elicits a motor evoked potential (MEP) of > 50 microvolts in 5 of 10 trials with a relaxed APB muscle. For ICF measurements, a subthreshold conditioning stimulus (CS) which is set to 80% of RMT precedes a suprathreshold test stimulus (TS), which is calibrated to produce an average MEP of 0.5 to 1.5 millivolt peak-to-peak amplitude in the contralateral APB muscle. Conditioning stimuli are delivered to the motor cortex prior to the TS in one of five random interstimulus intervals (ISIs): 2 msec, 4 msec for ICI; 10 msec, 15 msec, and 20 msec for ICF. Changes in TS MEP amplitude of each ISI are expressed as a percentage of the mean unconditioned MEP amplitude. Administration of tests is counterbalanced to prevent order effects. Please see Section 16, Appendix B for detailed methodology for collection of ICF and TMS neurophysiology measures.

5.3 Preparation and Administration/Implantation of Investigational Device

Administration of TMS treatment will be consistent with procedures outlined in the NeuroStar® User Manual and User training documentation.

Motor Threshold Determination Procedure – per NeuroStar User Manual

Participants in phase I (1 Hz vs. 10 Hz) will receive treatment parameters that have been safely and feasibly utilized in adult and adolescent studies. For 1 Hz and 10 Hz (Phase I) sessions, stimulation will occur at 120% magnetic field intensity relative to the participant's resting motor threshold (MT). In phase II (cTBS vs. iTBS) stimulations will occur at 80% of the magnetic field intensity relative to the participant's resting motor threshold.

Treatment

Participants in Phase I will be stratified based on ICF testing (high or low) at baseline. An ICF of >1.5 at baseline is considered "high" and an ICF ≤ 1.5 is considered "low". Based on our prior work and review of literature, a sample of depressed adolescents will have an even distribution of high and low ICF values. After stratification, adolescents are randomized to either LDLPFC 1 Hz rTMS with 2400 continuous pulses per session at 120% motor threshold or LDLPFC 10 Hz rTMS with 4 seconds on 36 seconds off for 2400 pulses each session at 120% of resting motor threshold. Hence sessions in each treatment arm with two different types of rTMS (1 Hz and 10 Hz) will have identical intensities (120% motor threshold) durations (40 minutes), number of pulses (2400), and treatment location (LDLFPC). Up to 50 nonresponders in Phase I will be offered to the opportunity to enroll in a Phase II. The decision to enroll in Phase II must be made by the subject no more than 30 days after the last day of Taper Week 3.We will deliver therapeutic rTMS sessions with a Neurostar XPLOR system magnetic stimulator. The research team will localize rTMS treatment sites with the Beam F3 method. Prior research demonstrates that this is a valid and reliable method for scalp location of the dorsolateral prefrontal cortex with comparable results to more expensive, time intensive, MRI-guided approaches. [87] Our prior research demonstrates that the Beam F3 method is a feasible and reliable method for rTMS treatment localization in adolescents.[69] Efficacy measures (CDRS-R) and TMS biomarkers will be collected at baseline and weekly. The TMS biomarker panel includes ICF, Motor threshold (MT) Short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and cortical silent period (CSP).

Participants in Phase II will be assigned to 2 weeks of cTBS if their intracortical facilitation measure (baseline assessment for TBS extension trial) is >1.5. Participants will be assigned to 2 weeks of iTBS if their ICF (baseline assessment for TBS extension trial) is $\leq$ 1.5. Extension trial TBS will be applied to the LDLPFC with the Beam F3 method. Participants receiving cTBS will receive 10 daily (5 sessions per week for two weeks) 120 second trains of uninterrupted TBS for 1800 pulses at 80% motor threshold. Participants receiving iTBS will receive 10 daily (5 sessions per week for two weeks) 2 second trains every 10 seconds for a total of 570 seconds for 1800 pulses at 80% motor threshold. Efficacy measures (CDRS-R) and TMS biomarkers will be collected at baseline, 1 week, and 2 weeks. The TMS biomarker panel includes ICF, Motor

threshold (MT) Short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and cortical silent period (CSP).

Safety Precautions for Seizures and Cardiogenic Syncope during Phase I and Phase II TMS sessions.

We will follow our standard lab procedures for screening to minimize the risk of seizures and syncope, monitoring during treatment sessions, and immediately addressing any induced seizures or syncope during TMS sessions.

With regard to the potential for seizures, all patients will be carefully screened for current or historical factors that may increase the risk of seizures (see Exclusion criteria). The Transcranial Magnetic Stimulation Adult Safety Screen (TASS) will be used to specifically query for any potential seizure or syncope risks. Neurologic disease which may increase the risk for seizures is part of the exclusion criteria.[74,75] Patients undergoing TMS in the proposed study will not be taking medications which could potentially lower their seizure threshold. All TMS sessions will be supervised by a physician located in the treatment suite and all personnel involved will be provided with training in the identification of potential prodromal signs or symptoms of a seizure, particularly the identification of more subtle behavioral events which may herald the occurrence of a frontal lobe seizure, such as changes in level of consciousness or inattention.

All TMS sessions will be conducted in the neurostimulation suite with access (within minutes) to medical facilities and life-support equipment in the event a seizure should occur.

Monitoring for Seizures during TMS sessions.

Seizure monitoring will be by continuous visual observation. This includes visual monitoring for signs of seizures and twitching of muscles, including the abductor pollicis brevis and the first dorsal interosseous muscles of the contralateral hand. The visual observation monitoring will include monitoring for behavioral events such as changes in level of consciousness or inattention. One or more research team members will be in the room with the participant at all times during TMS sessions for visual observation. At a minimum one TMS technician (with specialized onsite and national training on the delivery of TMS, visual monitoring for signs of a seizure, and training in seizure management) will be in the room with the participant at all times during the TMS session with a study physician in the TMS treatment suite.

Seizure Management

The TMS technician and physician investigators have the capability to manage a seizure if it were to occur. The Mayo Clinic Neurostimulation Lab has direct access (within minutes as it is in the same hospital) to emergency medicine services.

In the event of a seizure or syncopal event, the supervising physician will initiate appropriate clinical care as defined within the clinical rTMS practice. The Pediatric Code Team will also be called immediately. Attention must be taken to minimize the risk of aspiration, and when possible guiding the patient into the left lateral decubitus position is desirable. Because seizures

in the context of TMS are brief (typically <60 seconds) and without serious physical sequelae, efforts will be focused on preventing complications of the seizure rather than initiating any specific medication that is not required unless a seizure is prolonged. The Pediatric Code Team is a hospital-based pediatric rapid response team who will arrive within minutes with life-support equipment (oxygen, suction, blood pressure monitor, CPR equipment) and anticonvulsant/antiepileptic medications. The Pediatric Code Team and the supervising TMS study physician will escort the participant to the emergency medicine department upon stabilization (located in the same hospital as the TMS suite). To date, status epilepticus has never been described following rTMS.[47,74,75] However, in the rare event this occurs, the participant will be treated in the emergency medicine department with access (within minutes) to neurological consultation.

If a seizure occurs during the study, TMS treatment as part of the study will be discontinued. Ongoing monitoring of mood and neurocognitive symptoms per the study protocol will be offered. Any seizure will be reported to the Mayo Clinic IRB, Data and Safety Monitoring Board (DSMB), Neuronetics, and the FDA as required per agreements, regulations, and policies.

5.4 Packaging and Labeling

The NeuroStar XPLOR® Therapy System will be clearly labeled with the following warning:

"CAUTION – Investigational Device. Limited by Federal (or United States) law to investigational use"

5.5 Blinding of Study

In both phases of the study (phase I and phase II) participants and study team members completing clinical rating scales (Drs. Croarkin, Romanowicz, or other designated and trained clinical raters) will be blinded to treatment assignment. The study team member completing clinical rating scales will not be allowed in the treatment suite during therapeutic rTMS sessions (10 Hz, 1 Hz, iTBS, or cTBS). We will recruit adolescent participants with no prior TMS exposure or experience and maintain blind to rTMS treatment assignment (1 Hz or 10 Hz for phase I and cTBS or iTBS for phase II). A pre-post treatment expectancy and experience form will ask that participants and parents guess which treatment (1 Hz, 10 Hz, iTBS, or cTBS) was received.[88]

5.6 Receiving, Storage, Distribution and Return

5.6.1 Receipt of Investigational Devices

NeuroStar XPLOR® TMS Therapy System will be delivered to the Principal Investigator/Sponsor and installed by Neuronetics.

