Protocol Title: The effect of transcranial magnetic stimulation to the frontoparietal attention network on anxiety potentiated startle.

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Protocol Title: The effect of transcranial magnetic stimulation to the frontoparietal attention network on anxiety potentiated startle.
Abbreviated Title: The effect of TMS on anxiety.
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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:

(Per SOP 25, PIs or IRBs require investigators and staff working on a protocol to complete the following training, as applicable. Delete those that are not applicable. Indicate "None" if no training beyond the general training is required.)

- NIAID GCP course
- CITI GCP modules

Total requested accrual = 184 Healthy Volunteers

Project Uses Ionizing Radiation	■ No	o Yes
IND/IDE	■ No	o Yes
Durable Power of Attorney	■ No	o Yes
Multi-institutional Project	■ No	o Yes
Data and Safety Monitoring Board	■ No	o Yes
Technology Transfer Agreement	■ No	o Yes
Samples are being stored	■ No	o Yes

Flesch-Kincaid reading level of consent form:
Healthy Volunteer: 7.8

Précis:

Objective: To determine the effect of non-invasive brain stimulation on anxiety and anxiety-cognition interactions in healthy subjects. Toward this aim we will test the effect of transcranial magnetic stimulation (TMS) on two outcome measures: 1) Fear and anxiety during the threat of predictable and unpredictable shock (NPU threat test), and 2) Working memory (WM) related anxiety downregulation while performing the Sternberg WM task under threat of shock.

Study population: The study population will consist of up to 184 healthy volunteers between the ages of 18-50.

Design: This study will consists of two (sub-studies 1 and 2) or three (sub-study 3) outpatient visits (1 MRI, 1 or 2 TMS visits [2 for sub-study 3]). In this protocol we will explore the effect of TMS in three sub-studies in the TMS study visit. The sub-studies will contain either the NPU or the Sternberg task during the TMS visits. The first visit (MRI) will consist of the same procedures for all sub-studies. Each subject will be assigned to only one of the sub-studies.

Sternberg Task: Expose subjects to active or sham TMS to a region of the frontoparietal attention network during the Sternberg WM task. Subjects will have to maintain a series of letters in WM for a brief interval during blocks of safety and threat of shock.

NPU Task: Expose subjects to active or sham TMS to a region of the frontoparietal attention network during the NPU threat test. Subjects will be exposed to blocks in which they are either 1) safe from shock (neutral), 2) at risk of shock delivered only during a cue (predictable), or 3) at risk of shock presented randomly (unpredictable).

Outcome measures: In both studies the primary outcome measure will be anxiety-potentiated startle (APS), which is the increase in startle magnitude during periods of threat compared to periods of safety. We expect active, but not sham TMS to increase activity in the dlPFC, and therefore reduce APS in both studies.

Table of Contents

List of Abbreviations

APS = anxiety-potentiated startle
BOLD = blood oxygenation-level dependent response
CTDB = Clinical Trials Database
CTSS = Clinical Trial Survey System
dlPFC = dorsolateral prefrontal cortex
DoH = Declaration of Helsinki
EEG = electroencephalography
EMG = electromyographic
FDI = first dorsal interosseus
fMRI = functional magnetic resonance imaging
GDS = Genomic Data Sharing
ITI = intertrial interval
MEP = motor evoked potential
MRI = magnetic resonance imaging
MT = motor threshold
N = no threat
NPU = Threat test with neutral, predictable, and unpredictable conditions
P = predictable threat
rTMS = repetitive transcranial magnetic stimulation
TASS = TMS Adult Safety Screen
TMS = transcranial magnetic stimulation
U = unpredictable threat
WM = working memory

1. Introduction and Background

a. Background

The relationship between cognition and anxiety is complex and bidirectional (Robinson et al., 2013b; Vytal et al., 2012, 2013). Difficult tasks typically reduce anxiety, but performance on these tasks can suffer when individuals are in an anxious state. The mechanisms behind this bidirectional relationship are not yet known. Indeed, most prominent theories on the relationship between anxiety and cognition focus on the effect of anxiety on cognition, and largely ignore the effect of cognition on anxiety (Eysenck et al., 2007). As a result, the mechanisms underlying the reduction in anxiety during cognitive tasks remain largely unexplored. A better understanding of mechanisms involved in anxiety down regulation has clinical implications. For instance, individuals with anxiety disorders frequently suffer from attentional problems, such as being easily distracted and unable to focus on ongoing tasks. In fact, one of the core symptoms of generalized anxiety disorder (GAD) is "difficulty concentrating"(American Psychiatric Association, 2013). Such susceptibility to distraction has been linked to competition between task-relevant and task-irrelevant (e.g., worry) thoughts in working memory (WM) (Eysenck et al., 2007). To study this relationship in healthy subjects, it is possible to experimentally induce anxiety using the threat of shock paradigm.

Threat of shock is a well-validated (Grillon and Ameli, 1998; Lang et al., 1990), translational procedure (Grillon, 2008a; Grillon et al., 1994; Morgan et al., 1995; Robinson et al., 2012b), previously shown to increase state anxiety (Dunning et al., 2013; Hansen et al., 2009; Robinson et al., 2013b; Vytal et al., 2014), cause worry (Grillon, 2002, 2008b), and interfere with task performance (Balderston et al., 2015; Clarke and Johnstone, 2013; Lavric et al., 2003; Robinson et al., 2011, 2013a). In a typical threat of shock experiment, subjects are exposed to alternating blocks in which they are safe, or at risk for receiving an unpleasant electrical stimulation. Because the shock can be delivered infrequently, it is possible to superimpose a wide variety of tasks onto these alternating blocks, with little interference from the actual shock presentations, making this paradigm ideal for experimentally testing the effects of anxiety on cognitive processes, and their underlying neural mechanisms.

It is clear from resting state studies that threat of shock changes patterns of functional connectivity even in the absence of a cognitive task (McMenamin et al., 2014; McMenamin and Pessoa, 2015; Robinson et al., 2012a), and our data suggest that these resting state changes may be most robust in parietal regions of the frontoparietal attention network (FPN), potentially reflecting a state of threat induced hypervigilance (Balderston et al., 2017). Therefore, successful performance of a task during this altered baseline state may require additional cognitive control, when compared to non-threat periods. One candidate region that may be involved in this implicit emotion regulation is the dorsolateral prefrontal cortex (dlPFC). Indeed, the dlPFC has been repeatedly shown to play a role in WM (Altamura et al., 2007; Barbey et al., 2013; Curtis and D'Esposito, 2003; Feredoes et al., 2011; Geier et al., 2007), anxiety (Bishop, 2009; Forster et al., 2015; Nitschke et al., 2006; Peers et al., 2013; Shang et al., 2014), and emotional learning

(Carter et al., 2006). Studies with high anxious healthy controls have also implicated the dlPFC in anxiety/task interactions (Basten et al., 2011, 2012; Bishop, 2009; Eysenck et al., 2007; Fales et al., 2008; Telzer et al., 2008). In our pilot work, we show that even low load tasks activate the dlPFC under threat of shock, but that this activity is increased as cognitive load is increased.

Together these results suggest that the FPN plays a key role in the interaction between anxiety and cognition, however the nature of this role is unclear. We propose a novel model based on the frontoparietal attention network (FPN) [27]. According to our model, 1) elevated state anxiety enhances bottom-up attentional processes, mediated by the parietal cortex, and 2) top-down cognitive control processes, mediated by the right dlPFC, counteract this enhancement, reducing anxiety. This model is an extension and integration of the arousal theory of parietal cortex asymmetry, which states asymmetries in parietal activity reflect arousal processes [28], and the attention control theory, which states that anxious individuals cannot efficiently engage goal-directed attention [29]. Importantly, this model can be experimentally tested using TMS. TMS to the dlPFC was recently approved by the FDA as a second-line treatment for depression (Horvath et al., 2010; O'Reardon et al., 2007; Shajahan et al., 2002; Stern et al., 2007), and some studies have shown that TMS can reduce comorbid symptoms of anxiety in depressed individuals (Mantovani et al., 2013; O'Reardon et al., 2007; White and Tavakoli, 2015). TMS uses a strong magnetic current at the scalp to induce electrical currents in neurons just below the TMS coil. rTMS is capable of altering the resting membrane potentials of local neurons (Lefaucheur et al., 2014). rTMS at low frequencies (<5 Hz) tends to decrease local excitability, while rTMS at high frequencies (>5 Hz) tends to increase local excitability (Di Lazzaro et al., 2011). Therefore, 5 Hz rTMS should facilitate dlPFC activity, and according to our hypothesis this dlPFC stimulation in the absence of a task should inhibit threat-related processing, resulting in an overall decrease in anxiety.

