**Examining the mechanisms of anxiety regulation using a novel, sham-controlled, fMRI-guided rTMS protocol and a translational laboratory model of anxiety.**

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**NIH Grant Number** 1K01MH121777-01

**IRB Number:** 833320

**ClinicalTrials.gov Number** NCT03993509

**Current version** 06/21/2022 V-1.1
**Previous Versions** 10/28/2021 V-1.0
03/24/2021 V-0.9
10/05/2020 V-0.8
03/19/2020 V-0.7
10/11/2019 V-0.5
09/12/2019 V-0.4
06/12/2019 V-0.3
02/18/2020 V-0.6

## Contents

## List of Abbreviations

adverse event (AE)
analysis of functional neuroimages AFNI
anxiety potentiated startle (APS)
anxiety-potentiated-BOLD AP-BOLD
bed nucleus of the stria terminalis (BNST)
case report form (CRF)
central nervous system (CNS)
cognitive paired-associate stimulation (C-PAS)
continuous theta-burst stimulation (cTBS)
dorsolateral prefrontal cortex (dlPFC)
electromyographic (EMG)
echo planar imaging (EPI)
Ethics Committee (EC)
Food and Drug Administration (FDA)
Fear potentiated startle (FPS)
first dorsal interosseus (FDI)
fear-potentiated-BOLD (FP-BOLD)
Health Insurance Portability and Accountability Act of 1996 (HIPAA)
high-frequency rTMS (HF-rTMS)
independent components analysis (ICA)
intermittent theta-burst stimulation (iTBS)
intertrial interval (ITI)
low-frequency rTMS (LF-rTMS)
motor evoked potential (MEP)
motor threshold (MT)
magnetic resonance imaging (MRI)
National Institute for Neurological Disorders and Stroke (NINDS)
neutral (N)
neutral predictable unpredictable threat task (NPU)
principle investigator (PI)
predictable shock (P)
protected health information (PHI)
repetitive TMS (rTMS)
serious adverse event (SAE)
echo time (TE)
Beck Anxiety Inventory (BAI)
State Anxiety Scale (S-Anxiety)
transcranial magnetic stimulation (TMS)
TMS adult safety screen (TASS)
Theta-burst stimulation TBS
unanticipated problem (UP)
unpredictable shock (U)
working memory (WM)

## Study Summary

| | |
|---|---|
| **Title** | Examining the mechanisms of anxiety regulation using a novel, sham-controlled, fMRI-guided rTMS protocol and a translational laboratory model of anxiety. |
| **Short Title** | Effect of rTMS on anxiety |
| **IRB Number** | 833320 |
| **Phase** | Pilot Study |
| **Methodology** | Randomized, double-blinded, sham-controlled, cross-over design |
| **Study Duration** | 4 years |
| **Study Center(s)** | Single-center |
| **Objectives** | **Aim 1:** Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety using the unpredictable shock-threat paradigm.<br><br>**Aim 2:** Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety-related WM-deficits using the Sternberg WM paradigm during threat of shock.<br><br>**Aim 3:** Demonstrate target engagement by measuring BOLD responses evoked by TMS pulses to the right dlPFC during threat of shock. |
| **Number of Subjects** | 75 |

| | |
|---|---|
| **Main Inclusion and Exclusion Criteria** | Healthy volunteers, between 18 – 50 years old, free of psychological and neurological conditions, free of contra-indications for TMS and MRI |
| **Statistical Methodology** | **Aim 1:** Outcome measures. Fear potentiated startle (FPS) and Anxiety potentiated startle (APS) Statistical Analysis. The main analysis will be a 3 [stimulation: pre-stimulation vs. post-active vs. post-sham] by 2 [protocol: cTBS vs. iTBS] repeated-measures ANOVA.<br><br>**Aim 2:** Outcome measures. Accuracy and reaction time during the Sternberg Working memory paradigm. Statistical Analysis. The main analysis will be a 3 [stimulation: pre-stimulation vs. post-active vs. post-sham] by 2 [protocol: cTBS vs. iTBS] repeated-measures ANOVA.<br><br>**Aim 3:** Outcome measures. TMS-evoked BOLD activity during periods of acute fear and sustained anxiety. Statistical Analysis. BOLD maps will then be analyzed using a paired-sample t-test to compare BOLD responses evoked by active TMS and those evoked sham scalp stimulation. |
| **Data and Safety Monitoring Plan** | The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems (UPs), adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with IRB requirements and federal regulations. |

## BACKGROUND AND STUDY RATIONALE

This study will be conducted in full accordance all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including [as applicable include the following regulations as they apply 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and Good Clinical Practice. All episodes of noncompliance will be documented.

### Introduction

#### *1.1 Background and Relevant Literature*

Although anxiety disorders are the most commonly diagnosed class of disorders, with 1 in 5 individuals diagnosed annually, less than 50% of these patients receive treatment, and less than 15% receive minimally adequate treatment (Kessler & Chiu, 2005). Although extensive research has linked subcortical structures like the amygdala and bed nucleus of the stria terminalis (BNST) to the hyperarousal symptoms experienced by anxiety patients, hyperarousal is only one facet of the symptom profile shared across anxiety disorders. Much less is known about the cognitive symptoms (i.e. difficulty concentrating) experienced by anxiety patients. Given the low level of understanding of basic anxiety mechanisms, it is understandable that the most commonly used pharmacological treatments (e.g. benzodiazepines and SSRIs) for anxiety were developed more than 20 years ago, with little advancement since. Accordingly, there is a critical need for mechanistic research into the CNS mechanisms that mediate the cognitive symptoms experienced by anxiety patients. Without such research, treatment development for these disorders will continue to be slow.