Upon receipt of the NeuroStar and study treatment supplies, an inventory will be performed and a device accountability log completed by designated study staff. The designated study staff will

count and verify that the shipment contains all the items noted in the shipping invoice. Any discrepancies or damaged or unusable devices in a given shipment will be documented in the device accountability log. The Investigator at each study site will notify Neuronetics immediately of any discrepancies or damaged or unusable products.

5.6.2 Storage

The supplies and disposables required for each treatment will be stored in a supply station separate from clinical practice stock at each site.

5.6.3 Distribution of Study Device

All participants will be treated using a new single-use treatment disposable, or SenStar® Treatment Link, from their treatment site at the start of every new treatment. The treatment packs and SenStar Treatment Links will be stored in an area that is separate from clinical practice stock. Regular reconciliation of SenStar Treatment Links received from Neuronetics, SenStars used during study treatments, and SenStars remaining will be performed. This reconciliation of the inventory will be logged in the Device Accountability Log, signed, and dated. Any discrepancies noted will be documented, Neuronetics will be notified, and an investigation will be conducted to determine the cause of the discrepancy. Any SenStars disposed of for any reason other than use for the study will be documented in the Device Accountability Log at the time of disposal.

5.6.4 Return or Destruction of Study Device

NeuroStar devices and/or XPLORs that are loaned to the study site for purposes of this study will be returned to Neuronetics at conclusion of the study by Neuronetics personnel. SenStars® that are remaining in the site study research inventory at the completion of the study will be packaged and returned to Neuronetics.

5.7 Participant Compliance Monitoring

Non-adherence to the assigned treatment regimen is defined as missing ≥3 treatments in daily sequence, or missing more than 20% of the total number of treatment sessions occurring during the 6 weeks of acute treatment (does not include taper treatments) as outlined in the schedule of events (i.e., 100% compliance = 30 total treatments). However, if the participant does miss a treatment, the participant must not go more than 3 calendar days without a treatment session. Treatments missed during the taper visits are considered protocol deviations. Participants who are unable to comply with this schedule may be withdrawn from the investigational treatment portion of the study. If a participant receives more than the prescribed number of pulses (i.e. 2400 pulses of good contact), this would be considered an overdose and documented on the SAE form and reported to the IRB and US FDA.

5.8 Prior and Concomitant Therapy

5.8.1 Documenting Prior and Concomitant Therapy

Reasonable efforts will be made to determine all somatic therapies for depression received by the participant in the past 2 years. All other medications and therapies received within six months of study enrollment will also be determined. All relevant information will be recorded on the Participants CRF. The antidepressant treatment history form (ATHF) will be completed to quantify treatment history.

5.8.2 Permitted Concomitant Therapy

Zaleplon, zolpidem, or zopiclone (1 dose nightly) as needed for treatment emergent insomnia or lorazepam (up to 2 mg daily) for treatment emergent anxiety, may be administered for up to 14 doses during Phase I and an additional 5 doses during Phase II. The use of alternative hypnotics or anxiolytic compounds requires prior approval from the PI/Sponsor (Dr. Croarkin). Hormonal contraceptives are allowed if the participant has been on a stable dose for at least 3 months. Short-term treatments for headaches, allergies, colds, and flu symptoms will be allowed during the study provided the medications utilized have no established psychotropic effects that would be expected to confound interpretation of study outcome measures. These medications may include non-sedating, over-the-counter, or prescription antihistamines, analgesics and decongestants. All questions regarding the acceptability of specific medications must be approved by the PI/Sponsor (Dr Croarkin). Please see appendix A for further information.

5.6.3 Prohibited Concomitant Therapy

Any medication administered for the treatment of any psychiatric or neurologic disorder or any other known CNS active drugs, including herbal, over-the-counter, and homeopathic medications, MAOIs, other antidepressants, antipsychotics, stimulants and mood stabilizers are prohibited during this study (See Appendix A, Concomitant Medication Exclusion List). Use of Zaleplon, zolpidem, zopiclone or lorazepam for more than the permitted days (see above) or beginning a new regimen of hormonal contraception may lead to excluding the participant from the study.

6 Study Procedures

6.1 Phase I Visit 1 (Screening)

Table 2 describes all study assessments. Table 3 describes a schedule of events. The first visit is a screening visit. Informed consent and assent will be completed. A physical exam, vital signs, urine drug screen, and urine pregnancy test (if applicable) will be completed. Baseline assessments may be completed at this time as well in the interest of decreasing patient burden related to study procedures (see Table 3).

Once eligibility criteria are met and interest in the MRI/MRS is indicated, the subject will report to the University of Minnesota Center for Magnetic Resonance Imaging for the 3 and 7 Tesla MRI Scans. This procedure needs to be completed prior to the Baseline Visit.

6.2 Phase I Visit 2 (Baseline)

Assessments will be completed (see Table 3), TMS neurophysiology measures are collected, and the participant is stratified as described previously based on ICF **(ICF-15 % MEP amp)**

biomarker status. The participant is randomized to 1 Hz or 10 Hz rTMS. Based on participant preference, the baseline visit can be scheduled on a Monday to facilitate the initiation of investigational rTMS treatment (1 Hz or 10 Hz based on randomization). Otherwise, the participant will be scheduled to initiate the first investigational rTMS treatment day as appropriate based on the patient's schedule and staffing availability.

6.3 Phase I Daily Treatment Visits

On each treatment visit 1 Hz or 10 Hz rTMS will be delivered as described previously. Additionally, for the first week of Phase I treatment, a pre-treatment checklist will be completed by 2 members of study staff prior to initiating treatment to ensure treatments are performed properly and accurately. The 2nd member of study staff may assist telephonically if necessary. Participants will undergo weekly depression severity measures and TMS neurophysiological measures (see Table 3).

Table 2. Description of Assessments

Assessment	Description
Abnormal Involuntary Movement Scale (AIMS)	Collected at the screening visit and at weekly treatment visits, posttreatment, and 6-month follow up visit. This assessment is to screen and monitor twitching, trembling, and dyskinesias.
Antidepressant Treatment History Form (ATHF)	Collected at baseline to systematically document prior psychotropic medication exposure and for quantification of treatment resistance (number of failed antidepressant trials with an adequate dose and duration).
Adverse events	Participants will be evaluated for adverse events at each visit. Serious adverse events (SAEs) will be reported as they occur to the Mayo Clinic IRB and USA FDA (as per regulations). Systematic data collection focused on adverse events will include the Pediatric Adverse Event Rating Scale and Physical Symptom Checklist. These rating scales will be administered at baseline, weekly (during all rTMS, cTBS, and iTBS treatment weeks), at the 6 month follow up visit, and at study exit.
Auditory thresholds	Participants will undergo audiometry testing at baseline and upon completion of 6 week rTMS (1 Hz or 10 Hz) and once again (if applicable) after the two week open extension trial (of either cTBS or iTBS).
Beck Depression Inventory-II	This is a validated, 21-item, self-report measure with a range of 0-63. Collected at baseline, weekly during treatment, posttreatment, and 6 month follow up visit.
CDRS-R – Childhood Depression Rating Scale – Revised	This is a validated, 17-item, clinical rating tool to assess severity of depression. Parents provide input into 14 of the items. Total possible scores range from 17 to 113. Collected at baseline, weekly during treatment, posttreatment, and 6 month follow up visit.