One variant of the threat of shock paradigm is the so-called NPU paradigm, where subjects are exposed to simple cues during blocks of no threat (N), predictable threat (P), and unpredictable threat (U) (Schmitz and Grillon, 2012). During this paradigm, simple geometric shapes serve as cues (presented for 8 s). During the predictable threat blocks, these cues indicate that a shock may occur, while during the unpredictable and neutral blocks, these cues are not predictive of the shock. During the no threat blocks subjects are instructed that they are completely safe from shocks, and during the unpredictable blocks, subjects are instructed that they could receive a shock at any point. This paradigm provides measures of fear (e.g., fear-potentiated startle) associated with the threat cue in the P condition and of anxiety (e.g., anxiety-potentiated startle) in the N, P, and U contexts (no-cue periods). This paradigm is ideal to test the top-down inhibition hypothesis because it allows for the testing of both fear and anxiety, without adding additional cognitive demands. In addition, cue presentations during the NPU task activate the dlPFC (See below), which may reflect an implicit form of top-down emotion regulation.

The purpose of this protocol is to use TMS to determine the role of the FPN in anxiety reduction. According to our model, we have 2 hypotheses. First, suppressing activity in

the left parietal cortex with low frequency (1 Hz) rTMS should interfere with the bottom-up attentional processes associated with elevated state anxiety. Second, enhancing activity in the right dlPFC with high-frequency (10 Hz) rTMS should facilitate top-down cognitive control processes associated with anxiety reduction. Therefore the objective of this protocol is to test these hypotheses by altering FPN activity using non-invasive TMS, and measuring the effect of this altered FPN activity on anxiety using anxiety-potentiated startle (APS). Importantly, according to the top-down inhibition hypothesis strengthening activity the dlPFC with TMS alone (i.e. in the absence of a demanding cognitive task) should be sufficient to reduce anxiety, suggesting that we should see an effect in a passive task like the NPU paradigm. This is based on the following logic: 1) the dlPFC plays a key role in attention control (Basten et al., 2012; Cieslik et al., 2013; Fales et al., 2008; Harding et al., 2015; Peers et al., 2013), 2) anxiety patients are impaired in their ability to recruit this structure for cognitive control (Armstrong et al., 2011; Balderston et al., 2016b; Berggren and Derakshan, 2013a, 2013b; Braver et al., 2010; Coombes et al., 2009; Derryberry and Reed, 2002; Eysenck et al., 2007; Grillon et al., 2015b; Morrison and Heimberg, 2013; Najmi et al., 2012, 2014; Price et al., 2011; Reinholdt-Dunne et al., 2009, 2012). 3) cognitive tasks that occur during periods of elevated state anxiety recruit the right dlPFC (See Preliminary Results), 4) tasks that engage the right dlPFC reduce state anxiety (Balderston et al., 2016a; Vytal et al., 2012, 2013). However, it is possible that some level of prior engagement of the stimulation site is necessary for the stimulation to be effective (Luber et al., 2007, 2008, 2013). Therefore we will test the effect of TMS on two outcome measures: 1) Fear and anxiety during the NPU task, and 2) Working memory (WM) related decreases in anxiety during the Sternberg WM task.

b. Significance

Anxiety disorders affect approximately 18% of the population in a given 1 year period, placing a substantial burden on the national health care system (Kessler and Chiu, 2005). Anxiety patients suffer from cognitive deficits, which can severely impact their wellbeing (American Psychiatric Association, 2013). We have shown that these deficits seem to be a core feature of anxiety disorders, and that they may be mediated by impaired FPN functioning (Balderston et al., 2016b). The purpose of this project is to determine whether noninvasive stimulation of the FPN can reduce anxiety. Given that TMS is already approved by the FDA for treatment of depression, these results may provide a potential avenue for future treatment of anxiety disorders.

c. Preliminary data

The NPU threat test provides reliable measures of both fear and anxiety. In order to understand the relationship between anxiety and cognition, it is important to have a reliable method for experimentally manipulating state anxiety independent of cognitive tasks. Our lab has repeatedly done this using the NPU threat test (Alvarez et al., 2011; Davis et al., 2010; Grillon et al., 1994, 1998, 2004, 2006, 2008, 2011, 2012, 2015a; Robinson et al., 2012b; Schmitz et al., 2011; Schmitz and Grillon, 2012). As mentioned above, the NPU threat test consists of blocks of neutral, predictable, and unpredictable

threats. During this task, subjects are presented with cues, and these cues signal the shock only in the predictable conditions Consistent with our previous work, our current pilot data show that this task can differentiate between fear and anxiety at both the psychophysiological and the neural level. At the psychophysiological level, subjects show elevated startle to the cue compared to the ITI during the predictable condition, and elevated startle during both the cue and ITI in the unpredictable condition (Figure 1).

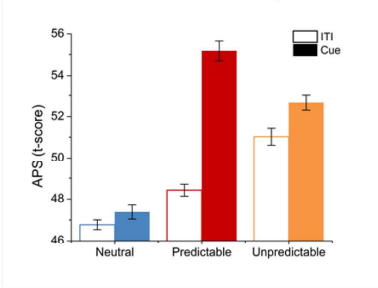

Figure 1: Typical pattern of startle data in the NPU paradigm

At the neural level, cues evoke an fMRI pattern of activity consistent with fear in the predictable but not the unpredictable condition (Figures 2-3). Importantly, cues during both the predictable and unpredictable conditions evoke activity in the dlPFC, a region important for cognitive control (See below).

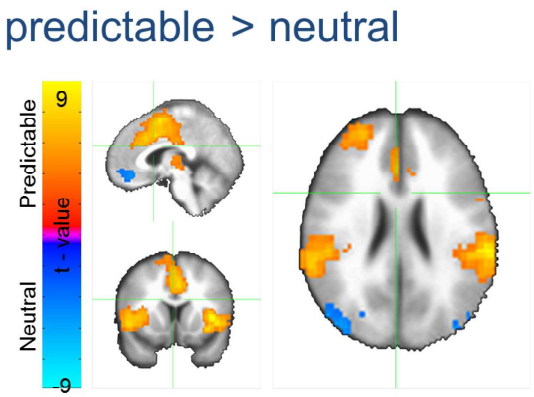

Figure 2: Cues in the predictable condition evoke neural activity in the fear network (dACC, anterior insula, thalamus).

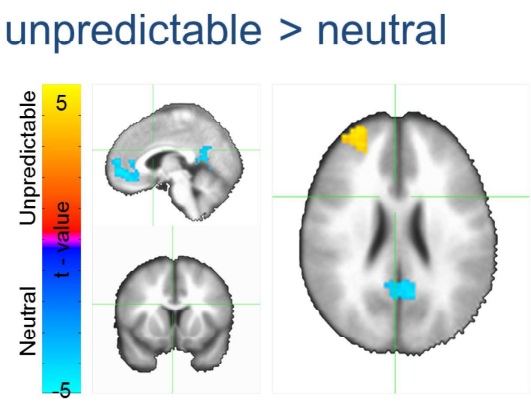

Figure 3: Cues in the unpredictable condition fail to evoke neural activity in the fear network.

Anxiety is reduced by WM load in the N-back WM paradigm: In our initial studies exploring the relationship between cognition and anxiety, we conducted the n-back task under threat of shock. In a typical n-back experiment, subjects are required to continuously monitor a series of item, and make judgments on each trial according to whether the current item matches that presented "N" trials previously. Increasing "N" increases the number of items a subject is required to maintain in WM, and therefore the cognitive load placed on the subject. In our n-back studies we found that threat reduces performance on this task under low load conditions (Figure 4). In addition, we found that under high load conditions, anxiety, as probed with the startle reflex (anxiety-potentiated startle or APS) was reduced (Figure 5). Because the n-back task is complex and involves

various cognitive processes, it is unclear which mechanism is responsible for the anxiety reduction at high loads in this task. Indeed, the n-back task is highly dependent on executive function, requiring simultaneous encoding, maintenance, and retrieval (Kane et al., 2007; Owen et al., 2005). Furthermore, during the 2- and 3-back task, subjects must also shift their attention away from representations of the intermediate to-be-maintained items, further engaging prefrontal cognitive control resources.

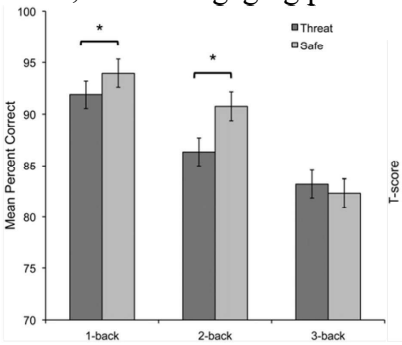

Figure 4: Threat reduces accuracy during verbal n-back task.