TMS is approved by the FDA as a second-line treatment for depression (Horvath, Mathews, Demitrack, & Pascual-Leone, 2010; O'Reardon et al., 2007; Shajahan et al., 2002; Stern, Tormos, Press, Pearlman, & Pascual-Leone, 2007), and some studies have shown that TMS can reduce comorbid symptoms of anxiety in depressed individuals (Mantovani, Aly, Dagan, Allart, & Lisanby, 2013; O'Reardon et al., 2007; White & Tavakoli, 2015). TMS uses a strong and alternating magnetic field at the scalp to induce electrical currents in neurons just below the TMS coil. Theta-burst stimulation (TBS), where high frequency bursts (50 hz triplets) are delivered at the theta frequency (5 Hz), is capable of altering the resting membrane potentials of local neurons (Lefaucheur et al., 2014). Continuous TBS (cTBS) tends to decrease local excitability, while intermittent TBS (iTBS) tends to increase local excitability (Lefaucheur et al., 2014). TMS treatment protocols for depression are based on the hypothesis that the left dlPFC is associated with positive thoughts, and is hypoactive in depression (Berman, Doran, Pickar, & Weinberger, 1993; Galynker et al., 1998; Miller, Crocker, Spielberg, Infantolino, & Heller, 2013), while the right dlPFC is associated with negative thoughts and is hyperactive in depression (valence model) (Davidson, 2004). Thus, successful rTMS treatments for depression typically involve either excitatory rTMS to the left dlPFC or inhibitory rTMS to the right dlPFC (Chen et al., 2013; Concerto et al., 2015; Fox, Buckner, White, Greicius, & Pascual-Leone, 2012; Horvath et al., 2010; Lefaucheur et al., 2014; Miniussi et al., 2005; Nauczyciel et al., 2011; O'Reardon et al., 2007; Shajahan et al., 2002; Stern et al., 2007) (or both), however other paradigms have also shown benefit (e.g. excitatory of both right and left dlPFC).

There is evidence that rTMS treatment protocols (based on the valence model) may be effective at treating anxiety symptoms comorbid with depression. For instance, excitatory stimulation to the left dlPFC (O'Reardon et al., 2007), inhibitory stimulation to the right dlPFC (Mantovani et al., 2013), and combined *right* inhibitory rTMS and *left*

excitatory rTMS to the dlPFC (White & Tavakoli, 2015) can reduce anxiety or panic in depressed individuals. However, several recent meta-analyses have concluded that there is little support for the efficacy of these treatments for anxiety reduction in non-depressed individuals (Lefaucheur et al., 2014; Pallanti & Bernardi, 2009; Pigot, Loo, & Sachdev, 2008; Zwanzger, Fallgatter, Zavorotnyy, & Padberg, 2009). One possible reason for this lack of support is that the underlying neuropathology mediating generalized anxiety and depression-related anxiety is fundamentally different (Baeken et al., 2010; Moulier et al., 2016; Zwanzger et al., 2014), suggesting a need for the development of novel, anxiety-specific rTMS treatment parameters. For instance, although excitatory stimulation to the left dlPFC facilitates retrieval of positive information in high anxious individuals (Balconi & Ferrari, 2012, 2013a, 2013b), excitatory stimulation to the left dlPFC does not reduce anxiety in non-depressed individuals (Moulier et al., 2016). Similarly, treatment with inhibitory stimulation to the right dlPFC enhances rather than suppresses subsequent visual processing of fearful stimuli (Zwanzger et al., 2014), while excitatory stimulation to the right dlPFC reduces subsequent amygdala activity to negative faces (Baeken et al., 2010). Taken together, these results suggest that the valence model may not be sufficient to explain the symptoms of anxiety in non-depressed individuals.

Instead, we propose a novel hypothesis based on the frontoparietal attention network (FPN; Figure 1) (Cole, Repovš, & Anticevic, 2014). According to our model, 1) elevated state anxiety enhances bottom-up attentional processes, and 2) top-down cognitive control processes, mediated by the right dlPFC, counteract this enhancement, reducing anxiety. This model is an extension of the attention control theory, which states that anxious individuals cannot efficiently engage goal-directed attention (Eysenck, Derakshan, Santos, & Calvo, 2007). Support for this model comes from the observations that 1) the right dlPFC plays a key role in attention control (Basten, Stelzel, & Fiebach, 2012; Cieslik et al., 2013; Fales et al., 2008; Harding, Yücel, Harrison, Pantelis, & Breakspear, 2015; Peers, Simons, & Lawrence, 2013), and 2) anxiety patients are impaired in their ability to recruit this structure for cognitive control (Armstrong, Zald, & Olatunji, 2011; Berggren & Derakshan, 2013a, 2013b; Braver, Cole, & Yarkoni, 2010; Coombes, Higgins, Gamble, Cauraugh, & Janelle, 2009; Derryberry & Reed, 2002; Eysenck et al., 2007; Grillon, Robinson, Mathur, & Ernst, 2015; Morrison & Heimberg, 2013; Najmi, Kuckertz, & Amir, 2012; Najmi, Amir, Frosio, & Ayers, 2014; Price, Eldreth, & Mohlman, 2011; Reinholdt-Dunne, Mogg, & Bradley, 2009, 2012). This model is also supported by recent studies and preliminary data from our lab that suggest, 1) cognitive tasks that occur during periods of elevated state anxiety recruit the right dlPFC, 2) tasks that engage the right dlPFC reduce state anxiety (Balderston, Quispe-Escudero, et al., 2016; Vytal, Cornwell, Arkin, & Grillon, 2012; Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013), 3) anxiety patients are less able to recruit the right dlPFC during demanding cognitive tasks (Balderston, Vytal, et al., 2016). Therefore, according to our model, we expect that enhancing activity in the right dlPFC with excitatory iTBS should facilitate top-down cognitive control processes associated with anxiety reduction. In addition, data suggest that activating the stimulation site via task engagement can facilitate the effect of rTMS (Luber et al., 2007, 2008, 2013), suggesting that rTMS during a challenging cognitive task may be more effective at anxiety reduction than rTMS alone.

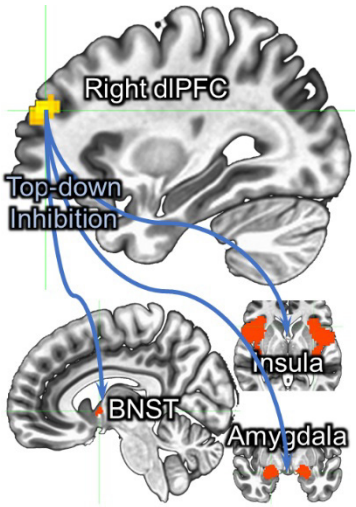


**Figure 1.** R dlPFC may regulate anxiety through top-down inhibition of regions in the fear/anxiety circuit.

This research is significant because upon completion, we expect to have direct evidence for a causal role of the right dlPFC in anxiety regulation, complete with evidence

of target engagement through simultaneous TMS/fMRI, and a novel application to anxiety. This research will lay the groundwork for my long-term career objective, which is to develop an independent, NIH-funded research program employing psychophysiology, neuroimaging, and neuromodulation in healthy and anxious participants aimed at developing a circuit-level understanding of the neurobiological mechanisms underlying anxiety.