Assessment	Description
CGI-S– Clinical Global Impressions Severity Scale	A standard measure of symptom severity and treatment response. Collected at baseline, weekly during treatment, posttreatment, and 6 month follow up visit.
Center for Magnetic Resonance Research Safety Screen (CMRR)	This assesses subject safety to go in the magnet room and undergo 3T and 7 MRI/MRS. This will be completed at Mayo Clinic and CMRR
C-SSRS – Columbia Suicide Severity Rating Scale	This is collected to assess lifetime suicidal ideation and behavior prior to participation, reassessed weekly during 6 week trial of 1 Hz vs 10 Hz rTMS (phase I) and if applicable 2 week extension trial of cTBS or iTBS (phase II) during treatments, posttreatment, and 6 month follow up visit.
CTQ – Childhood Trauma Questionnaire	A 28 item self-report scale used to screen for a history of adversity. Collected at baseline.
Edinburgh Inventory for Handedness	Used to assess handedness of all Participants. Collected at baseline.
Mini International Neuropsychiatric Interview (M.I.N.I)	A structured psychiatric interview (M.I.N.I. for age 18 and M.I.N.I Kid for ages 12-17). Collected at baseline.
NIH Toolbox Cognition Battery	http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox Cognition measures: http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/cognition The NIH Toolbox Cognition Battery, recommended for ages 7+, consists of tests of multiple constructs. It yields individual measure scores and the following summary scores: Education, Handedness, Attention (Flanker Inhibitory Control and Attention Test), Episodic memory (Picture Sequence Memory Test, and Auditory Verbal Learning Test), Executive Function (Dimensional Change Card Sort Test, Flanker Inhibitory Control and Attention Test), Language (Picture Vocabulary Test, Oral Reading Recognition Test), Processing Speed (Pattern Comparison Processing Speed Test), Working Memory (List Sorting Working Memory Test). Collected at baseline, posttreatment, and 6 month follow up.
PDS – Pubertal Development Scale and Tanner Staging	A self-report questionnaire and line-drawings used to evaluate level of pubertal development. Collected at baseline.
Pre/Post Treatment Expectations and Experience Questionnaire (Adolescent and Parent)	Assesses adolescent and parent blinding to treatment arm (1 Hz or 10 Hz) and expectations for treatment outcome. Collected at baseline and posttreatment.
SHAPS-Snaith-Hamilton Pleasure Scale	This is a 14 item, self-report measure of validated measure of hedonic capacity. Collected at baseline.

Assessment	Description
Pediatric Adverse Event Rating Scale	This is a 51 item; self-report measure collected at baseline, weekly during rTMS treatments, posttreatment, and at 6 month follows up visit for adverse event monitoring.
Physical Symptoms Checklist	This is a 46 item measure to be collected by the study team (under the supervision of the PI) at baseline, weekly during rTMS treatments, posttreatment, and at 6 month follow up visit for adverse event monitoring.
SIT-R3 – Slosson Intelligence Test – Revised	A rapid, validated index of intellectual ability. Its items cover six cognitive domains: Information, Comprehension, Quantitative, Similarities and Differences, Vocabulary, and Auditory Memory. Collected at baseline, if PI the determines there are concerns about cognitive functioning.
TASS – Transcranial Magnetic Stimulation Safety Screen	This safety screen is completed prior to TMS for confirmation patient safety. Collected at baseline.
UCSD Brief Assessment of Capacity to Consent (UBACC)	This will be given to patients that are 18 years of age who are interested in participating in the MRI/MRS scans at CMRR of age during informed consent. This assesses the patient's understanding of the MRI/MRS portion of the study.
Vital Signs	(Pulse, blood pressure, height, and weight) will be collected at baseline, upon completion of 6 week rTMS (1 Hz or 10 Hz) once again (if applicable) after two week open extension trial (of either cTBS or iTBS), and at 6 month follow up visit.
YMRS-Young Mania Rating Scale	This is an 11 item measure used to assess for manic symptoms. The YMRS scale will be completed at baseline and repeated during the course of study participation if symptoms of mania occur in a study participant. If the YMRS is positive, A MINI/MINI-Kid will be repeated to evaluate for mania. If the study participant meets full DSM-5 criteria for mania or hypomania he or she will be discontinued from the study and followed clinically.
TMS Neurophysiology Measures (MT, SICI, ICF, LICI, CSP)	Single (motor threshold and cortical silent period) and paired-pulse (intracortical facilitation, short-interval cortical inhibition, long-interval cortical inhibition). TMS neurophysiology measures will be collected in depressed adolescent participants at baseline, weekly during six weeks of rTMS treatment with 1Hz or 10 Hz rTMS (phase I), posttreatment, and weekly for 2 weeks for depressed adolescent participants in a, trial of cTBS or iTBS (phase II).

Table 3 Phase I Assessments

Phase I	**1-Week Prestudy**		**6-Week Treatment (1 Hz vs. 10 Hz)**						**3-Week Post-Treatment Taper**			**6-Month Follow Up**
Week	Wk –2 to -1[a] (Screening)	Wk 0[a] (Baseline)	Wk 1[b]	Wk 2[b]	Wk 3[b]	Wk 4[b]	Wk 5[b]	Wk 6[b,c]/ET	Wk 1	Wk 2	Wk 3	
Day(s)	-7[a]	0[a]	1-5	8-12	15-19	22-26	29-35	36-42	43-49	50-56	57-63	
Informed Consent/Assent/HIPAA	X											
CMRR Safety Screen (prior to MRI/MRS)	X							X				
UCSD Brief Assessment of Capacity to Consent (UBACC) (only patients age 18 participating in MRI/MRS scans at CMRR)	X											
MRI/MRS Study(Before Baseline Appt.)	X							X				
Transcranial Magnetic Stimulation Adult Safety Screen (TASS)	X											
Antidepressant Treatment History Form (ATHF)	X											
Abnormal Involuntary Movement Scale (AIMS)	X		X	X	X	X	X	X	X	X	X	X
MINI / MINI-Kids	X											
CTQ		X										
PDS		X										

Phase I	**1-Week Prestudy**		**6-Week Treatment (1 Hz vs. 10 Hz)**						**3-Week Post-Treatment Taper**			**6-Month Follow Up**
Week	Wk –2 to -1[a] (Screening)	Wk 0[a] (Baseline)	Wk 1[b]	Wk 2[b]	Wk 3[b]	Wk 4[b]	Wk 5[b]	Wk 6[b,c]/ET	Wk 1	Wk 2	Wk 3	
Day(s)	-7[a]	0[a]	1-5	8-12	15-19	22-26	29-35	36-42	43-49	50-56	57-63	
SHAPS		X										
Pre/Post-TEEQ-P		X						X				
Pre/Post-TEEQ-A		X						X				
Efficacy Assessments												
BDI-II	X	X				X		X[c,d]	X		X[d]	
CDRS-R	X	X	X	X	X	X[d]	X	X[c,d]	X	X	X[d]	X
CGI-S	X	X				X		X[c,d]	X		X[d]	X
Neuropsychological Assessments												
NIH Toolbox		X						X[c,d]				X
SIT-R3		X										
YMRS[d]		X										
C-SSRS	X	X	X[d]	X[d]	X[d]	X[d]	X[d]	X[c,d]	X[d]	X[d]	X[d]	X
Safety Assessments												
Physical examination	X											
Vital Signs	X							X[c]	X			
Urine drug screen	X											
Pregnancy test[e]	X											
Audiometry assessment		X						X[c]				X
Physical Symptom Checklist		X	X	X	X	X	X	X				X
Pediatric Adverse Event Rating Scale		X	X	X	X	X	X	X				X
Adverse Events	X ---------- X											
Prior/Concomitant Treatment	X ---------- X											

Phase I	**1-Week Prestudy**		**6-Week Treatment (1 Hz vs. 10 Hz)**						**3-Week Post-Treatment Taper**			**6-Month Follow Up**
Week	Wk –2 to -1[a] (Screening)	Wk 0[a] (Baseline)	Wk 1[b]	Wk 2[b]	Wk 3[b]	Wk 4[b]	Wk 5[b]	Wk 6[b,c]/ET	Wk 1	Wk 2	Wk 3	
Day(s)	-7[a]	0[a]	1-5	8-12	15-19	22-26	29-35	36-42	43-49	50-56	57-63	
Motor Threshold Determination[g]		X										
TMS Treatment Session (daily × 5 weekdays/week)			X----X[b]	X----X[b]	X----X[b]	X----X[b]	X----X[b]	X---X[b]				
TMS neurophysiology measures (MT, SICI, ICF, LICI, CSP)		X	X	X	X	X	X	X				
Post-Treatment Taper TMS Session(s) (3X/Wk1, 2X/Wk 2, 1X/Wk 3)									X	X	X	

a. A maximum of 7 days may elapse between the screening and baseline visits; a maximum of 5 days may elapse between the baseline visit and the first treatment day of Week 1. All baseline assessments must be completed before 1st TMS treatment.