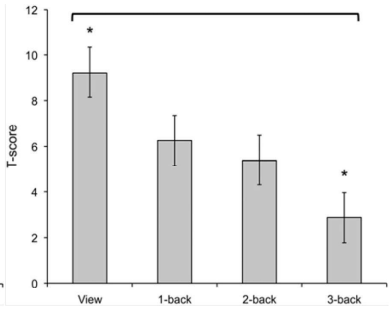

Figure 5: APS is reduced during 3-back relative to view condition.

Anxiety is reduced by WM load in the Sternberg WM paradigm: Therefore, in an effort to better understand the psychological mechanisms underlying the load related anxiety reduction, we followed up the N-back studies with the Sternberg WM paradigm (Sternberg, 1966). In the Sternberg WM paradigm, subjects are presented with a series of letters, and required to maintain this series of letters in WM for a brief maintenance interval. After the maintenance interval, subjects are presented with a response prompt, which consists of a letter and a number. The letter is chosen from the study series, and the number corresponds to a position in the series. At the prompt, subjects are instructed to identify whether the position of the letter in the series matches the number. By varying the number of to-be-encoded letters in a series, it is possible to increase WM load. Because simple rehearsal is sufficient to maintain these letters (Altamura et al., 2007), this task does not require the engagement of central executive resources (Baddeley, 1992).

In these follow-up studies, we required subjects to perform the Sternberg task during periods of safety and threat. We increased WM load by manipulating the number of letters in this series, and probed subjects anxiety with the startle reflex during the safe or threat blocks. Consistent with the previous n-back studies, we found that increasing WM load reduces anxiety (APS), when anxiety is probed during the maintenance period, but not the ITI (Figure 7). This is true even though the Sternberg task typically does not engage central executive processes, suggesting that this acute anxiety down-regulation can be caused by WM components other than the central executive. However, unlike the previous n-back studies, performance was not impaired under threat of shock (Figure 6), suggesting that the central executive processes, rather than maintenance processes were impacted by anxiety in the n-back task.

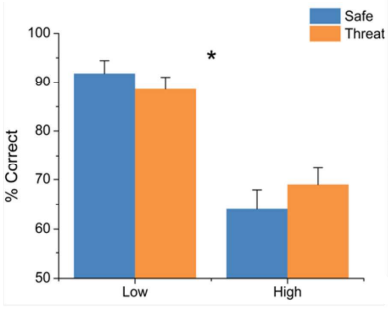

Figure 6: Sternberg WM performance is not affected by threat.

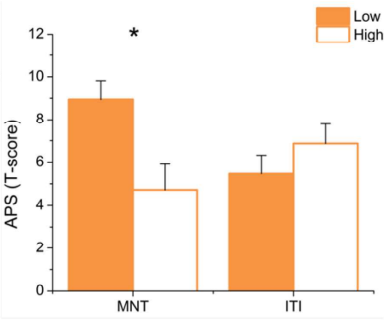

Figure 7: APS is reduced during the maintenance period of high load Sternberg WM trials.

Threat of shock activates the dlPFC: In addition, in several recent studies we found that threat of shock evoked activity in the dlPFC. In Study 1 (n = 37), subjects completed the NPU task. In Study 2 (n = 36), subjects underwent an incidental picture encoding session, where pictures of common household items were presented during blocks of safety and (unpredictable) threat. Subjects were simply instructed to indicate whether the item presented on each trial could typically be found inside a house or outside a house. In Study 3 (n = 21), subjects completed maintenance and manipulation trials in the Sternberg working memory paradigm. On each trial, subjects were presented with a series of letters, followed by a brief maintenance interval, followed by a response prompt. On maintenance trials, subjects were instructed to maintain the series of letters in working memory in their current order. On manipulation trials, subjects were instructed to sort the trials in alphabetical order. In all studies we found a significant cluster of activity over the right dorsolateral prefrontal cortex (dlPFC) as a function of threat. Across studies this region showed increased cue-evoked activity during threat blocks relative to safe blocks (Figures 8-10).

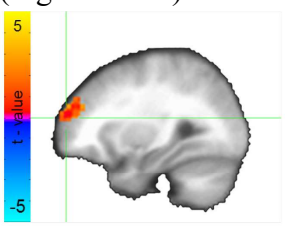

Figure 8: Increased cue-evoked activity during unpredictable vs. neutral periods in the NPU task.

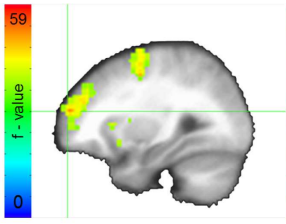

Figure 9: Increased activity during threat compared to safe in a picture encoding task.

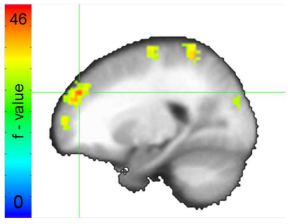

Figure 10: Increased activity during threat compared to safe in the Sternberg WM task.

Anxiety patients show impaired performance and dlPFC activity during N-back: In a recent study we sought to determine whether WM-related deficits in clinical anxiety are chronic, or whether they arise due to threat-related processing (e.g. worry). In the latter case, one would expect that experimentally-inducing threat would exacerbate the deficits in anxiety patients. We tested WM performance during threat of shock and safety in anxiety patients and healthy controls, while recording brain activity with functional magnetic resonance imaging (fMRI). Subjects performed a spatial N-back working memory (WM) task comprising 4 levels of difficulty (i.e., cognitive load), 0-back, 1-back, 2-back and 3-back conditions. In this study we found that patients were impaired

relative to controls across all levels of the N-back task, and that this performance impairment was independent of the threat manipulation (Figure 11). Similarly, the task-positive BOLD activity in the dlPFC was reduced in patients relative to controls (Figure 12). These results suggest that poor engagement of the prefrontal cortex (particularly dlPFC) in clinical anxiety reflects a core component of the disorder rather than a transient effect of threat-related processing (Basten et al., 2011; Bishop, 2009; Telzer et al., 2008).

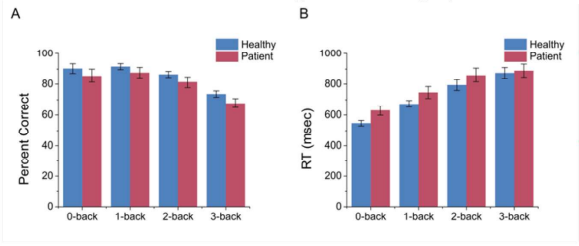

Figure 11: Anxiety patients show reduced accuracy and increased RT during N-back task.

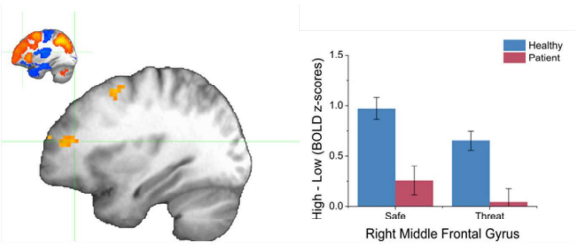

Figure 12: Anxiety patients show reduced WM related dlPFC activity.

Threat of shock fundamentally alters the IPS. In two recent studies, we exposed subjects to threat of shock while recording brain activity using MEG or fMRI (See Figure 2). In the MEG study, subjects received startle probes, and we sampled the MEG activity during the 2 seconds prior to the probe. When we compared the power from safe blocks to the power from threat blocks using a paired-sample t-test, we found a significant decrease in power during the threat blocks compared to the safe blocks in the alpha band over parietal sensors, suggesting an increase in cortical excitability (Klimesch et al., 2007). We used a beamformer to localize the source of this power decrease, and found a large cluster of voxels in the IPS showing a decrease in alpha power in the threat blocks compared to the safe blocks.

In the fMRI study, we assessed functional connectivity during threat blocks vs. safe blocks using the whole brain global brain connectivity (GBC) approach. This allowed us to assess functional connectivity from threat to safe across the entire brain, without specifying a seed-region. This approach allows us to identify the regions of the brain that show the most significant changes in connectivity with the rest of the brain as a function of our task design, and has previously been used to identify network hubs at the whole-brain level (Cole et al., 2010). We found a significant increase in GBC in the threat blocks compared to the safe blocks bilaterally in the IPS, showing that this region increased its connectivity with the rest of the brain during threat blocks, suggesting that this region functions as a network hub for threat-related attentional processes.