# 2 Study Objectives

**Aim 1:** Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety using the unpredictable shock-threat paradigm. Our hypothesis, consistent with our pilot data, is that strengthening circuits in the right dlPFC will facilitate anxiety regulation. Accordingly, we expect excitatory but not inhibitory TBS (compared to sham) to reduce induced anxiety.

**Aim 2:** Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety-related WM-deficits using the Sternberg WM paradigm during threat of shock. Our hypothesis is that the rTMS will strengthen right dlPFC circuits, which will rescue the anxiety-related WM deficits. Accordingly, we expect that 10 Hz rTMS (compared to sham) will improve accuracy on the Sternberg WM task during threat.

**Aim 3:** Demonstrate target engagement by measuring BOLD responses evoked by TMS pulses to the right dlPFC during threat of shock. Our hypothesis is that direct stimulation of the right dlPFC will reduce ongoing anxiety-related activity. Accordingly, we expect active but not sham TMS pulses to the right dlPFC to evoke BOLD deactivations in regions of the fear/anxiety network (i.e. amygdala, BNST, anterior insula).

# 3 Investigational Plan

## *3.1 General Design*

Aims 1 and 2 of this project will be tested using a between-subjects design where 1 group of healthy volunteers will receive 4-day courses of active and sham cTBS stimulation to the right dlPFC, while the other group will receive 4-day courses of active and sham iTBS stimulation to the right dlPFC. In Aim 1, we will test the effects of this stimulation on anxiety during the NPU threat task. In Aim 2, we will test the effects of this stimulation on anxiety-related WM deficits using the Sternberg WM paradigm. In addition, we will also administer several self-report questionnaires to assess their baseline (pre-stimulation) anxiety. Subjects will be randomly assigned to groups, and the order of stimulation (active vs. sham) will be counterbalanced across subjects. Aim 3 will be tested using a within-subjects design where this same group of subjects will receive single-pulse active and sham stimulation to the right dlPFC during the NPU threat task while in the MRI scanner. BOLD activity to the TMS pulses will be the primary outcome measure.

Subjects will undergo 12 study visits spaced over a 4-week period (Figure 3). Subjects may also be offered an optional 13$^{th}$ visit where TMS/fMRI is conducted. On Week 1,

subjects will undergo a pre-stimulation behavioral visit where they complete the NPU threat task and the Sternberg WM paradigm, and a pre-stimulation MRI visit where they undergo all the structural and functional imaging needed to identify the optimal target of stimulation for the course of TMS used in Aims 1 and 2. This information will be used to perform neuronavigation during the study visits to ensure accurate target stimulation. Subjects will complete the TMS/fMRI scans needed for Aim 3 during the optional visit. On Weeks 2 and 4, subjects will undergo 5 visits. The first 4 visits will be stimulation visits where they receive either active or sham stimulation while doing the Sternberg WM task. On the 5th visit, they will return for the post-stimulation test visit, where they will complete the NPU threat task and the Sternberg WM paradigm.

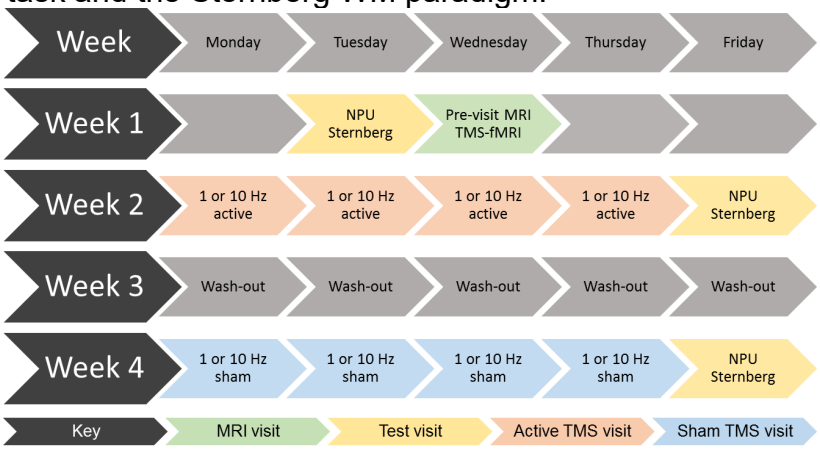


**Figure 3:** Timeline showing subject visits in the proposed project.

### *3.1.1 Screening Phase*

Participants will be recruited from the community via paper or web ads, flyers, and listserv announcements. Participants will be recruited without any preference based on gender, race, religion, or other social variables. All recruitment materials (paper or web ads, flyers and listserv announcements) will be IRB approved prior to use.

Study members authorized by the IRB will obtain informed consent from the participant. All study investigators obtaining informed consent complete formal informed consent training. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. Consent forms will be signed in the presence of a witness.

Subjects will be informed that they can withdraw from the study whenever they wish. The experiment will also be stopped for any subject who exhibits signs of distress during any phase of the study. Subjects will be constantly monitored by the study staff. The subject will be withdrawn at any time if unable to follow the rules for participation in this study. In the event of unanticipated problems or serious side effects, the principal investigator will consider whether the study should continue.

## *3.2 Study Endpoints*

**Aim 1:** Determine the effect of a 1-week course of rTMS TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety using the unpredictable shock-threat paradigm. Our hypothesis, consistent with our pilot data, is that strengthening circuits in the right

dlPFC will facilitate anxiety regulation. Accordingly, we expect excitatory but not inhibitory TBS (compared to sham) to reduce induced anxiety.

**Aim 2:** Determine the effect of a 1-week course of rTMS TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety-related WM-deficits using the Sternberg WM paradigm during threat of shock. Our hypothesis is that the rTMS will strengthen right dlPFC circuits, which will rescue the anxiety-related WM deficits. Accordingly, we expect that iTBS (compared to sham) will improve accuracy on the Sternberg WM task during threat.

**Aim 3:** Demonstrate target engagement by measuring BOLD responses evoked by TMS pulses to the right dlPFC during threat of shock. Our hypothesis is that direct stimulation of the right dlPFC will reduce ongoing anxiety-related activity. Accordingly, we expect active but not sham TMS pulses to the right dlPFC to evoke BOLD deactivations in regions of the fear/anxiety network (i.e. amygdala, BNST, anterior insula).

## 4 Study Population and Duration of Participation

Participants will be recruited without any preference based on gender, race, religion, or other social variables, and participants will not be excluded on the basis of sex/gender, racial, or ethnic affiliation. We exclude pregnant women because of the unknown effects of the TMS pulses, shocks, and fMRI on the developing fetus. We exclude non-English speakers since not all the instruments and tests we use are translated and validated in Spanish or other languages.