b. The first visit during each week of treatment should occur on a Monday, with daily treatment sessions occurring on Monday through Friday of each week.

c. Participants who prematurely discontinue should complete all Week 6 procedures within 2 days after their last TMS treatment session.

d. Efficacy and neuropsychological assessments to be performed after last TMS treatment session on last day of the treatment week when assessments are required. YMRS to be completed if symptom indicating mania occurs. If positive, a MINI/MINI-Kid must be repeated to determine if Participant meets full DSM-5 criteria for mania or hypomania. If positive, participant to be discontinued from the study and followed clinically.

e. If Participant is female of childbearing potential, a urine pregnancy test will be performed at screening. Note that parents or guardians will not be told the results of the pregnancy test without the participant's permission. But if the participant's doctor believes that being pregnant may cause serious health problems, they may need to tell the parent/guardian the pregnancy test results.

f. Adverse events occurring prior to randomization will be recorded as part of each patient's medical history. Those AEs occurring following the first TMS treatment session through 30 days after last TMS treatment session will be collected.

g. In addition to the indicated day, Motor Threshold Determination (MT) may be repeated at any time during the course of the active TMS treatment sessions based on clinical assessment of the supervising physician.

Medication treatment for emergent insomnia and emergent anxiety is permitted (refer to protocol for usage allowance). Refer to Appendix A for the Concomitant Medication List.

h. The following assessments will be completed once only for healthy controls at the Mayo Clinic site: Urine testing (drugs and pregnancy if female), vitals (blood pressure, pulse, height and weight), GUID (demographics) form, physical examination record, Transcranial Magnetic Stimulation Adult Safety Screen, M.I.N.I. Kid, Children's Depression Rating Scale, Columbia - Suicide Severity Rating Scale, Childhood Trauma Questionnaire, Clinical Global Impressions, Center for Magnetic Resonance Research Screening Form, Edinburgh Handedness Inventory, Scale for Pubertal Development, Beck Depression Inventory-II, Authorization to Release Information (if applicable)

Table 4 Phase II Assessments

Week	Week 1	Week 2	6 Month Follow-up
Days	**1-5**	**8-12**	
Informed Consent/Assent	**X**		
Motor Threshold	**X**		
CDRS-R	**X**	**X**	**X**
CGI	**X**	**X**	**X**
C-SSRS	**X**	**X**	**X**
TMS neurophysiology measures (MT, SICI, ICF, LICI, CSP)	**X**	**X**	
AIMS	**X**	**X**	**X**
Vital Signs	**X**	**X**	
Physical Symptom Checklist	**X**	**X**	**X**
Pediatric Adverse Event Rating Scale	**X**	**X**	**X**
Audiometry assessment		**X**	**X**
NIH toolbox		**X**	**X**
Adverse Events[f]	**Daily**	**Daily**	
Prior/Concomitant Treatment	**Daily**	**Daily**	

Every treatment day of Phase II treatment a pre-treatment checklist will be completed by 2 members of study staff prior to initiating treatment to ensure treatments are performed properly and accurately. The 2nd member of study staff may assist telephonically if necessary.

7 Statistical Plan

7.1 Sample Size Determination

Sample Size Estimation and Power Analysis for the Primary Aim of the Study

Using a putative estimate of effect size (*d*~0.61) as it relates to the Primary Aim, an *a priori* sample size estimation and power analysis was performed for the repeated measures design in the proposed study. The results suggest that a sample size of 25 participants per group (N=120) achieves 80% power, at a 0.05 alpha level (two-tailed), to detect standardized group mean differences (Cohen's *d*) as small as 0.61 in a 2-treatment by 2-biomarker status between-Participants group design with 6 (within-Participants) repeated measurements of the primary outcome (depression severity) having a compound symmetry covariance structure, when the assumed within-Participant correlation between observations (depression severity) on the same Participant is ≤0.50 (0.50 was based on guidance from our preliminary rTMS work). We anticipate some dropouts (~20% based on treatment discontinuation results from our previous rTMS studies). Thus, ~120 participants will be enrolled to allow for this expected rate of attrition with the intent of capturing a range of evaluable data for 100 participants. The Power Analysis and Sample Size (PASS) 2016 software, version 14.0.7, was used to carry out the sample size and power analysis. [89]

We recognize that to definitively evaluate the relationship between rTMS Treatment (low frequency 1 Hz rTMS vs. high frequency 10 Hz rTMS) by biomarker status (high baseline ICF vs. low baseline ICF) on depression severity—since this relationship has not been previously studied, we have no previous findings to guide effect size selection for the present effort, and the current study would be the first of its kind to examine this effect in depressed youth—we will possibly need a larger sample size than the 100 participants put forth in this proposal. The selection of 100 participants was, in part, resource-driven. The sample size is designed, in part, to detect a moderate effect and to establish a *proof of concept* so as to expand to a subsequent, larger-scale multi-center study. Nevertheless, the current proposal will permit an evaluation of whether receiving low frequency (1 Hz) rTMS vs. high frequency (10 Hz) rTMS treatment according to patients' baseline biomarker status (ICF) shows initial evidence of a relationship with improvement in depression severity in a depressed adolescent population.

7.2 Statistical Methods

Statistical Methods
All statistical analyses will be carried out using SAS software, version 9.4. The level of significance will be set at $\alpha = .05$ (two-tailed) and, to address multiple testing (where applicable), p-values will be adjusted using the False Discovery Rate.

Primary Aim
Data Analysis Plan for Primary Aim 1 (Hypotheses 1a−1d)

Primary Continuous Outcome: CDRS-R Total Score
Depression severity (measured by CDRS-R total) over the 6-week study period will be the primary continuous outcome measure. A linear mixed model analysis of repeated measures will be used to evaluate the rTMS Treatment (low frequency 1 Hz rTMS vs. high frequency 10 Hz rTMS) by biomarker status (high baseline ICF vs. low baseline ICF) interaction effect on depression severity (CDRS-R total) over 6 weeks of rTMS in depressed adolescents. The model will contain fixed effects terms for rTMS treatment, biomarker status, time, rTMS treatment × biomarker status interaction, and baseline CDRS-R total score as a covariate. We will consider the inclusion of pubertal state and treatment resistance (as defined by Antidepressant Treatment History Form [ATHF] score) during the study period as additional covariates in the mixed model. Restricted maximum likelihood estimation, Type 3 tests of fixed effects, and generalized least squares will be used, with the Kenward-Roger correction applied to the appropriate and best fitting covariance structure. The sandwich (robust covariance matrix) estimator will also be considered. Least squares (LS) means will be estimated as part of the mixed model. Tests of Simple Effects will be examined so as to partition the interaction LS means effects; i.e., simple group main effects of biomarker status at each level of rTMS treatment (Hypotheses 1a, 1b) and simple group main effects of rTMS treatment at each level of biomarker status (Hypotheses 1c, 1d) will be examined. Cohen's *d* and Hedges' *g* will be calculated and interpreted as the effect size estimator for the between-Participants group main effects. A separate linear mixed model repeated measures analysis similar to that described above will also be implemented over 6 weeks of acute rTMS treatment and throughout the 6-month follow-up period. The PROC

MIXED procedures (mixed model approach) in SAS software will make use of all available data from all participants from the efficacy analysis set, and provide a robust mechanism for handling data that are assumed missing at random (see sensitivity analysis section below for MNAR).

Secondary Aim
Data Analysis Plan for Secondary Aim 2 (Hypotheses 2a−2d)

Secondary Continuous Outcome: Intracortical Facilitation (ICF)
Intracortical Facilitation over the 6-week study period will be the secondary continuous outcome measure. A within-Participants linear mixed model analysis of repeated measures will be used to examine the change in ICF over 6 weeks of rTMS treatment in depressed adolescents. We will consider the inclusion of pubertal state and treatment resistance (as defined by ATHF score) during the study period as a covariate in the mixed model. A mixed model analysis will be conducted on ICF for those who receive 6 weeks of low frequency (1 Hz) rTMS treatment (Hypothesis 2a) and, in a separate model, for those who receive 6 weeks of high frequency (10 Hz) rTMS treatment (Hypothesis 2c). Restricted maximum likelihood estimation, Type 3 tests of fixed effects, and generalized least squares (LS) will be used, with the Kenward-Roger correction applied to the appropriate and best fitting covariance structure. The sandwich (robust covariance matrix) estimator will also be considered. LS mean contrasts (between ICF measurement time periods) from the mixed model will also be examined. A separate linear mixed model analysis similar to that described above will also be implemented over 6 weeks of acute rTMS treatment and throughout the 6-month follow-up period. A nonlinear mixed model will be considered (e.g., exponential decay/growth model) to estimate ICF over the 6 weeks of rTMS treatment (if the pattern of ICF values suggests that a nonlinear model is deemed necessary).