TMS pulses evoke transient eyeblink responses due to stimulation of the scalp: In a recent pilot, we tested the effect of TMS pulses on eyeblink responses. Given that our primary outcome measure is startle, we wanted to ensure that stimulation of the scalp muscles did not interfere with our ability to record startle evoked eyeblinks. We found that TMS pulses (in a 5 Hz train) indeed induce twitches in the orbicularis oculi muscle. However, these twitches were small, and the EMG signal recovered within 150 ms of the TMS pulse (See Figure 13). When comparing the TMS evoked eyeblink to the acoustic starlte reflex, it is clear that the TMS evoked eyeblink has an earlier onset, and a shorter duration than the acoustic startle reflex. This difference is likely due to the origin of the two blinks. The TMS evoked blink arises due to direct stimulation of the orbicularis

oculi, while the blink evoked by the startle probe arises through the CNS via engagement of the acoustic startle reflex arc (Blumenthal et al., 2005).

At a more mechanistic level, we can identify 3 possible mechanisms whereby the TMS pulse may affect the acoustic startle response: 1) The TMS pulse may interfere with the ability of the acoustic startle reflex to activate the fibers innervating the orbicularis oculi. We do not believe this will be an issue because orbicularis oculi EMG activity returns to baseline within 150 ms of the TMS pulse, which is well below the minimum interval between the TMS pulse and the subsequent startle probes; 2) The TMS pulse may trigger prepulse inhibition, which is the reduction of startle due to an unexpected preceding stimulus. We do not believe this will be an issue because prepulse inhibition is maximal at 120 ms and no longer exist after ~500 ms, which again is well below the minimum interval between the TMS pulse and the subsequent startle probes (Hoffman and Fleshler, 1963; Larrauri and Schmajuk, 2006); 3) The TMS pulse may reduce the predictability of the startle probe, thereby reducing the likelihood/amplitude of the startle response. We do not believe this will be an issue because we startle probes in many of our other experiments are somewhat predictable (e.g. the NPU threat paradigm; See Schmitz & Grillon, 2012). We also plan to vary the interval between the TMS pulse and the startle probe using timing parameters that have shown to be effective in previous studies (Balderston et al., 2015, 2016a). In addition, if such an effect would occur, it would be balanced across conditions.

We have made every possible effort to account for the non-specific effects of scalp stimulation by TMS on our dependent measure. First, it is important to note that our dependent measure in these studies is not the raw startle magnitude, rather it is the change in startle magnitudes during periods of safety and threat, and that the stimulation will be balanced across these conditions. Therefore, any non-specific effect of scalp stimulation on blink magnitude should be eliminated in the difference score. Furthermore, the current generated by the e-stim placebo coil is similar in magnitude, duration, and distribution to the current induced by the TMS pulse (Borckardt et al., 2008; Gonz, Strauss, & Schwerdtfeger, 2011; Rossi et al., 2007; Sheffer et al., 2013). In addition, electrically and magnetically stimulated blinks are nearly identical to each other in topology (Blumenthal et al., 2005). Together, these observations suggest that any non-specific effects of scalp stimulation on blink magnitude should be similar for the sham and TMS conditions.

These results suggest that it will be possible to record startle evoked eyeblinks given a sufficient interval between the TMS train and startle probe.

Summary: Our preliminary data show the following:
1) Anxiety interferes with WM performance, only during a task that requires central executive resources.
2) WM load is sufficient to reduce anxiety whether or not the WM task engages central executive resources.
3) Even during low load tasks, threat of shock activates the dlPFC.
4) Anxiety patients show impaired performance and reduced dlPFC activity relative to healthy controls during the n-back task.
5) Threat of shock increase cortical excitability in the parietal cortex, and this hyperexcitability may mediate the hypervigilance seen in anxious patients.

Based on these preliminary findings, we propose the following model, which is consistent with the top-down inhibition hypothesis. According to this model, individuals in an anxious state experience worrisome thoughts that can potentially distract them from ongoing tasks (especially those that require central executive processes). When task demands are high, cognitive control processes (mediated by the dlPFC) filter out these worrisome thoughts. By filtering out these worrisome thoughts, these cognitive control processes reduce anxiety, as well as the impact of anxiety on ongoing task performance. Individuals who are unable to engage these cognitive control processes are less able to reduce their anxiety and are therefore more likely to be negatively impacted by their anxiety.

2. Study Objectives

a. Primary objectives

The primary objective of this study is to determine whether altering activity in the FPN with TMS will reduce anxiety either alone (during NPU) or during a low load cognitive task (Sternberg WM).

3. Subjects

a. Description of study populations

The study will be conducted in 184 healthy volunteers. The participants will be adult males and females, aged 18 to 50. We will begin with a 10 subject pilot phase to determine the minimum interval between the TMS probe and the startle probe necessary to recover usable APS data. The target number of completers for each experiment will be N=48 for a total of 144 completers. We anticipate that about 15% of subjects (N=121) will either terminate the study during the task because of discomfort. An additional 9 subjects (3 per experiment) will be included for piloting. To account for this projected attrition rate, we aim for an accrual of 184 medically and psychiatrically healthy volunteers including the 10 pilot subjects. The participants will be adult males and females, aged 18 to 50. The accrual ceiling is 184. Subjects may participate in multiple sub-studies, and will be consented each time they are asked to participate in a given substudy. NIH employees may participate. NIMH employees and staff and their immediate family members will be excluded from the study per NIMH policy.

b. Inclusion criteria

- Ages 18-50
- Subjects able to give their consent
- Right handed

c. Exclusion criteria

- Non-English speaking individual
- Any significant medical or neurological problems (e.g. cardiovascular illness, respiratory illness, neurological illness, seizure, etc.)
- Current or past Axis I psychiatric disorder(s) as identified with the Structured Clinical Interview for DSM-IV, non-patient edition (SCID-np)
- Active or history of active suicidal ideation.
- Evidence of a first-degree relative with history of psychosis or bipolar disorder; specifically, participant will know diagnosis or treatment in order to confirm presence of disorder.
- Alcohol/drug problems in the past year or lifetime alcohol or drug dependence according to the Structured Clinical Interview for DSM-IV.
- Current use of medications that act on histamine (i.e. diphenhydramine), dopamine (methylphenidate), norepinephrine (buproprion), serotonin (sertraline), or acetylcholine (amitryptiline) receptors. Subjects will be excluded on this basis if they either 1) take these medications on a chronic basis, or 2) if they have taken the drug within 5 half-lives of the drug metabolism, determined by the medical professional at the time of screening.
- History of seizure (childhood febrile seizures are acceptable and these subjects may be included in the study),

- History of epilepsy in self or first degree relatives, stroke, brain surgery, head injury, cranial metal implants, known structural brain lesion.
- Increased risk of seizure for any reason, including prior diagnosis of increased intracranial pressure (such as after large infarctions or trauma), or currently taking medication that lowers the seizure threshold (table below).
- Pregnancy, or positive pregnancy test.
- Neurological syndrome of the arm (e.g., carpal tunnel syndrome, cubital tunnel syndrome, etc.)
- Positive urine toxicology screen during the screening visit.
- IQ <80
- Employee or staff of NIMH or are an immediate family member of a NIMH employee, staff, or NIMH contractors.
- Allergy to lidocaine or topical anesthetics (participants in sub-study 3 only).
- Any medical condition that increases risk for fMRI or TMS:
    - Any metal in their body which would make having an MRI scan unsafe, such as pacemakers, stimulators, pumps, aneurysm clips, metallic prostheses, artificial heart valves, cochlear implants or shrapnel fragments, or if you were a welder or metal worker, since you may small metal fragments in the eye.
    - Participants who are uncomfortable in small closed spaces (have claustrophobia) and would feel uncomfortable in the MRI machine
    - Patients who have difficulty lying flat on their back for up to 60 min in the scanner
    - History of hearing loss

4. Study Design and Methods

a. Study overview

Sub-studies 1 and 2 will consist of 2 outpatient visits (1 MRI visit,1 TMS visit), each lasting up to 3 hours each. Sub-study 3 will consist of 3 outpatient visits (1 MRI visit, 2 TMS visits). Healthy volunteers will be assigned to one of the three sub-studies. All visits will be located in the Clinical Center at the NIH in Bethesda, MD. The MRI visit will take place in the NMR center and the TMS visits will take place on the 7th floor inpatient unit. The visits will be separated by ~1 week (no more than 2 weeks), according to the subject's availability. Each participant will sign the consent form during the MRI visit.