### *4.1 Inclusion Criteria*

- Subjects must be 18-50 years old
- Able to give their consent
- Right-handed

### *4.2 Exclusion Criteria*

- Non-english speaking
- Any significant medical problems
- Current or past Axis I psychiatric disorder(s),
- Active or history of active suicidal ideation
- Alcohol/drug problems in the past year or lifetime alcohol or drug dependence
- Medications that act on the central nervous system
- History of seizure
- History of epilepsy or other neurological problems
- Increased risk of seizure for any reason
- Pregnancy
- Any medical condition that increases risk for fMRI or TMS
- Any metal in their body which would make having an MRI scan unsafe
- Any sort of medical implants
- Hearing loss

- Claustrophobia

### *4.3 Subject Recruitment*

Participants will be recruited from the community via paper or web ads, flyers, and listserv announcements. Participants will be recruited without any preference based on gender, race, religion, or other social variables. All recruitment materials (paper or web ads, flyers and listserv announcements) will be IRB approved prior to use.

In order to facilitate the enrollment of interested subjects in multiple studies at the Center for Neuromodulation in Depression and Stress (CNDS), we utilize a general pre-screening form (either administered by phone or self-report depending on participant preference) center-wide as the majority of our projects closely relate, in both purpose and eligibility criteria.

### *4.4 Duration of Study Participation*

The study will consist of 12 study visits and take place over a period of approximately 4 weeks depending upon subject availability. Subjects may also be offered 3 optional visits. TMS/fMRI will be conducted during the first optional visit. The NPU will be administered before and after cTBS during the second and third optional visits. If subjects choose to participate in the optional visits. Their study participation will be extended approximately 1 week, according to their schedule. Each session will last approximately 2-3 hours.

### *4.5 Total Number of Subjects and Sites*

The target number of completers will be N=56. We anticipate that about 20% of subjects (N=11) will terminate the study during the task because of discomfort and another 10% (N = 5) to withdraw due to scheduling issues. An additional 3 subjects per experiment (n=3) will be included for piloting.

### *4.6 Vulnerable Populations:*

All subjects must be able to provide their own consent. This study is above minimal risk and we do not want to enroll participants who do not understand the risk/benefit ratio of the study, particularly when there is no benefit to the participants. For this reason we exclude people with IQ lower than 80. We exclude and rule out pregnant women because of the unknown effects of the shocks and of the fMRI on the developing fetus. We exclude non-English speakers since not all the instruments and test we use are translated and validated in Spanish or other languages. Based on our experience, we made the decision that 50 would be the upper age limit to prevent age-related changes in brain structure and function from confounding our analyses. Patients will not be taken off medications for the purpose of the study.

## 5 Study Procedures

Does your study use MRI? (CAMRIS is the appropriate contact for all studies involving MRIs)

☒ Yes ☐ No

Check of all that apply:

☐ 1.5T MRI
☒ 3T MRI
☐ 7TMRI

Does the MRI use investigational sequences and/or coils?
(See Experimental Device Clause)

☒ Yes ☐ No ☐ Unsure (if unsure you need to contact CAMRIS)

**Experimental Device Clause** -- Some of the pulse sequences and/or RF coils are not FDA approved but are considered to pose no more than minimal risk.

Does your study include pregnant women?
(See Pregnancy Clause and Justification)

☐ Yes ☒ No

**Pregnancy Clause** -- Although there are no known risks related to MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no possible benefit from participating in this protocol for a pregnant woman, we will exclude pregnant women.

Does the MRI require the use of Contrast Agents?
(See Contrast Risks)

☐ Yes ☒ No

Does your study involve the exposure to radiation, radiotracers and/or radiological imaging modalities?

☐ Yes ☒ No (If No, no RRSC review is needed)

Will any of the radiation exposure result from procedures that are or could be performed solely as a result of a subject's participation in the research protocol?

☐ Yes ☒ No

Ultrasound

☐ Yes ☒ No

Will your study be using CT Scans? (CACTIS is the appropriate contact for studies involving CT scans)

☐ Yes ☒ No

### *5.1 Study Intervention Phase*

The following procedures will be carried out on each study visit. See section 5.1.7 for a detailed description of each procedure.

#### 5.1.1 Lab Visit 1

Consent
Motor threshold testing
White noise habituation
Shock workup
NPU
Sternberg working memory task

#### 5.1.2 MRI visit

Basic MRI scans

#### 5.1.3 TMS visits (1-4)

Either cTBS or iTBS

#### 5.1.4 Lab Visit 2

White noise habituation
Shock workup
NPU
Sternberg working memory task

#### 5.1.5 TMS visits (5-8)

Either cTBS or iTBS

#### 5.1.6 Lab Visit 3

White noise habituation
Shock workup
NPU
Sternberg working memory task

### 5.1.7 Optional MRI visit

NPU TMS/fMRI

### 5.1.8 Optional Pilot visits

NPU (1 run)
cTBS
NPU (1 run)

### 5.1.9 Procedures

**TMS motor threshold testing:** The resting motor threshold (MT) will be determined in order to find the safe TMS dosing level. MT is defined as the minimum magnetic flux needed to elicit a threshold EMG response in a target muscle in 5 out of 10 trials. MT is the standard in the field for measuring cortical excitability and to reduce seizure risk. MEPs for the contralateral first dorsal interosseus (FDI) muscle will be measured with EMG. The scalp region producing the largest amplitude MEP will be identified. At that scalp location, the lowest TMS intensity able to elicit 5 MEP's of ≥50μV in peak-to-peak amplitude in 10 trials at this site will be determined using a descending method of limits procedure.

**Sternberg working memory task:** On each WM trial, subjects will see a series of 4 letters presented singularly (encoding period) that will be followed by a brief interval where subjects are required to maintain these letters (maintenance period). At the end of the maintenance period, subjects will be prompted to make a response based on the task instructions (response period; Sternberg, 1966). The response prompt will consist of a letter and a number. The letter will be chosen from the study series, and the number will correspond to a position in the series. The subjects will indicate whether the position of the letter in the series matches the number.

There will be 2, 14 min runs of the experiment. Within each run, there will be 4 blocks (2 threat, 2 safe). Within each block, there will be 2 types of trials based on the delivery of startle probes. On each trial a startle probe will be presented either during the maintenance period or the ITI. There will be 6 trials per condition per block. There will be a total of 3 shocks per run presented at random points during the threat blocks.