A linear mixed model repeated measures analysis similar to that described above will also be used to examine the relationship between measures of ICF (as the response variable) and depressive symptoms (CDRS-R total as a time-varying covariate) over the 6 weeks of rTMS treatment. We will consider the inclusion of pubertal state and treatment resistance (as defined by ATHF) during the study period as a covariate in the mixed model. The parameter estimates (regression coefficients) will be interpreted from the solution for fixed effects in the mixed model analysis for those who receive 6 weeks of low frequency (1 Hz) rTMS treatment (Hypothesis 2b) and for those who receive 6 weeks of high frequency (10 Hz) rTMS treatment (Hypothesis 2d). A separate linear mixed model analysis similar to that described above will also be implemented over 6 weeks of acute rTMS treatment and throughout the 6-month follow-up period. A nonlinear mixed model will be considered to estimate ICF over the 6 weeks of rTMS treatment from CDRS-R total (if the pattern of ICF and CDRS-R total suggests that a nonlinear model is deemed necessary).

Data Analysis Plan for Exploratory Aim 3 (MRI/MRS)

A within-participants linear mixed model analysis of repeated measures will be used to examine baseline differences between depressed adolescents and healthy control adolescents (Hypotheses 1 and 2); and the change in cortical MRS measures of GABA and Gln/Glu over 6 weeks of rTMS treatment in depressed adolescents. Separate mixed models will be conducted on cortical GABA and Gln/Glu for those who receive 6 weeks of low frequency (1 Hz) rTMS treatment

(Hypotheses 3 and 4) and for those who receive 6 weeks of high frequency (10 Hz) rTMS treatment (Hypotheses 5 and 6). A nonlinear mixed model will be considered (if the pattern of GABA and Gln/Glu values suggests that a nonlinear model is deemed necessary).

Data Analysis Plan for Exploratory Aim4

Moderator Effects of Anhedonia and Adversity on Treatment Outcome of Depression Severity
The multiple mediation/moderation path analytic model, as described in Preacher and Hayes will be used to estimate the direct effect as well as the total and specific indirect effects of rTMS treatment on depression severity (at the 6-week endpoint) through each of the potential baseline moderators (anhedonia and adversity) operating in parallel and, via a separate model, in serial, while controlling for any potential demographics/patient characteristics (not designated as mediators/moderators), including pubertal state and treatment resistance (as defined by ATHF score). A test of the indirect effects will be conducted by bootstrapping standard errors and confidence intervals (95% bias-corrected) from 5,000 bootstrap samples. The procedures of the PROCESS computational SAS macro developed by Hayes will be used to implement the path analytic-based multiple mediation/moderation model.

Sensitivity Analysis
In this section we present a brief sensitivity analysis plan to address incomplete response patterns according to a missing not at random mechanism. First, we will follow the outcome-based model framework of for longitudinal data with non-random missingness along with a model-based approach proposed for modeling both the treatment response and the missing data process.[90] That is, if deemed necessary, we will build longitudinal models for the data which includes a model for the dropout (or missing data) process so as to assess the sensitivity of the results about the dependence between dropout (or missingness) and treatment response. Additionally, if deemed necessary, we will consider implementing the semi-parametric shared parameter model approach as described in Tsonaka et al. (2009) to handle nonmonotonic nonignorable missingness for mixed-effects models. A Pattern-Mixture Model will also be considered (if necessary). [90] The results from such (non-random dropout/missingness) models will then be compared with the results of the proposed standard methods (dropout or missing at random models) so as to assess the sensitivity of the results to the effect of dropouts (or incomplete response patterns according to a missing not at random mechanism).

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Adverse Device Effect (UADE)
A UADE is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device if that effect, problem or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants.

Adverse Effect (Event)

Any untoward medical occurrence in a participant involved in clinical study of an investigational device; regardless of the causal relationship of the problem with the device or, if applicable, other study related treatment(s).

Associated with the investigational device: there is a reasonable possibility that the adverse effect may have been caused by the investigational device.

Life-threatening adverse effect: Any adverse effect that places the participant, in the view of either the investigator or the sponsor, at immediate risk of death from the effect **as it occurred**. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse effect: An adverse effect is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- death
- a life-threatening AE
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect.

Unanticipated adverse effect: Any adverse effect, the nature, specificity, severity, or frequency of which is not consistent with the risk information in the clinical study protocol or elsewhere in the current IDE application.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as an unanticipated adverse device effect unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery will **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the Participant is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the research team will instruct each participant to report any subsequent event(s) that the participant, or the participant's personal physician, believes might reasonably be related to participation in this study.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Unanticipated Problems Involving Risk to Participants or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets all of the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the Participant or others (including individuals who are not research Participants). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the Participants or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- Related: A problem or event is "related" if it is possibly related to the research procedures.

An *adverse event* is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a person who has received NeuroStar TMS therapy (1 Hz, 10 Hz, cTBS, or iTBS). The event need not be causally related to the NeuroStar device or the present clinical trial. An AE includes, but is not limited to:

- Any clinically significant worsening of a pre-existing condition;
- An AE occurring from overdose (i.e., a dose higher than that described in the protocol) of a NeuroStar TMS device, whether accidental or intentional;
- An AE occurring from abuse (e.g., use for non-clinical reasons) of a NeuroStar TMS device;
- An AE that has been associated with the discontinuation of the use of a NeuroStar TMS device; a *preexisting condition* is a clinical condition (including a condition being treated)

that is diagnosed before an informed consent form is signed and is documented as part of the Participant's medical history.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or frequency will be used to determine whether an event is a treatment-emergent AE (TEAE). An AE is considered to be a TEAE if (1) it was not present when the active phase of the study began and is not a chronic condition that is part of the Participant's medical history, or (2) it was present at the start of the active phase of the study or as part of the Participant's medical history, but the severity or frequency increased during the active phase.

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

Life threatening refers to immediate risk of death as the event occurred or use or continued use of the device or other medical product might have resulted in the death per the reporter. A life-threatening event does not include an event that, had it occurred in a more severe form, might have caused death, but as it actually occurred, did not create an immediate risk of death.

Hospitalization is to be considered only as an admission. Hospitalization or prolongation of a hospitalization constitutes an AE to be classified as serious.

Note that hospitalizations planned before the start of the study, for a preexisting condition that has not worsened, do not constitute an SAE (e.g., elective hospitalization for surgery due to a preexisting condition that has not worsened during the study).

If there is any doubt whether the information constitutes an SAE, the information should be treated as an SAE for the purpose of this study.

8.2 Recording of Adverse Events

All adverse events occurring during the study, including those not meeting the criteria of an Unanticipated Adverse Device Effect (UADE) will be recorded on the appropriate case report form on the day of the occurrence. Records of these events will be maintained and reports submitted to the FDA and IRB according to the regulatory requirements. Expected clinical adverse events and nonsignificant (not serious) clinical adverse events will not be reported. Expected clinical adverse events and anticipated adverse device effects are those listed in Section 1.4.1. The PI/Sponsor (Dr. Croarkin) and research team will follow all AEs, SAEs, and other reportable events until the event has subsided or values have returned to baseline, or in case of permanent impairment, until the condition stabilizes.

8.3 Reporting Procedures

At each required study visit, all AEs that have occurred since the previous visit will be recorded in the adverse event record of the Participant's CRF. The information recorded should be based on the signs or symptoms detected during the physical examination and clinical evaluation of the

Participant. In addition to the information obtained from those sources, the Participant should be asked the following nonspecific question: "How have you been feeling since your last visit?" Signs and symptoms should be recorded using standard medical terminology. On the case report form the treating physician will record the event, sign with their initials and enter the current date. After the treatment is completed, this process will be repeated. The PI or a Co PI will review the Adverse Events on a daily basis and will determine the event to be "Clinically Significant" or "Not Significant." This process can be completed by phone, email or face to face.