MRI visit. The MRI visit will be used to identify single-subject FPN targets for TMS. Subjects participating in multiple sub-studies will complete the MRI visit only when participating in their first sub-study, unless the MRI visit needs to be repeated. First subjects will complete the TMS Adult Safety Screen (TASS) (Keel et al., 2000), to ensure that they meet the inclusionary criteria for TMS. Only subjects eligible for TMS will complete the MRI visit. Subjects in Sub-study 3 will also receive TMS sensation screen. During this screen, subjects will receive a single train of electric scalp stimulation, and asked whether they would be willing to tolerate this level of scalp stimulation during the TMS sessions.

Next, subjects will be setup to go into the MRI scanner. A T1, a T2, and a diffusion weighted echo-planar series will be collected to assist with targeting (Fox, 2009). After, the subject will undergo 1 run of resting state, and 1 run of the Sternberg WM task without shock, to identify single-subject FPN targets for TMS. This visit will be the same for subjects in both sub-studies.

Sub-studies 1 and 2 TMS visit. During the TMS visits, subjects will first be prepped for the procedure. Shock, and physiology electrodes will be attached (See below for details). Subjects will undergo the shock workup and startle habituation procedures. Next subjects will undergo motor threshold testing to determine the proper TMS dose. Afterward the subjects will receive stimulation TMS while completing the task (WM or NPU). Stimulation type will be manipulated within subjects, across trials. Subjects will receive active or sham TMS to the left IPS during random intervals of the NPU task, or during a random selection of WM trials.

Sub-study 3 TMS visits. During the TMS visits, subjects will first be prepped for the procedure. Shock, and physiology electrodes will be attached (See below for details). Subjects will undergo the shock workup and startle habituation procedures. Next subjects will undergo motor threshold testing to determine the proper TMS dose (Visit 1). Afterward the subjects will undergo the NPU task pre and post delivery of offline rTMS. The procedure of the TMS visits will be identical with the following 2 exceptions. First, the motor threshold testing only needs to be completed once, so this will take place on the first but not the second TMS visit. Second, the subject will receive active stimulation during 1 TMS visit will and sham TMS during another visit. The order of the visits will be counterbalanced across subjects.

b. Recruitment

Participants will be recruited under the screening protocol 01-M-0254: "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers." All recruitment materials (paper or web ads, flyers and listserv announcements) will be IRB approved under this protocol prior to use.
IRB-approved advertisements may be placed in local print and online publications of newspapers, magazines and support or health care organizations, the may include the Washington Post, Express, Washington Parent, Washingtonian, Bethesda Magazine, Washington Examiner, Military papers, NAMI Advocate, campus newspapers, the NIH Record/CC News, and the AADA. Web ads will direct readers to the study specific link on the NIMH Join a Study website.

Notecards and/or flyers may be posted on bulletin boards at local establishments including grocery stores, coffee shops, community centers, college campuses, NIH Clinical Center, libraries, or placed in advocacy group offices or in doctor's office waiting rooms with approval of the venue or in accord with their policy. They may be made available at outreach exhibits, speaking engagements, and professional meetings

with approval of the venue or in accordance with their policy. Clinicians who are contacted will be provided with information to disseminate to patients as they see fit. We will explain to them that individuals interested in participating in our studies will need to initiate contact with our group and that we will not make this initial contact. Notecards and/or flyers may be given directly to those requesting study information.

IRB-approved text ads will be sent from Twitter Accounts such as NIH Record to the accounts' Twitter followers. IRB-approved text ads may also be placed on Craigslist.

IRB approved ads will be posted on listservs with the permission of the moderator and IRB required statement on how the receiver was identified. Listservs may include provided by Public Relations and Public Liaison office (e.g. NIMH Outreach Partnership Updates, Club PCR) or local groups (e.g. Community Service Announcements – City of Gaithersburg, Montgomery County Providers, Howard County Providers, Frederick Providers Council, etc.). We will not post/send directly to the listserv. Rather, an email with information about our study information attached will be sent to the administrator of the listserv, which will include the following disclaimer:

"You are receiving this message because your email address is included in the above listserv. The purpose of this message is to inform you of our NIMH research studies. The moderator of the listserv has permitted its use for this distribution."

We will use the Public Relations and Public Liaison (PRPL) list of volunteers. Recruitment efforts will also include the research match website organized by PRPL. We will also use Research Match as a recruiting method. IRB-approved text ads will be sent from Twitter and Facebook accounts such as the NIH Record to the accounts' followers, and may also be placed on Craigslist under the "Volunteer" category.

There is no direct solicitation of employees/staff by supervisors nor co-workers. We understand that all recruitment materials, including those specific for NIH (e.g. for IRTA list-serv) must be IRB approved.

Any new advertisements or changes to existing advertisements will be submitted to the IRB for approval prior to publication. The written advertisements will be used in color as submitted, or may be printed in black and white. The color of the ads may vary. Color changes will not be used to change the emphasis of an ad. The size of the ads may vary, but all parts of the ads, including fonts and pictures, will be changed proportionately to the rest of that ad. Disproportionate changes in size will not be used to change the emphasis of an ad. Email addresses and phone numbers provided on the advertisements may be changed to the NIH email or phone number of other staff on this protocol following any staff changes or changes in the individual responsible for referrals. We anticipate to accrue 2 subjects a week. We anticipate we can have a successful accrual rate with these methods.

c. Screening

All of the screening procedures are conducted under protocol 01-M-0254. After an initial phone screen, eligible participants will sign a screening consent form that will enroll them in a medical and mental screening session. Participants who are cleared to continue are invited to participate in the study. The 01-M-0254 screening protocol uses the following measures to evaluate if they are indeed eligible for the study:

- Subject demographic information
- Structured Clinical Interview for DSM-IV-TR non-patients edition (non-patients only)
- Medical history and physical examination
- Vital signs (sitting blood pressure and pulse), height, weight
- Concomitant medication and pharmacotherapy history
- Family history method to identify medical or psychological history in first-degree relatives that are listed as exclusion criteria
- Wechsler Abbreviated Scale of Intelligence (WASI) (Weschler, 1999)
- Inclusion/exclusion criteria
- Self-administered Questionnaires (Affective Control Scale, Adverse Life Events, Anxiety Sensitivity Index, Mood and Anxiety Symptom Questionnaire, Penn State Worry, State-Trait Anxiety Inventory)
- Pregnancy test (urine beta HCG test) for women of child bearing age
- Urine toxicology screen

Participants who are cleared to continue are invited to participate in the study. Screening results are regularly discussed with the research team at the weekly clinical meetings. Information obtained under the 01-M-0254 screening protocol may be used for research data in this protocol. Dr. Ernst, Medical Advisory Investigator, will ultimately be medically responsible for the enrollment of each subject in the study.

d. Study procedures

Pilot phase: We will begin with an ~10 subject pilot study to determine the parameters necessary to recover usable APS data following a TMS probe. In this pilot study we will present startle probes following no stimulation (control), a TMS pulse, and a sham pulse (with electrical stimulation of the scalp). Pulses will be administered at 100% of the MEP threshold, and delivered to the motor cortex site used to determine the motor threshold, so as to minimize the potential for CNS-based startle reduction. We will then measure the effect of this stimulation on raw startle magnitude, and APS. Importantly, we will vary the duration between the TMS/sham pulses and the acoustic startle probe to identify the minimum pulse/probe interval needed to recover usable APS data. This minimum threshold, anticipated to be ~1 second, will be used in all future studies.

General: As mentioned in "Study overview," subjects in sub-studies 1 and 2 will participate in 2 outpatient visits (1 MRI visit and 1 TMS visit). where they will be assigned to one of two sub-studies (NPU or WM) for the TMS visit. Subjects in sub-study 3 will participate in 3 outpatient visits (1 MRI visit and 2 TMS visits). Visits will be separated by ~1 week (no more than 2 weeks), according to the subject's availability.

Prior to the study visits, the participants will have been screened and cleared under the 01-M-0254 screening protocol. Healthy volunteers will first participate in the MRI visit in the NMR center. The consent form will be signed at the beginning of the MRI visit. Immediately after the consent form is signed, the subject will complete the TMS safety screen (TASS). Subjects who do not meet the criteria for TMS will be withdrawn from the study at this time. Subjects participating in multiple sub-studies will complete the MRI visit only when participating in their first sub-study, unless the MRI visit needs to be repeated. Subjects in Sub-study 3 will also receive TMS sensation screen. During this screen, subjects will receive a single train of 4 mA electric scalp stimulation matching the frequency and duration of the study visit TMS. No actual TMS will be delivered during this visit. The subjects will then be asked whether they would be willing to tolerate this level of scalp stimulation during the TMS sessions. Subjects who are unable or unwilling to continue will be withdrawn from the study at this time. Subjects will be compensated for the MRI visit whether or not they wish to discontinue after the tolerability screen. Next, we will collect at T1, a T2, and a diffusion-weighted echo-planar image using an available 3T scanner. Next subjects will undergo a single run of resting state and a single run of the Sternberg task, without shocks, probes, or TMS pulses to serve as functional localizers to identify a FPN stimulation site for TMS. Subjects who need clinical scans will receive those as well. All procedures are solely for research purposes.