**NPU Task:** The instructed fear paradigm that will be implemented uses administration of predictable and unpredictable shocks to generate phasic and sustained forms of potentiated startle. We will use the NPU threat procedure as described in (Schmitz & Grillon, 2012). The experiment consists of three different conditions: no shock (N), predictable shock (P), and unpredictable shock (U), each lasting approximately 150 sec. In the N condition, no shocks will be delivered. In the P condition, shocks will be administered predictably, that is, only in the presence of a threat cue. In the U condition, the shocks will be unpredictable. In each 150-sec condition, an 8-sec cue will be presented four times. The cues will be different geometric colored shapes in each

condition (e.g., a blue square for N, a red circle for P, and a green star for U) presented on a computer monitor in front of the subjects. The cue will signal the possibility of receiving a shock only in the P condition. It has no signal value in the N and U conditions. Instructions will also be displayed on a computer monitor to inform subjects of the nature of current condition by showing the following information throughout the testing procedure: “no shock” (N), “shock only during shape” (P), or “shock at any time” (U). During each predictable and unpredictable condition, one shock will be administered during the cue in the predictable condition and in the absence of a cue in the unpredictable condition.

There will be 2, 12 min runs during each test visit. Within each run, there will be 8 blocks (2 predictable, 2 unpredictable, 4 neutral). There are twice the number of neutral blocks to ensure that safe blocks alternate with threat (predictable or unpredictable) blocks. Startle probes will be presented either during the cue period, or the ITI. During the predictable and unpredictable blocks, there will be 2 trials per condition. Given that there will be twice as many neutral blocks, during these blocks there will only be 1 trial per condition. There will be a total of 3 shocks per run presented during either the ITI (unpredictable) or cue (predictable) periods.

**White noise habituation:** The acoustic startle stimuli will be a 40-ms burst of white noise (103 dB) with instantaneous rise time. Auditory stimuli will be delivered binaurally via headphones. The eyeblink component of the startle response will be measured by recording electromyographic (EMG) activity of the left orbicularis oculi muscle. The session will begin with a startle habituation phase with 9 startle stimuli presented alone to reduce startle reactivity.

**Shock workup:** Electric shocks will be delivered through two tin disk electrodes located on the median nerve of the right wrist with a current constant stimulator. During each test visit prior to the experiment, the subject will undergo a shock workup procedure to identify an appropriate shock level. During the workup, subjects will receive brief (100 ms) presentations of the shock starting at a low level (2 mA), which will be increased in intensity gradually, until it reaches a level that they rate as uncomfortable but tolerable. Shocks will be administered at that level during the visit. The subject will also be informed that they are free to withdraw from the experiment if they later determine that the shock level is too high.

**Self-report Questionnaires:** Subjects will be asked to fill out self-report questionnaires during the experiments (i.e. TMS adult safety screen (Keel, Smith, & Wassermann, 2000), the State Anxiety Scale (S-Anxiety)(Spielberger, 1987), the Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988), Retrospective Threat Questionnaire). During the test visits, subjects will be asked to retrospectively rate their fear and anxiety levels using an analog scale during each condition (Retrospective Threat Questionnaire). Retrospective measures of fear and anxiety will be collected on an analog scale ranging from 0 (not at all anxious) to 10 (extremely anxious). This analog scale has been a reliable measure of anxiety in our past studies. The State Anxiety Scale (S-Anxiety) (Spielberger, 1987) a 20-item self-administered rating scale with measures of mood.

**cTBS:** Subjects will receive a continuous train of triplet 50 Hz bursts, repeated at 5 Hz. They will receive a total of 600 pulses per session. Consistent with the iTBS condition, TMS will occur during the Sternberg WM paradigm.

**iTBS.** Subjects will receive 20 trains of triplet 50 Hz bursts, repeated at 5 Hz; 2 s on and 8 s off for a total of 600 pulses per session and a total duration of 3 min. and 9 s). Stimulation will occur while subjects are doing the Sternberg WM paradigm, which should facilitate the efficacy of the stimulation by taking advantage of the cognitive paired-associate stimulation (C-PAS) principle (Luber et al., 2017). The timing of the Sternberg task will be jittered so that each rTMS train will be administered during the maintenance interval of a WM trial.

**Basic scans**: During the MRI visit, we will collect at T1, a T2, and a diffusion-weighted echo-planar image using an available 3T scanner. Next subjects will undergo a two runs of the Sternberg task, without shocks, probes, or TMS pulses to serve as functional localizers to identify a dlPFC. Subjects may also undergo a resting state scan.

**NPU TMS/fMRI.** A Magventure MagPro 100X stimulator with a MRi-B91 figure-8 coil will be used. The TMS coil will be placed on the head over the target. TMS intensity will be 100% of the motor threshold (MT). For sham stimulation, a 4 cm spacer will be placed between the subject's scalp and the TMS coil. As with the study visits, subjects will have Neutral, Predictable, and Unpredictable periods. During the neutral periods, they will be safe from shocks. During the predictable periods, they can receive shocks but only when there is a cue present. During the unpredictable periods, they will be at risk for shock during the entire duration of the block. Rather than probing their ongoing fear and anxiety with the startle probes, we will replace the startle probes with single TMS pulses to the right dlPFC. This will allow us to causally examine the effect of right dlPFC activity (induced by the TMS pulse) on the neural activity that mediates fear (during the predictable cue) and anxiety (during the unpredictable cue and ITI). Importantly, by replacing the startle probes with TMS pulses, it will be possible to directly compare the TMS-evoked BOLD responses to the pattern of startle responses collected during the MRI/pre-stimulation visit. Subjects will have 1 run of active stimulation and one run of sham stimulation. Subjects may receive an additional 1 run of active stimulation at a control site in over the parietal cortex.

### *5.2 Subject Withdrawal*

Subjects will be informed that they can withdraw from the study whenever they wish. Sample shocks will be administered prior to the study. At this point, subjects will be explicitly asked if they wish to continue. The experiment will also be stopped for any subject who exhibits signs of distress during any phase of the study. The subject will be withdrawn at any time if unable to follow the rules for participation in this study.

### *5.3 Early Termination Visits*

If a subject is withdrawn from the study or decides to discontinue, they will be compensated for the visits they completed, and a note will be placed in their CRF.