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

Causal relationship options and definitions are as follows:

- *Definitely related*: Event can be fully attributable to administration of the investigational device.
- *Probably related*: Event is most likely to be explained by administration of the investigational device, rather than the Participant's clinical state or other agents/therapies.
- *Possibly related*: Event is as likely explained by administration of the investigational device, as by the Participant's clinical state or other agents/therapies.
- *Probably not related*: Event is most likely to be explained by the Participant's clinical state or other agents/therapies, rather than the investigational device.
- *Definitely not related*: Event can be fully explained by the Participant's clinical state or other agents/therapies, rather than the investigational device.

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

- Temporal relationship between the investigational device/protocol and the AE
- Biologic plausibility of relationship
- Participants' underlying clinical state or concomitant agents/therapies
- Where applicable, whether the AE abates on discontinuation of the investigational device (dechallenge)
- Where applicable, whether the AE reappears on repeat exposure to the investigational device (rechallenge)

SAEs that are not investigational device-related may nevertheless be considered by the participating Investigator or the medical monitor (or designee) to be related to the conduct of the clinical study, i.e., to a Participant's participation in the study.

For this study, the treatment follow-up period for adverse events is defined as 30 days following the last study visit. Follow up will be documented in the Participants study file.

Per Mayo Clinic policy: "All health care providers in the State of Minnesota are mandated reporters under the Minnesota Maltreatment of Minors Act." All study staff are made aware of this policy and will remain in compliance with associated reporting procedures when necessary.

8.3.1 Sponsor-Investigator Reporting, Notifying Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

8.3.2 Sponsor-Investigator Reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unanticipated adverse device effects according to the required reporting timelines, formats and regulations.

Additionally, per FDA request, all blurred vision adverse events will be reported to the FDA within 5 business days.

The sponsor-investigator will submit a completed **FDA Form 3500A** to the FDA's Center for Devices and Radiological Health for any observed or reported adverse effect that is determined to be an unanticipated adverse device effect. A copy of this completed form will be provided to the DSMB and all participating sub-investigators.

The completed FDA Form 3500A will be submitted to the FDA as soon as possible and, in no event, later than 10 working days after the sponsor-investigator first receives notice of the adverse effect.

If the results of the sponsor-investigator's follow-up evaluation shows that an adverse effect that was initially determined to not constitute an unanticipated adverse device effect does, in fact, meet the requirements for reporting; the sponsor-investigator will submit a completed FDA Form 3500A as soon as possible, but in no event later than 10 working days, after the determination was made.

For each submitted FDA Form 3500A, the sponsor-investigator will identify all previously submitted reports that that addressed a similar adverse effect experience and will provide an analysis of the significance of newly reported adverse effect in light of any previous, similar report(s).

Subsequent to the initial submission of a completed FDA Form 3500A, the sponsor-investigator will submit additional information concerning the reported adverse effect as requested by the FDA.

Reporting Process
Unanticipated Adverse Device Effect reports will be submitted on FDA Form 3500A.
The contact information for submitting reports is:

Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993-0002

8.3.3 Deviations from the investigational plan.

The sponsor-investigator shall notify Mayo IRB (see 21 CFR 56.108(a) (3) and (4)) of any deviation from the investigational plan to protect the life or physical well-being of a Participant in an emergency. Such notice shall be given as soon as possible, but in no event later than 5 working days after the emergency occurred. Except in such an emergency, prior approval by the sponsor-investigator is required for changes in or deviations from a plan, and if these changes or deviations may affect the scientific soundness of the plan or the rights, safety, or welfare of human Participants, FDA and IRB notification in accordance with 21 CFR 812.35(a) also is required.

8.4 Unblinding Procedures (Breaking the Blind)

While the safety of the participant always comes first, it is still important to seriously consider if unblinding the study therapy is necessary to ensure a Participant's safety. In the event of a serious adverse device effect, the Investigator will carefully assess whether breaking the blind will critically affect how a Participant is treated in response to the adverse effect and whether this knowledge outweighs the implications to the scientific soundness of the study. In the case of most serious adverse effects, treatment would be discontinued and symptoms treated symptomatically. In these instances, having this information would not significantly alter the treatment of the adverse effect(s).

As an additional safeguard against bias, the DSMB has been charged with making the final recommendations for breaking the study blind

If the decision to break the blind is made immediately upon learning of the adverse event, this information will be reported to the FDA and reviewing IRB at the time of initial adverse event reporting. If the unblinding occurs after the initial reporting, the FDA will be notified of the action within ten working days from the time of breaking the blind. The reviewing IRB will be notified according to their reporting guidelines if the decision is made to break the study blind after the initial reporting.

8.5 Stopping Rules

Specific occasions when study treatment may be stopped are explained Section 4.4.1 in the discussion of Anticipated Risks.

Because of the anticipated low level of adverse events of TMS, the DSMB will be charged with reviewing adverse events at least every six months. Serious adverse events will be reviewed on a monthly basis, unless a more urgent review is requested. Only under extreme circumstances or if it were determined that a high level of side effects was due to TMS, would the DSMB be charged with breaking the study mask.

Specific Study Stopping Rules
The study will be stopped should any of the following occur:

- Two participants experience seizures
-One or more participant experiences status epilepticus
-One or more participant attempts or commits suicide during treatment weeks in Phase I or II.
-Two or more Serious Adverse Events in one or more participants.
-Two or more participants have blurred vision associated with rTMS treatment (confirmed by an ophthalmologist.)

8.6 *Medical Monitoring*

The PI/sponsor (Dr. Croarkin) has oversight and accountability for the overall safety of the study participants. PI/sponsor safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Independent Data and Safety Monitoring Board

A Data and Safety Monitoring Board will be assembled to oversee the safety of the study, Participants, and the scientific validity and integrity of data collected as part of the study. This DSMB will include members from academic sites independent of the study sites and will consist of at least one non-study, board-certified psychiatrist and one biostatistician.

The responsibilities and decision points for the DSMB will be captured in the DSMB charter.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study Participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed Participant authorization informing the Participant of the following:

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

In the event that a Participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of

Participant authorization. For Participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the Participant is alive) at the end of their scheduled study period.

9.2 Source Documents

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

All clinical notes and discussions regarding the patient will be appropriately captured, signed and documented.

9.3 Case Report Forms

An eligibility form will be completed for each Participant enrolled into the clinical study. As needed, an adverse event worksheet will be completed for study Participants experiencing AEs. The site investigator or designated study staff will review, approve, and sign/date each completed CRF; the signature serving as attestation that all clinical and laboratory data entered on the CRF are complete, accurate, and authentic. Data will be entered into an excel database.

9.4 Data Management

a) Data is entered into an excel spreadsheet
b) Study data will be reviewed regularly by the Investigator and Study Coordinator for the following:
    a. Participant inclusion criteria has been met
    b. Transcription of data is accurate and complete
    c. Units of measure are recorded appropriately
c) Details of the study treatments including treatment parameters, such as percent of MT for each treatment, will be retained within the TMS device by the TrakStar software. In addition, a daily summary of the treatment will be created and stored in the Participant's source document.
d) Case files will be created for each Participant where completed visit CRFs will be stored. Any adverse event CRFs will also be stored in the case file.

9.5 Data Security and Confidentiality

a) Non-electronic source document data will be stored in a locked cabinet in a secure office. Only authorized study staff will have access.
b) Electronic data will be stored on a secure database. Only authorized users will have access. All users will have unique identifiers and passwords. Sharing of log-in information is not permitted.

9.6 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include Participant case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted], whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by the Mayo Clinic Office of Research Regulatory in accordance with standard operating procedures.

10.2 Auditing and Inspecting

The sponsor-investigator will permit study-related monitoring, audits, and inspections by the IRB, the monitor, and government regulatory agencies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The sponsor-investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

Participation as a sponsor-investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

Although this study is considered more than minimal risk to adolescents, TMS treatments (1 Hz, 10 Hz, cTBS, and iTBS) offer a potential direct benefit for the individual Participant. In addition, the schedule of mood assessments and daily assessment during the interventional portion of the study for any adverse events has been developed for the purpose of monitoring the Participant's well-being.