During the TMS visit, the subject will complete a packet of pre-experiment questionnaires. Next the subject will be connected to the equipment. The stimulation device, as well as the electrodes for shock and autonomic measures will be attached, and the subject will be given headphones for the white noise delivery. Subjects will undergo TMS motor threshold testing (during first TMS visit only for sub-study 3). Once the subject is prepped for the experiment, they will undergo startle habituation, and the shock workup. After the shock workup, subjects will complete the task while receiving TMS. Once the task is complete, the subject will complete retrospective questionnaires. For sub-studies 1 and 2, each subject will be assigned to one of two tasks (Sternberg WM or NPU). The TMS visit will be the same for subjects in both sub-studies with the exception of being assigned to one of the two tasks. For sub-study 3, subjects will participate in the NPU task pre and post TMS delivery, which will occur during a run of the Sternberg WM task (for active TMS targeting).

Sub-study 1, Sternberg task: The session will begin with a startle habituation phase with 9 startle stimuli presented alone to reduce startle reactivity. 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 positon of the letter in the series matches the number.

There will be 4, 14 min runs of the experiment (2 active and 2 sham). Within each run, there will be 4 blocks (2 threat, 2 safe). During 1 block each of safe and threat, subjects

will receive a continuous train of 1 Hz stimulation to the left IPS. 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, resulting in a total of 192 trials across the entire experiment (2 [active vs. sham] x 2 [threat vs. safe] x 2 [maintenance vs. ITI] x 2 [TMS vs. no TMS] x 2 [run repetitions] x 2 [block repetitions] x 3 [trial repetitions]). Trials will last approximately 17 seconds. There will be a total of 3 shocks per run presented at random points during the threat blocks.

Sub-study 2, 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 and 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 4, 14 min runs of the experiment (2 active and 2 sham). 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. During half of the blocks (1 predictible, 1 unpredictable, 2 neutral), subjects will receive a continuous train of 1 Hz stimulation to the left IPS. Within each block, there will be 2 types of trials based on the delivery of startle probes. Startle probes will be presented either during the cue period, or the ITI. During the predictable and unpredictable blocks, there will be 4 trials per condition. Given that there will be twice as many neutral blocks, during these blocks there will only be 1 trial per condition. The result will be a total of 192 trials across the entire experiment (2 [active vs. sham] x 2 [neutral vs. predictable vs. unpredictable] x 2 [maintenance vs. ITI] x 2 [TMS vs. no TMS] x 2 [run repetitions] x 4 [block*trial repetitions]). Trials will last approximately 17 seconds. There will be a total of 3 shocks per run presented during either the ITI (unpredictable) or cue (predictable) periods.

Sub-study 3, NPU task: There will be 1 pre-TMS and 1 post-TMS NPU run during each TMS 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. The result will be a total of 192 trials across the entire experiment (2 [active vs. sham] x 3 [neutral vs. predictable vs. unpredictable] x 2 [maintenance vs. ITI] x 2 [pre-TMS vs. post-TMS] x 8 [block*trial repetitions]). There will be a total of 3 shocks per run presented during either the ITI (unpredictable) or cue (predictable) periods.

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.

rTMS targeting: The TMS target will be individualized for each subject as the location of greatest fMRI activation in the DLPFC found in the MRI session in response to the Sternberg task. The fMRI image contrasting responses to high and low set sizes will be overlaid on the subject's structural MRI, and the location in the DLPFC that most activated in that contrast will be chosen as the target for TMS. We will follow the method developed in Herwig et al. (2003): after initial processing, voxel-wise analysis will be performed. In the right DLPFC, voxels of which effects survive p values of < 0.05 in an F test will be accepted when found in clusters of 50 or more. Continuing to follow Herwig et al., when DLPFC contains greater than one cluster with higher activity, the region chosen will be based on a more conservative analysis adjusting the threshold upwards until only one cluster remains. Conversely, when no clusters appear in DLPFC at more conservative significance levels, the threshold will be adjusted downwards until one cluster appears. It should be noted that this liberal thresholding procedure is being used for localization of activation only, and is not a statistically rigorous method of correction. Further, if no cluster emerges at a $z=1.5$ ($p<.05$) level, the coordinates for the significant activation in DLPFC for this contrast in the published group data will be substituted. To identify the target of stimulation for the left IPS, the same approach will be applied. However, rather than using the task-based data, we will calculate global brain connectivity from the resting state data, and extract the IPS location with the greatest global brain connectivity. TMS coil placement will be performed using Brainsight (Rogue Research, Inc., Montreal Canada), a frameless stereotaxic system for neuronavigation, which can co-register scalp positions directly onto the individual's structural MRI scan (with fMRI scan overlaid). This system offers real-time three-dimensional display of cortical localization as the TMS coil is moved across the scalp, and permits the targeting of individual sites of activation found by fMRI with millimeter resolution.

TMS: Subjects will be brought to the Noninvasive Neuromodulation Unit Laboratory (Building 10, room 7-5485) for the experimental sessions. They will be seated comfortably in a padded chair designed for TMS procedures, and hearing protection will be worn. A Magventure MagPro 100 stimulator will be used. A Figure 8 TMS coil will be placed on the head over the targeted cortical location, and trains of rTMS. rTMS intensity will be 100% of the individual's EMG-determined resting motor threshold, adjusted for scalp-to-cortex distances at motor cortex and target cortex using the subject's T1. For online targeting, a continuous train of 1 Hz stimulation will be applied on a per block basis according to the design of each experiment. 1 Hz was chosen because rTMS at frequencies below 1 Hz tends to decrease local excitability (Di Lazzaro et al., 2011). For offline targeting, 4 second trains of 10 Hz rTMS will be applied at a maximum frequency of 2 trains per minute for approximately 20 minutes. Stimulation will take place during the Sternberg WM paradigm, which should engage the target dlPFC circuits, thus increasing the efficacy of the TMS. 10 Hz was chosen because it is a frequency often used in offline stimulation protocols used to treat depression (Horvath et al., 2010; O'Reardon et al., 2007; Shajahan et al., 2002; Stern et al., 2007). For sham stimulation, we will use a placebo coil with the exact same characteristics as the active coil. However, the placebo coil will have a magnetic shield that will provide a field reduction of ~80%, and the coil has an integrated e-stim for delivering small current pulse synchronous to the TMS pulse. The e-stim placebo coil administers a brief electric pulse to the scalp to replicate the current induced by the TMS pulse in the active condition. Importantly, the current generated by the e-stim placebo coil is similar in magnitude, duration, and distribution to the current induced by the TMS pulse (Borckardt et al., 2008; Gonz et al., 2011; Rossi et al., 2007; Sheffer et al., 2013). In addition, although electrically and magnetically stimulated blinks differ in topology from acoustically generated blinks, they are nearly identical to each other in that respect (Blumenthal et al., 2005).

Startle Reflex: 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 as we have done in all of our previous psychopharmacology studies.

Electric Shock: 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 the TMS 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 experiment. 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.

Autonomic measures: In addition to the acoustic startle reflex, we will also record the heart rate and the skin conductance for exploratory purposes. These measures will not

add discomfort to the subjects. The heart rate will be monitored with two disposable electrodes on the ribcage midway between the waist and the armpit. The skin conductance will be measured using two (Ag-AgCl) electrodes in conjunction with a .05M NaCl electrolyte. Electrodes will be placed on the distal phalange of the index and second finger of the left hand. These measures will not add discomfort to the subjects.

Self-report Questionnaires: Subjects will be asked to fill out self-report questionnaires during the experiments (i.e. TMS adult safety screen (Keel et al., 2000), the State Anxiety Scale (S-Anxiety)(Spielberger, 1987), the Beck Anxiety Inventory (Beck et al., 1988), Retrospective Threat Questionnaire). During the TMS visit, 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. The non-analog measures from these reports may be completed by the subject on the Clinical Trial Survey System (CTSS) online system. AIs may then collect data from the Clinical Trials Database (CTDB) for the purposes of this study. Subjects may enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. There are no actionable items with use of CTSS requiring real-time monitoring.