# 6 Statistical Plan

## 6.1 Primary Endpoints

**Aim 1: Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety using the Neutral, Predictable, and Unpredictable (NPU) shock-threat paradigm.**

**Outcome measures.** Fear potentiated startle (FPS) will be calculated by subtracting the blink magnitude during the predictable inter-trial interval (ITI) from the predictable cue. Anxiety potentiated startle (APS) will be calculated by subtracting the blink magnitude during the neutral ITI from the unpredictable ITI. Online anxiety ratings will be processed similarly.

**Statistical Analysis.** The main analysis will be a 3 [stimulation: pre-stimulation vs. post-active vs. post-sham] by 2 [protocol: cTBS vs. iTBS] repeated-measures ANOVA. In addition to the main factors considered, we may also co-vary out several individual difference measures (e.g. baseline anxiety via self-report questionnaires, counterbalance order, overall stimulation accuracy, and Sternberg working memory (WM) performance to assess target engagement).

**Aim 2: Determine the effect of a 1-week course of TBS treatment (Continuous vs. Intermittent; right dlPFC target) on anxiety-related WM-deficits using the Sternberg WM paradigm during threat of shock.**

**Outcome measures.** During each Sternberg trial, subjects will see a series of 5 or 8 letters presented singularly (encoding period) that will be followed by a brief interval where subjects are required to maintain these letters (maintenance period). At the end of the maintenance period, subjects will indicate whether the position of the letter in the series matches the number. Accuracy will be scored as the percent correct for each trial type.

**Statistical Analysis.** The main analysis will be a 3 [stimulation: pre-stimulation vs. post-active vs. post-sham] by 2 [protocol: cTBS vs. iTBS] repeated-measures ANOVA. As with Aim 1, we may also include several other individual difference measures as covariates in this analysis.

**Aim 3: Demonstrate target engagement by measuring BOLD responses evoked by TMS pulses to the right dlPFC during threat of shock.**

**Outcome measures.** Standard Multi-echo fMRI preprocessing will be conducted using the AFNI software package. Steps include: slice-time correction, despiking, volume registration, TE-dependent ICA denoising (to remove non-BOLD artifacts), masking, blurring, scaling, motion scrubbing, T1-EPI alignment, normalization (using MNI template). At the first level, variable duration mini-blocks will be used to model BOLD activity during the maintenance interval of the Sternberg trials. Motion parameters and all other events will be included in the model as regressors of no interest.

**Statistical Analysis.** Whole-brain maps will be created for contrasts mirroring those used to create FPS and APS in Aim 1. FP-BOLD and AP-BOLD maps will then be analyzed using a paired-sample t-test to compare BOLD responses evoked by active TMS and those evoked sham scalp stimulation. Monte Carlo simulations will be conducted to cluster-correct the results using a voxelwise p-value threshold of 0.001, a cluster alpha of 0.05, and a realistic spatial autocorrelation function to model the noise. This is the standard approach implemented in AFNI following the influential Eklund et al. 2016 paper. As with Aim 1, we may also include several other individual difference measures as covariates in this analysis.

### *6.2 Sample Size and Power Determination*

Because we have several outcome measures, and because there is little known about how TMS will affect these outcome measures, we are conducting a single power analysis to determine sample size, based on a moderate effect size of (f=0.5). If we set power at 0.8 and use a corrected two-tailed alpha of 0.025 we would need 26 subjects per group to detect a main effect. Assuming a 15% unusable data rate and 3 pilot subjects, we will need 63 total subjects. However, this will be updated prior to enrollment based on the best available information at the time. To ensure that we are adequately powered across the study arms, and to account for additional dropout due to scheduling issues, we plan to recruit up to 75 participants.

## 7 Safety and Adverse Events

### *7.1 Definitions*

#### 7.1.1 Adverse Event

*An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:*

- *results in study withdrawal*
- *is associated with a serious adverse event*
- *is associated with clinical signs or symptoms*
- *leads to additional treatment or to further diagnostic tests*
- *is considered by the investigator to be of clinical significance*

#### *7.1.2 Serious Adverse Event*

***Serious Adverse Event***

*Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:*

- *fatal*
- *life-threatening*
- *requires or prolongs hospital stay*
- *results in persistent or significant disability or incapacity*
- *a congenital anomaly or birth defect*
- *an important medical event*

*Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.*

*All adverse events that do not meet any of the criteria for serious should be regarded as* ***non-serious adverse events****.*

### *7.2 Recording of Adverse Events*

All adverse events will be recorded by the study team via the adverse event source document, and summarized in the enrollment log. All adverse events will be reported by the study staff to the PI within 4 days.

### *7.3 Relationship of AE to Study*

Relatedness to the research of all serious adverse events will be determined by the medical advisor.

### *7.4 Reporting of Adverse Events, Adverse Device Effects and Unanticipated Problems*

#### *7.4.1 Follow-up report*

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The PI is responsible for ensuring that all SAE are followed until either resolved or stable.

#### *7.4.2 Investigator Reporting*

The medical advisor will determine whether the event represents a severe adverse event (SAE) or an unanticipated problem (UP). SAEs and UPs will be reported to the IRB and DSMB within 7 days. Mild and Moderate adverse events will be logged and reported to the IRB annually and the DSMB quarterly.

### *7.5 Stopping Rules*

The study will be suspended if there is any UP or SAE until IRB reviews the event and approves continuation.

#### 7.5.1 Data and Safety Monitoring Plan

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems (UPs), adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the medical advisor.

In addition, the PI will maintain a full FDA regulatory binder tracking subject enrollment, qualifications of investigators, IRB correspondence, inclusion/exclusion criteria, and all source data. All essential data will be recorded in the electronic case report form by the study staff. Acknowledgement of incomplete data records will be noted in the screening and enrollment log. Pilot subjects, subjects who withdraw, or subjects who experience an adverse event are expected to have incomplete data records, and will be excluded from the final analysis.

Site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by an in-house individual trained by the office of clinical research.
- The first subject will be monitored. Then 30% of all subsequent subjects will be monitored.
- Monitored subjects will be selected randomly during the yearly monitoring visit conducted prior to continuing review.
- The following data will be reviewed for all monitored subjects:
    - Informed consent and documentation of consent
    - Verification of eligibility
    - SAE (Serious Adverse Events) reported to IRB
    - Primary and secondary outcome measures
- Details of clinical site monitoring are documented in a Monitoring Plan (MP). The MP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits or compliance reviews may be conducted by the Office of clinical Research to ensure that monitors are following the MP.