This protocol and any amendments will be submitted to a properly constituted local IRB, in agreement with local legal prescriptions, for formal approval of the study at each participating site. The decision of the IRB concerning the conduct of the study at MUSC will be documented in writing to the regulatory sponsor before commencement of this study.

All families and Participants for this study will be provided a consent/assent form describing this study and providing sufficient information for Participants to make an informed decision about their participation in this study. This consent/assent form will be submitted with the protocol for review and approval by the site IRB for the study. The formal consent of a Participant, using the approved IRB consent/assent form, must be obtained before that Participant undergoes any study procedure. This form must be signed and dated by the Participant, the Participant's legally-authorized representative, and the individual obtaining informed consent/assent.

12 Study Finances

12.1 Funding Source

The study will be funded by a National Institute of Mental Health Grant 1R01MH113700. Neuronetics, Inc. will provide grant-in-kind support for NeuroStar magnetic stimulators and disposable SenStar Shields.

12.2 Participant Stipends or Payments

Participants will be remunerated $10 per visit to assist with costs related to travel.

13 Publication Plan

The PI/Sponsor (Dr. Croarkin) holds the primary responsibility for publications of results from the proposed study. The PI/Sponsor will register the study on ClinicalTrials.gov (*https://register.clinicaltrials.gov/*) prior to participant recruitment and enrollment. Results will be posted to ClinicalTrials.gov within 12 months of final data collection form the primary outcomes.

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15. Appendix A Concomitant Medication List

CONCOMITANT MEDICATION LIST

This list should not be considered exhaustive. Drugs that are categorized into one of the drug categories listed in the table below will be treated similarly.

CLASS: Drug	PRN	Chronic
ANTIASTHMA DRUGS		
Theo-Dur (theophylline)	**N**	**N**
Bronchodilators		
Alupent (metaproterenol), Proventil (albuterol), Ventolin (albuterol)	**Y**	**Y**
Steroids		
Inhaled (Beclovent [beclomethasone], Azmacort [triamcinolone], Vanceril [beclomethasone])	**Y**	**Y**
Oral	**Y**	**Y**
Leukotriene antagonists		
Accolate (zafirlukast), Zyflo (zileuton), Singulair (monoleukast)	**N**	**Y**
ANTICOAGULANTS		
Coumadin (warfarin)	**N**	**Y** (w/ stable protime)
ANTICHOLINGERGICS		
Cogentin (benztropine), Artane (trihexyphenidyl), etc.	**N**	**N**
Phenylpropanolamine (PPA) and pseudoephedrine	**N**	**N**
ANTIHISTAMINE and DECONGESTANTS		
Sedating		
Anticholinergic (Benadryl [diphenhydramine], chlorpheniramine, brompheniramine, Atarax [hydroxyzine]) (not for psychiatric indications)	**N**	**N**
Non-sedating		
Allegra (fexofenadine), Claritin (loratadine), Zyrtec (cetirizine)	**Y**	**Y**
Hismanal (astemizole)	**N**	**N**
ANTI-INFECTIVE AGENTS		
Antibacterial antibiotics (Penicillin, sulfa, etc.)	**Y**	**Y**
Antifungal agents *(ketoconazole, fluconazole, etc.)*		
Topical creams and ointments	**Y**	**Y**
Oral antifungals (single-dose for vaginitis OK)	**Y**	**N**
Antiviral agents		
Zovirax (acyclovir), Famvir (famcyclovir), Valtrex (valacyclovir)	**Y**	**Y**
Antiretroviral drugs (e.g. AZT, protease inhibitors)	**N**	**N**
ANTI-NEOPLASTIC AGENTS	**N**	**N**
SKELETAL MUSCLE RELAXANTS		
Lioresal (baclofen), Flexeril (cyclobenzaprine), Parafon Forte (chlorzoxazone), Robaxin (methocarbamol), Soma (carisoprodol)	**N**	**N**

CLASS: Drug	PRN	Chronic
ANALGESICS AND ANTIPYRETICS		
Nonsteroidal anti-inflammatory agents (aspirin, ibuprofen, naproxen)	**Y**	**Y**
Opiate Agonists / Partial Agonists (including Ultram [tramadol])	**N**	**N**
Miscellaneous analgesics & antipyretics (acetaminophen, paracetamol)	**Y**	**Y**
CARDIOVASCULAR DRUGS (All drugs in this category must be stable for at least 3 months)		
CARDIAC DRUGS		
Calcium Channel Blockers for coronary disease or angina (see also "Antihypertensives") (Calan [verapamil], Cardizem [diltiazem], Norvasc [amlodipine], Procardia [nifedipine])	**N**	**Y**
Antiarrhythmics		
Cordarone (amiodarone), lidocaine (note: local anesthesia injection OK), Mexitil (mexiletine), Norpace (disopyramide), Procan (procainamide), quinidine, Tambocor (flecainide)	**N**	**N**
Digoxin (blood levels must be monitored)	**N**	**Y**
ANTI-HYPERTENSIVE AGENTS (All anti-hypertensives must be stable dose x 1 month)		
ACE Inhibitors (Vasotec, Capoten, Zestril)	**N**	**Y**
Angiotensin receptor blockers (Cozaar)	**N**	**Y**
Calcium Channel Blockers	**N**	**Y**
Diuretics	**Y**	**Y**
Phentolamine	**N**	**N**
Beta-Adrenergic Blockers *(see also* ***Anti-Migraine****)*		
Low lipid solubility/CNS penetration (Corgard [nadolol], Tenormin [atenolol])	**Y**	**Y**
High lipid solubility/CNS penetration (Inderal [propranolol], Lopressor [metaprotereno]), (low lipid solubility preferred)	**Y**	**Y**
Peripherally-acting alpha adrenergic agents		
Cardura (doxazosin), Hytrin (terazosin), Minipress (prazosin)	**Y**	**Y**
Centrally-acting alpha adrenergic agents		
Aldomet (methyldopa), Catapres (clonidine)	**N**	**N**
All others *(quanethidine, reserpine, etc.)*	**N**	**N**
ANTILIPEMIC AGENTS		
Niacin (no slow-release preparations), Lipitor (atorvastatin), Lopid (gemfibrozil), Mevacor (lovastatin), Zocor (simvastatin)	**N**	**Y**
VASODILATING AGENTS		
Hydralazine	**N**	**Y**
minoxidil (topical OK)	**N**	**C**
ANTI-CONVULSANTS	**N**	**N**

CLASS: Drug	PRN	Chronic
PSYCHOTHERAPEUTIC AGENTS		
Antidementia drugs (donepezil, etc.), Antidepressants, Antipsychotics (Thorazine, Prolixin, etc.), Miscellaneous Psychotherapeutic Agents	**N**	**N**
Anxiolytics (per protocol)	**Y**	**N**
Stimulants	**N**	
Sedatives and Hypnotics		
Barbiturates, Benzodiazepines (except those allowed per the protocol), Other hypnotic agents	**N**	**N**
Ambien (zolpidem; per protocol), chloral hydrate (per protocol), Sonata (zaleplon; per protocol), Lunesta (eszopiclone; per protocol), Rozerem (ramelteon; per protocol)	**Y**	**N**
ANTIMANIC AGENTS		
Anticonvulsants, lithium	**N**	**N**
HORMONES & SYNTHETIC SUBSTITUTES		
Corticosteroids (methylprednisolone, prednisone)	**Y**	**N**
Mineralocorticoids (Florinef [fludrocortisone])	**N**	**N**
Topical steroids (creams, ointments, eyedrops)	**Y**	**Y**
pregnenolone	**N**	**N**
Androgens	**N**	**N**
DHEA, testosterone, danazol	**N**	**N**
Contraceptives		
Hormonal *(dose must be stable for at least 3 months)*: Oral, Vaginal Ring, Patch, Injections, Norplant (levonorgestrel)	**N**	**Y**
Intrauterine device	**Y**	**Y**
Latex condom, diaphragm and or cervical cap with spermicide	**Y**	**Y**
Estrogen replacement *(postmenopausal, stable dose for ≥3 months)*	**N**	**Y**
Thyroid *(stable dose for ≥3 months and thyroid function tests within normal limits. Clinical significance to be determined by the physician)*	**N**	**Y**
ANTIDIABETIC AGENTS		
Oral hypoglycemic agents	**N**	**Y**
Insulin's	**Y**	**Y**
DERMATOLOGIC AGENTS		
Topical steroid preparations, Rogaine (topical minoxidil), Miscellaneous creams, ointments, etc.	**Y**	**Y**
VITAMINS	**Y**	**Y**
OVER-THE-COUNTER SUPPLEMENTS		
chromium picolinate, DHEA, melatonin, pregnenolone, St. John's Wort	**N**	**N**