Urine Tests: Participants will also undergo a urine pregnancy prior to the visits.

e. End of participation

Participants may be discharged after the end of the procedures. Adverse effects will be followed up by the medically responsible physician or nurse practitioner and medical care will be provided as needed.

Individual research results are not shared with the participants. Medical information will be shared with the primary provider upon request if there are abnormal findings upon physical exam, brain scan, or from laboratory findings.

5. Management of Data and Samples

a. Storage

All electronic records will be kept confidential according to NIH Clinical Center's standard policies.. Participants' names and other personal identifying information will be stored in electronically secured databases at the study sites. These databases are 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.

b. Data sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) Policy. Data may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

No additional considerations are applicable.

a. Research with investigational drugs or devices

rTranscranial Magnetic Stimulation investigational device is used for this protocol. The NSR device is not used for FDA approved treatment and used off-label as an investigational device. MagPro x100 manufactured by MagVenture was used for this research study with 510K #K091940. FDA information can be found here: https://www.accessdata.fda.gov/cdrh_docs/pdf9/K091940.pdf

b. Gene therapy

Not applicable.

7. Risks and Discomforts

a. Procedures

Questionnaires: 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.

Psychophysiological 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. The risk is equivalent to that of an EEG recording.

Topical anesthetics: We will offer a topical lidocaine cream to the individuals receiving frontal TMS. This may cause skin redness, swelling, or skin irritation. These symptoms are typically minor and temporary.

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.

Auditory startle stimulus: 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 1000 of subjects with no adverse reactions. The auditory stimulus may trigger a migraine.

TMS: In 2008, the FDA approved the use of high frequency rTMS in the treatment of depression. In 2009, a safety guideline was published whereupon the authors systematically reviewed the thousands of healthy subjects and patients who have undergone rTMS in order to allow better assessment of relative risk (Rossi et al., 2009). The relative infrequency of adverse events using rTMS was noted include the rTMS intensity and timing parameters considered safe, training, and planning for and managing emergencies. We will follow these guidelines, and have incorporated them into our screening and session procedures.

Although there are no known long-term health risks to the use of TMS when operated within consensus safety guidelines (Rossi et al., 2009), the greatest potential short-term risk in the use of TMS is seizure. The Rossi et al. (2009) consensus safety report stated that "the occurrence of seizures has been extremely rare, with most of the few new cases

receiving TMS protocols exceeding previous guidelines, often in patients under treatment with drugs which potentially lowered the seizure threshold." As Rossi et al. delineate, "rare" means that up to the end of 2008, 16 cases of seizure related to TMS had been reported, out of tens of thousands of TMS sessions over the last two decades. Seven occurred before safety parameters were established in 1997. These seizures appeared to be the result of excessive stimulator intensity, pulse frequency and train duration and too short inter-train intervals, in various combinations, and resulted in the establishment of safety guidelines for each parameter. Higher frequencies of TMS are most closely associated with seizure risk. While high frequency TMS will be employed in this protocol (5 Hz rTMS), the parameters to be used are well within suggested safety limits.

Aside from seizure, other adverse effects of TMS include headache, vasovagal syncope and hearing effects. Stimulation over superficial scalp tissue can result in headaches, typically of a muscle-tension type, and local muscle aches, especially with high-frequency TMS. They usually develop during or immediately after the stimulation, may last for minutes to hours following the end of the stimulation, and usually respond promptly to single doses of over the counter pain medications. Cramped conditions extended in time within frameless stereotaxic apparatus can also lead to head and body aches. Both are usually managed easily with over-the-counter analgesics. The sensation of the TMS pulses on the scalp can trigger vasovagal syncope (i.e. fainting) in some individuals, but these episodes typically resolve without the need for follow-up (Gillick et al., 2015; Kesar et al., 2016; Riedel et al., 2016). In addition, hearing loss and tinnitus may result from TMS. The clicking sound made by the TMS coil at high intensities may not sound extremely loud, but this is deceptive: subjective loudness is a function of both sound intensity and duration, and the duration of a TMS pulse is less than a millisecond. The sound intensity can be quite high, exceeding 100 dB and federally-recommended safety levels. It has been recommended that all participants wear ear protection with TMS exposure (Rossi et al., 2009).

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 at the NIMH. Potential risk of heart rhythm disturbances exists for patients with a history of heart rhythm abnormalities or those who have certain types of pacemakers. A substantial risk to persons who have metallic objects inside their bodies exists, as the magnet in the scanner can cause these to move. Pregnant women should not undergo MRI because of the possible harmful effects to the fetus.

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. fMRI scanner is very noisy for this reason participants wear ear plugs, or headphones that are designed to reduce the noise impact of the scanner. The enclose space of the scanner can be uncomfortable. Participants have access to a panic button at all times and can press this to stop the scan and be removed from the scanner.

b. Procedures to Minimize Risks

Electric Shock: Shock will be delivered at a level that is judged by the subject as uncomfortable but tolerable. 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: Seizure is a theoretical risk with rTMS. In the Rossi et al. report it was stated that: "The occurrence of seizures has been extremely rare, with most of the few new cases receiving rTMS protocols exceeding previous guidelines, often in patients under treatment with drugs which potentially lowered the seizure threshold." As Rossi et al. delineate, "rare" means that 16 cases (out of tens of thousands of rTMS sessions over the last two decades) of seizure related to rTMS have been reported. Eight occurred before safety parameters were established in 1997. Of the other eight reports, six occurred either when the safe rTMS parameters were exceeded or other safety guidelines ignored, and the actual occurrence of a seizure has been questioned in the other two (i.e., convulsive syncope or pseudoseizure may have occurred). In a workshop convened by the National Institute for Neurological Disorders and Stroke (NINDS) in 1996 (including the PI here, Dr. Lisanby, and one of the investigators, Dr. Luber), researchers in the field agreed upon a set of *rTMS consensus safety guideline*s, including recommended stimulation parameters and contra-indications (Wasserman, 1998), and these consensus guidelines have been updated (Rossi et al., 2009). Widespread adherence to the 1996 guidelines has resulted in the virtual elimination of inadvertent seizures in rTMS studies (Rossi et al., 2009). The levels of stimulation used in this protocol are well within safety guidelines (Rossi et al., 2009; Wasserman, 1998).

We will screen subjects for known risk factors for seizure with rTMS (using the TASS questionnaire) after consent during the MRI visit (Keel et al., 2000). Personnel who administer rTMS are trained to recognize a potential seizure event and to act as "first responders" in order to administer appropriate initial care. All study personnel have undergone first-aid and CPR training, and seizure-specific training. The major physical signs the study personnel will look out for in detecting a potential seizure include chewing movements, convulsions/tremor/shaking, difficulty talking, a blank stare, eyes rolling up, and profuse sweating. If any of these signs are observed, study personnel will stop the research procedure and inquire whether the subject feels okay. If the subject is unresponsive (and therefore likely experiencing a seizure), first-aid will be supplied. The first-aid response consists of making sure the subject is physically safe for the duration of the seizure. This involves moving the subjects out of the TMS chair and onto the floor lying down on his or her left side. The subject will be kept lying down on his or her left side, while the staff call call a code blue, as per NIH Clinical Center policy. Resources available in the laboratory include a first-aid kit and immediate phone access. A seizure constitutes a reportable adverse event, and will thus be immediately reported to the IRB via the Safety Events Form mechanism.

The most commonly reported side effect of rTMS is headache. This headache is typically of a muscle-tension type. It usually develops during or immediately after the

stimulation and may last for minutes to hours following the end of the stimulation. It is typically limited to the day of stimulation, and usually responds promptly to single doses of over the counter pain medications. Neck pain or scalp pain may also occur. This is especially true for TMS to frontal regions. For this we may offer a topical lidocaine cream to reduce the scalp pain for sub-study 3 which employs frontal TMS. Both are usually managed easily with over-the-counter analgesics. We will also give subjects a chance to experience the sensation of the frontal TMS for sub-study 3 via electric scalp stimulation, and they will have the opportunity to discontinue if they find the sensation unpleasant.

Subjects will be monitored continuously during TMS administration. In the event of a syncopal episode, all study procedures will be stopped. The subject will then be given first aid by the study staff, and evaluated by the LIP in charge.