# 8 Study Administration, Data Handling and Record Keeping

## *8.1 Subject Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Access to PHI will be limited to study staff, and allowed on an as needed basis. All protected health information (PHI) will be maintained using an institutionally secured and managed network drive. Transportation of any PHI will be conducted via either a Penn-approved encrypted portable drive, or via a Penn-approved secure encrypted file transfer service. All PHI will be deleted upon study close.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

### *8.2 Data Collection and Management*

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

### *8.3 Subject Privacy*

All subject interactions including screening, consent discussion, and study procedures will occur in a private location with only the subject and necessary study staff present. All electronic records will be kept confidential according to standard policies. Participants’ names and other personal identifying information will be stored in electronically secured databases. These databases will be password protected and only study personnel will be given a password. Results will be published as group data without the use of characteristics that would identify individual subjects. No biological samples will be collected.

## 9 Study Monitoring, Auditing, and Inspecting

### *9.1 Auditing and Inspecting*

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including expertise in affective neuroscience, transcranial magnetic stimulation, and biostatistics. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet quarterly to assess safety and efficacy data on each arm of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to Dr. Balderston who is the Sponsor/Investigator

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

## 10 Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

### *10.1 Risks*

**Clinical interview and assessments:** There is minimal medical risk in completing the questionnaires. Some of the questions may make the participants feel uncomfortable or anxious. Participants may refuse to answer any question or to stop a test at any time and for any reason.

**Auditory startle stimulus (i.e. loud noises):** The auditory stimuli that will be used in the startle studies are 40-ms duration 102 dB white noise. Auditory startling sounds of much higher intensities are frequently used in startle studies. Sounds of higher intensities and longer duration are also widely used in aversive conditioning in human subjects, where they serve as unconditioned stimuli. The short duration (40 ms) of these sounds makes them safe (i.e., there is no danger of hearing impairment). In addition, a white noise is safer than a pure tone. The PI has been involved in similar studies and collaborations involving over 200 subjects with no adverse reactions. The auditory stimulus may trigger a migraine.

**Psychophysiological (Blink) recording**: The psychophysiological measures that will be obtained are non-invasive, requiring the administration of no needles, drugs, or dyes. Little discomfort is expected. During electrode placement, the possibility of skin irritation from contact with the saline electrode paste exists. However, this is unlikely as the salt concentration of the paste is similar to that of human sweat.

**Electric shock**: The shocks will be delivered through two disk electrodes located on the subject's left wrist. The PI has extensive experience with shocks. The shock is generally described by subjects as anxiogenic and uncomfortable. The mean rating of aversiveness on a scale of 1 (not painful at all) to 10 (extremely painful) is about 5. Over 95% of subjects who experienced the shock chose to participate in the experiment.

In very rare occasions, subjects have experienced symptoms that may be related to the shock. A participant with a condition called "cubital tunnel syndrome," a repetitive motion injury similar to carpal tunnel syndrome, indicated worsening of his syndrome over the months subsequent to his participation. Another participant reported pain in her arms for several hours after testing. The pain was no longer present the next day. It is unclear whether these symptoms were due to the shocks. Nevertheless, subjects with neurological symptoms of the wrist and arms will be excluded from the study.

**MRI:** MRI is widely regarded as a safe, noninvasive procedure for visualization of brain tissue. The risks involved with fMRI are the same as those involved in standard anatomic MRI, since these three procedures rely on the same physical properties of brain tissue. This study will be performed on an FDA approved 3T scanner. MRI at 3 Tesla is a routine clinical procedure, and issues regarding radio frequency deposition, time varying magnetic fields, and the static field at 3 Tesla do not require detailed discussion.

**Likely/ Common:**

- Because the MRI scanner is a narrow space, subjects may experience claustrophobia, or a fear of enclosed spaces and/or anxious feelings accompanied by fast heart rate or shortness of breath.
- Additionally, the scanner produces a loud repetitive knocking noise during the study that some people find bothersome. To lessen the noise, earplugs will be provided.

**Rare:**

- The MRI scanner has a strong magnet which attracts certain metals. As a result, the MRI will not be performed on anyone having these types of metal in their body. This includes metallic fragments and certain implanted medical devices, such as: Pacemakers, Internal Cardiac Defibrillators, Insulin Pumps, and other medical devices. Implanted medical devices and metallic foreign fragments inside the subject may pose a risk if they were to enter the MRI magnet room. Therefore, questions regarding medical and work history will be asked prior to the exam to ensure subjects do not have any of these metallic fragments in their body
- Flying Objects: The known risks associated with this study are minimal. The greatest risk is a magnetic object flying through the air toward the magnet and hitting the subject. To reduce this risk we require that all people involved with the study remove all magnetic metal from their clothing and all magnetic metal objects from their pockets. No magnetic metal objects are allowed to be brought into the magnet room at any time except by approved personnel. To prevent any injury to patients and staff and any damage to the MRI scanner, subjects will be asked to remove all jewelry and clothing containing metal before they enter the MRI scan room. Also, since the MRI magnet will erase credit cards, they must not be taken into the scan room. In addition, once they are in the magnet, the door to the room will be closed so that no one inadvertently walks into the room.
- Some dyes in tattoos and permanent eyeliner contain metals which may heat up during the MRI scan. This can cause the area with the tattoo to become irritated and swollen.
- It is possible that during the course of the research study, the research staff may notice an unexpected finding. Should this occur, the finding will be considered by the appropriate personnel and the PI will inform the subject. These possible findings may or may not be significant, but could lead to anxiety about the condition and to further evaluation by their physician.
- Although there are no known risks related to MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no possible benefit from participating in this protocol for a pregnant woman, we will exclude pregnant women. All women of childbearing potential will be asked to

confirm before entering the MRI scanner that they are not pregnant at the time. Implantable contraceptives are generally very safe for MRI, but the MRI technician may ask the subject additional questions before entering the MRI suite to ensure their safety.

**TMS:** When used in accordance with the safety guidelines, there are no known long-term risks associated with TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). A recent consensus safety report summarized the likelihood and factors contributing to the recorded occurrences of seizure (Rossi et al., 2009). As of 2008, there were only 16 cases of seizure reported in the preceding 20 years of research. Seven of these occurrences occurred prior to the development of rigorous safety guidelines implemented in 1997, which govern stimulator intensity, pulse frequency, train duration, inter-train intervals. According to the consensus report, when used according to the safety guidelines the occurrence of seizure is rare, and the few new cases involve "TMS protocols exceeding previous guidelines, often in patients under treatment with drugs which potentially lowered the seizure threshold" (Rossi et al., 2009). While there are no known long-term adverse effects reported with the use of this device, there may be unforeseen risks in the long-term that are currently unknown.