CLASS: Drug	PRN	Chronic
GASTROINTESTINAL DRUGS		
Cisapride (propulsid)	**Y**	**Y**
Antiemetics		
Over-the-counter (e.g., Emetrol [dextrose], Coke syrup)	**Y**	**Y**
Phenothiazines & related drugs (Compazine [prochloroperazine], Phenergan [promethazine], Reglan [metoclopramide], Tigan [trimethobenzamide]), Anzemet (dolasetron), Kytril (granisetron), Zofran (ondansetron)	**Y**	**N**
Marinol (dronabinol),	**N**	**N**
Antacids		
AlternaGel (aluminum hydroxide), Amphojel (aluminum hydroxide), Basaljel (aluminum carbonate), Bicitra (sodium citrate), Maalox (calcium carbonate), MagOX (magnesium oxide), Mylanta (magnesium hydroxide), Tums (calcium carbonate), UroMag (magnesium oxide)	**Y**	**Y**
Antidiarrhea agents		
Arco-Lase (trizyme), Furoxone (furazolidone), Motofen (difenoxin), Sandostatin (octreotide acetate)	**N**	**N**
Imodium (loperamide), Lomotil (diphenoxylate), Pepto-Bismol (bismuth subsalicylate)	**Y**	**N**
Cathartics & Laxatives		
Colace (docusate), enemas, Metamucil, Perdiem, Senokot, Soflax, Unifibre	**Y**	**Y**
Dialose (docusate)	**Y**	**Y**
Antisecretory agents		
H2 Blockers	**N**	**N**
Axid (nizatidine), Pepcid (famotidine), Zantac (ranitidine)	**Y**	**Y**
Proton pump inhibitors		
Prevacid (lansoprazole), Prilosec (omeprazole)	**Y**	**Y**
Miscellaneous gastrointestinal drugs		
Carafate (sucralfate)	**Y**	**Y**
Reglan (metoclopramide)	**N**	**N**
Levsin (L-hyoscyamine sulfate)	**Y**	**Y**
ANTI-MIGRAINE		
DHE 45 (dihydroergotamine), Imitrex (sumatriptan), methysergide, Midrin (isometheptene mucate), Zomig (zolmitriptan)	**N**	**Y**
Fiorinal (butalbital, ASA, caffeine), Cafergot (ergotamine)	**Y**	**Y**
Nonsteroidal anti-inflammatory drugs (ibuprofen, naproxen, Toradol, etc.)	**Y**	**Y**
Beta-blocking drugs		
Corgard (nadolol), Tenormin (atenolol), Other beta-blockers (e.g., Inderal [propranolol])	**Y**	**Y**
SKIN & MUCOUS MEMBRANE AGENTS	**Y**	**Y**

CLASS: Drug	PRN	Chronic
MISCELLANEOUS DRUGS		
Accutane, All over-the-counter diet pills, Ionamin (phentermine resin), Lioresal (baclofen), Wellbutrin (bupropion), Zyban (bupropion)	**N**	**N**
Allopurinol, colchicine	**Y**	**Y**
Fosamax (alendronate)	**N**	**Y**
Gold compounds, hydroxychloroquine, and methotrexate for severe arthritis	**N**	**Y**
Smoking cessation aids (Nicorette, nicotine patches)	**Y**	**Y**
Chantix (varenicline)	**N**	**N**
ENDNOTES		
N = Never to be used in this study. Y = Can be used in this study for either PRN or Chronic use as indicated in the table.		
C = Call for Sponsor approval. Approval for these medications is generally granted if the patient is otherwise acceptable, including stable medical condition, no undue risk, no other "marginal" considerations in the medical history or concomitant medications. Or may be used during the long-term maintenance phase with certain restrictions.		

16 Appendix B ICF and TMS Measures

Collection of ICF and TMS Neurophysiology Measures

1) The subject is greeted and seated comfortably.

2) Details of the subject's session will be recorded in the study file.

3) Subjects, technicians, and the study physician (who will always be present) will be provided with earplugs, which are to be worn at all times. Subjects will be instructed to turn off all cell phones and pagers. Eyeglasses will be removed. Subjects should not have any metal above their neckline (with the exception of orthodontics), specifically hairpins or large metallic earrings. Subjects will be instructed to sit quietly with their eyes open throughout the measurements.

4) The TMS coil will be wiped with an alcohol swab before and after each session.

5) EMG set up includes a 1k gain, HPF 30, and LPF 500 or 1000. When the operator is doing calibration setting, the isolated bioamp must be on the lowest setting. When collecting data the operator must ensure that the switch of the bioelectric amplifier is on.

Connecting EMG Leads:

a. EMG leads are connected to the right hand.
b. The white wire goes in the red circle.
c. The black wire goes in the green circle.
d. The green wire goes in the white circle (this is the ground).
e. Place one electrode on abductor pollicis brevis (white wire).
f. Place one lead on the middle joint of the index finger (black wire).
g. Place ground lead on the hand that you are measuring (green wire).
h. On the computer, the red line refers to the right hand.

6) At the start of each session, the operator must check the plastic casing of the stimulating coil for any signs of external damage. If any cracks are visible in the housing, the stimulating coil must not be used and should be serviced.

7) Connect the coil to the main Magstim unit. Switch on, select the desired stimulating power by using the output power knob, and press the run switch on the front panel of the Magstim to arm the unit. The Magstim will charge and the "ready" display will be illuminated.

8) Position the stimulating coil on the desired area to be stimulated. Press and hold down one or both of the safety switches at the baseline of the stimulating coil. Triggering the Magstim results in a magnetic pulse being delivered by the stimulating coil, stimulating the nerves beneath it.

9) When necessary, reposition the stimulating coil and/or modify the stimulating power level to suit the requirements for the next stimulus. Meanwhile, the Magstim will have recharged and can be triggered once again in the normal manner.

10) When the tests have been completed, press the "stop" switch on the front panel of the Magstim and replace the stimulating coil on the coil hanger. The Magstim will then revert to standby mode and cannot be triggered until it is armed once more.

11) The TMS coil is held tangential to the subject's head at 45 degrees.

12) Single-pulse measures (MT and CSP):

    a. When giving pulses, always wait 5-10 seconds between pulses.
    b. Start at 30% intensity and administer a few pulses to acclimate the subject to the test conditions.
    c. Increase to 40% intensity and then by 10 at a time to get motor responses, and then decrease intensity.
    d. Administer 10 successive pulses at each intensity. If 6 of 10 pulses are not sufficient, this is the threshold point. If 5 of 10 pulses elicit a motor response, decrease the intensity until 6 of 10 do not elicit a motor response.
    e. Measure optimal site laterally and posterior from the vertex.
    f. Single-pulse measures are performed with submaximal, tonic, hand muscle contraction. This reduces variability in the CSP (Mathis et al. 1998, Bajbouj et al. 2006).
    g. The duration of the CSP is measured from the onset of the corticospinal mediated EMG response, to the end of the silent period. The end is the point at which averaged tonic EMG activity reaches the amplitude of mean EMG activity prior to TMS.

13) Paired-pulse measures (ICI and ICF):

    a. The figure of eight coil and the bistim module are utilized for these measurements.
    b. Two stimulations are given in succession, as described previously (Kujirai et al. 1993, Nahas et al. 2007). The first pulse is the conditioning stimulus. The second pulse is the test pulse.
    c. Stimulations for ICI are spaced 1-5 msec apart, and 100-200 msec apart.
    d. Stimulations for ICF are spaced 7-20 msec apart.
    e. Complete 10 trials of the test stimuli, each administered 10 seconds apart. Peak to peak amplitudes of the conditioned response are averaged and expressed as a percentage of the average of the test response amplitudes.