**Likely/ Common:**

- The most common side effect of TMS is a mild headache, jaw pain, or other facial discomfort, which approximately 25% of patients experience. We will demonstrate TMS at the initial screening visit to make sure the subject is comfortable receiving this procedure. The TMS that will be used in this research is not expected to carry any more than the limited potential for these risks listed above.

**Rare:**

- Subjects may experience temporary and local bruising, swelling or pain from the swim cap and/ or muscle activation by TMS.
- Although uncommon, some subjects have experienced nausea during the experiment.
- In patients with epilepsy, TMS could result in a seizure. Patients with stroke may also be at increased risk for a seizure due to brain scarring. Therefore, those with history of epilepsy or stroke will be excluded from TMS studies. For a typical healthy person, producing a seizure from TMS in this experiment is very unlikely.
- The TMS device produces a clicking sound. Although studies have found no hearing impairments as a result of this sound, some subjects' experience a mild temporary effect on their hearing. To minimize this possibility, you will be given protective earplugs.
- Objects such as watches and credit cards should be removed as these could be damaged.
- The effects of TMS on a fetus are unknown. Therefore, we require that females of child-bearing potential attest at the time of participation that they are not pregnant.

**Alternatives to participation:** The studies proposed in this project do not involve an intervention, so the participants will not directly benefit from the research. Therefore, there are no alternatives to participation. However, participants will be free to withdraw from the

study without consequence.

**Protections against risk**

**Electric Shock:** Shock will be delivered at a level that is judged by the subject as unpleasant but not painful. Study shock levels will be determined before the test begins. The subject may stop the experiment at any time if they find the discomfort to be too great.

**TMS:** To minimize the likelihood of seizures we will 1) conform to the guidelines set in the workshop convened by the National Institute for Neurological Disorders and Stroke (NINDS) in 1996 (Rossi et al., 2009), and 2) screen subjects for risk factors using the TMS adult safety screen questionnaire (Keel et al., 2000). In addition, we will train all personnel to recognize potential seizures, and administer appropriate care. In the event that the TMS operator observes symptoms of a seizure he or she will 1) stop the protocol and inquire about the subject's well-being, 2) apply first-aid if the subject is unresponsive, 3) ensure the subject is physically safe for the duration of the seizure, 4) call 911. If the subject develops a headache during or immediately after the stimulation, this will be managed with over-the-counter pain medication. To minimize the likelihood of hearing loss or tinnitus, subjects will wear hearing protection for the duration of the rTMS session. Risks to the unborn children of pregnant women receiving MRI and TMS are unknown. Pregnant women will be excluded as per IRB policy.

**MRI:** The potential risks related to MRI will be minimized as follows: 1) Claustrophobia associated with MRI will be reduced by explaining the nature of the procedure in detail prior to subject enrollment; and 2) a possible history of any intraocular, intra-aural, intracranial, or intrathoracic metal will exclude the subject from the study. Earplugs will be given to each subject to wear during the scan to minimize discomfort and prevent any adverse effects on hearing resulting from the scanning procedure.

A radiology technologist (or trained individual authorized to run the scanner) and a clinician will be present throughout the MRI study in case medical emergencies would arise. During MRI scanning, the subject can communicate with the control room personnel via an intercom at the operating console. Thus, the subject can be removed immediately from the MRI scanner, if necessary.

### *10.2 Benefits*

Participants in the experiments proposed during the project will be compensated for their time and travel, but will not directly benefit from the proposed research.

### 10.3 Risk Benefit Assessment

Although anxiety disorders are the most commonly diagnosed class of disorders, with 1 in 5 individuals diagnosed annually, less than 50% of these patients receive treatment, and less than 15% receive minimally adequate treatment (Kessler & Chiu, 2005). Although extensive research has linked subcortical structures like the amygdala and bed nucleus of the stria terminalis (BNST) to the hyperarousal symptoms experienced by anxiety patients, hyperarousal is only one facet of the symptom profile shared across anxiety disorders. Much less is known about the cognitive symptoms (i.e. difficulty concentrating) experienced by anxiety patients. Given the mismatch between the existing literature and the anxiety phenotype in patients, it is understandable that the most commonly used pharmacological treatments (e.g. benzodiazepines and SSRIs) for

anxiety were developed more than 20 years ago, with little advancement since. Accordingly, there is a critical need for mechanistic research into the CNS mechanisms that mediate the cognitive symptoms experienced by anxiety patients. Without such research, treatment development for these disorders will continue its stunted progress. Once completed, we expect this research to yield direct evidence for a causal role of the right dlPFC in anxiety regulation, complete with evidence of target engagement through simultaneous TMS/fMRI, and a novel application to clinical anxiety.

### 10.4 Informed Consent Process / HIPAA Authorization

Consent will be obtained by research coordinators, postdoctoral fellows, a graduate student or the principal investigator. Because this study does not involve treatment, coercion is not a concern. Consent will be obtained in a private room where study staff members can explain the purpose of the fMRI and what it will add to our knowledge of TMS, neuroscience and cognitive processes. They will explain that participating is completely voluntary and that not participating will not change access to treatment in any way. The potential participant will be given the option to consider study enrollment and will not be forced to make a decision the same day. If they decide to participate, a combined consent and HIPAA form will be signed by research staff and the patient. The patient will be reminded before and after enrolling, and before any research procedure that their participation is optional and has no impact on the care they can expect.

## 11 Study Finances

### *11.1 Funding Source*

This study is financed through a grant from the US National Institute of Health.

### *11.2 Conflict of Interest*

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

### *11.3 Subject Stipends or Payments*

Study compensation is based on the following schedule:

- Week 1
  - Lab Visit 1 $25
  - MRI Visit $50
- Week 2
  - TMS Visit 1 $25
  - TMS Visit 2 $25
  - TMS Visit 3 $25
  - TMS Visit 4 $25
  - Lab Visit 2 $25
- Week 3

    - No Visits
- Week 4
    - TMS Visit 5 $25
    - TMS Visit 6 $25
    - TMS Visit 7 $25
    - TMS Visit 8 $25
    - Lab Visit 3 $25
- Completion Bonus
    - Upon Completion $175
- Optional MRI visit $50
- Optional Pilot visit 1 $75
- Optional Pilot visit 2 $75

**Total Compensation $700**

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## 13 Attachments

- Consent Form