As noted in Rossi et al. (2009), Loo and colleagues reported found mild and transient changes in auditory threshold in two depressed patients following a 2-4 week rTMS course of rTMS (Loo et al., 2001). Cases of tinnitus have been reported after rTMS treatments. In addition, recently in a study investigating the effects of rTMS on symptoms of depression, a patient experienced moderate to severe tinnitus after an rTMS session in which hearing protection was not used. Rossi et al. recommended that hearing protection always should be worn during rTMS application, and that individuals with cochlear implants not receive rTMS. Hearing protection will be worn by all subjects during rTMS procedures. Individuals with cochlear implants will be excluded from participation.

Risks to the unborn children of pregnant women receiving MRI and TMS are unknown. Pregnant women will be excluded as per CC 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. Females of childbearing potential will undergo either serum or urine pregnancy testing to rule out pregnancy no more than 24 hours prior to each MRI session before the scanning procedures are initiated. 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.

8. Subject Safety Monitoring

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. Subjects receiving the frontal TMS will also be given a chance to experience the sensation of the TMS via electric scalp stimulation. Subjects will be given the opportunity to discontinue after this sensation screen. 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 via closed circuit video by the AI for MRI sessions. An AI is present during TMS sessions. A credentialed staff member (RN, NP or MD) will assess the subject at the conclusion of the study or at any time if significant side effects develop. The subject will be withdrawn at any time if unable to follow the rules for participation in this study.

9. Outcome Measures

a. Primary outcome measures

The primary outcome measures will be APS reduction as a function of stimulation type, active or sham stimulation.

10. Statistical Analysis

a. Analysis of data/ study outcomes

Sternberg. APS scores will created by subtracting the startle magnitude during the safe blocks from the startle magnitude during the threat blocks. These scores will be analyzed using a 2 [block: active vs. sham] x 2 [stimulation: TMS vs. no TMS] x 2 [interval: maintenance vs. ITI] repeated measures ANOVA.

NPU. APS scores for the cue and ITI will be created by subtracting the startle magnitude during the safe blocks from the startle magnitude during the unpredictable blocks. FPS scores will be calculated by subtracting the startle magnitude during the predictable ITI from the startle magnitude from the predictable cue. These scores (APS cue, APS ITI, FPS cue) will be analyzed using a 2 [block: active vs. sham] x 2 [stimulation: TMS vs. no TMS] repeated measures ANOVA.

b. Power analysis

In our Sternberg pilot experiment, we obtained the effect size (f=0.72) for our observed WM load-related decrease in anxiety. Assuming a somewhat smaller effect size (f=0.5) due to regression to the mean, if we set power at .95 and experiment-wise, two-tailed alpha at 0.05. Based on these parameters, we will need 48 subjects per experiment. Assuming a 15% unusable data rate and 3 subjects to initially adjust the experimental procedure, we will need 58 subjects per experiment.

11. Human Subjects Protection

a. Subject selection

This study will be conducted in healthy males and females between the ages of 18-50. Participants will be recruited without any preference based on gender, race, religion, or other social variables. We have attempted to make our subject selection as equitable as possible.

Justification for exclusion of children

We want to test this paradigm in adults first to examine the scientific questions in the non-developing brain. If the findings are according to hypotheses, we would consider examining these questions in the pediatric population.

Justification for inclusion of other vulnerable subjects

This study will include only healthy volunteers.

Justification for exclusion of other vulnerable subjects

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, or otherwise deemed to lack capacity to consent.

Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. This study collects sensitive information (e.g. drug and alcohol use, specific medical diagnoses). The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. Information about sensitive information (e.g. drug and alcohol use, specific medical diagnoses) will be in the participant's NIH medical record.

Justification for exclusion of non-English speaking subjects

We exclude non-English speakers since not all the instruments and test we use are translated and validated in Spanish or other languages.

Justification for exclusion of subjects over 50 years of aged

As people get older, their startle response decreases (Le Duc et al., 2016). The upper age limit will be 50 years old to minimize the confounding effects of age.

Justification for exclusion of non-right-handed subjects

We have chosen to exclude individuals who are not right-handed because our hypothesis is based on pilot data showing lateralized patterns of activity in the prefrontal cortex. Given that our pilot data was recorded in right-handed individuals, it is unclear whether the results generalize to non-right-handed individuals.

Justification for exclusion of subjects with a history of drug or alcohol abuse or dependence

We will exclude subjects with a lifetime history of drug or alcohol dependence or abuse in order to select participants whose CNS physiology will not be confounded by changes induced by substance use.

Justification for exclusion of NIMH staff

We will exclude NIMH employees and staff to prevent bias from previous or current professional relations.

Justification for exclusion of pregnant or breast-feeding women

We exclude and rule out pregnant women via a pregnancy test because of the unknown effects of the shocks and of the fMRI on the developing fetus.

Justification for sensitive procedures

Electric shocks are used as the stressor in this study. Our use of electric shocks stems from our experience that electric shocks are among the most efficient ways to induce anxiety in the laboratory setting. Because of this advantage along with the fact that such

shocks have been very well tolerated by 100's of past participants in our psychophysiology experiments, we decided to again use electric shocks in our neuroimaging investigations described herein. TMS will be used to stimulate the dlPFC. According to our previous research, this should reduce anxiety potentiated startle. We chose TMS because it allows us to stimulate the dlPFC online, during the task, which is not possible with other methods like tDCS.

Safeguards for vulnerable populations and sensitive procedures

We assess pregnancy via urine test within 24 hours of any MRI session and exclude all participants who are pregnant. Neither participation nor refusal to participate as a subject will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. The NIH Information Sheet on Employee Research Participation will be available to employees considering enrollment. All staff administering sensitive procedures will be trained and competency to administer procedures verified by the PI or AI.

b. Qualifications of investigators

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

Christian Grillon, Ph.D. is the Unit Chief of the Affective Psychophysiology, National Institute of Mental Health. Dr. Grillon received his B.S. and Ph.D. from the University of Paris XI, France. He completed his post-doctoral training at UC Irvine and UC San Diego. Before joining the NIMH in September of 2001, he was Associate Professor at Yale University School of Medicine. His research is reflected in over 90 articles and focuses on the neurobiology of anxiety and anxiety disorders, and the psychophysiology of emotion. His role also will include supervising the experiment design and the post doctorate and post baccalaureate training award students who run the study, and analyzing the data. He will be able to obtain consent.

Nicholas Balderston, Ph.D. is a postdoctoral fellow in the Section on the Neurobiology of Fear & Anxiety. He completed an undergraduate degree in psychology at the University of West Florida. He then joined the lab of Dr. Fred Helmstetter in the psychology department at the University of Wisconsin-Milwaukee, where he investigated the role of awareness in fear conditioning using psychophysiology and fMRI. For his dissertation he received a fellowship to collaborate with Dr. Catherine Tallon-Baudry in the laboratoire de Neurosciences Cognitives at the École Normale Supérieure, where he investigated trace fear conditioning with magnetoencephalography. Dr. Balderston will be involved in experimental design, subject testing and data analysis for this protocol. Dr. Balderston is qualified to obtain informed consent.

Monique Ernst, M.D., Ph.D. will act as the Principle Investigator and the Medical Advisory Investigator. She is a credentialed staff clinician at the NIH Clinical Center and an experienced psychiatrist who has worked in clinical research for more than a decade. Dr. Ernst will be involved in the clinical oversight of the study, supervising the study design, and implementation of the study. She will provide clinical coverage and monitor side effects. She will be able to obtain consent.

Sarah Lisanby, M.D. is Director of the Division of Translational Research (DTR) at the NIMH, and directs the Noninvasive Neuromodulation Unit (NNU) of the Experimental Therapeutics and Pathophysiology Branch Research Unit (ETPB). Previous to her appointment at the NIMH, she had been serving as Chair of the Department of Psychiatry and Behavioral Sciences at Duke University School of Medicine, and has 20 years of experience in brain stimulation research. She successfully ran a DARPA-funded study to use TMS to remediate working memory deficits using the Sternberg task from the present protocol. Her role will be to oversee the use of the NNU resources, and to ensure that all procedures related to TMS administration are conducted properly. She will be able to obtain consent.

Bruce Luber, Ph.D. is Staff Scientist in the NNU of the ETPB at the NIMH. Dr. Luber has 20 years of experience as lead in numerous studies using TMS and other brain stimulation techniques, especially as they have to do with elucidating the mechanisms of cognition, perception, memory and executive function. He has specialized in using TMS to produce cognitive enhancement, and has run a number of NIH funded studies to develop techniques to remediate working memory deficits in aging. With Dr. Lisanby, he has developed fMRI-guided TMS paradigms to specifically study the working memory task used in the present protocol. His role will be to train users of the TMS equipment, and to ensure that the equipment is used properly. He will be able to obtain consent.

Thomas Radman, Ph.D. is a staff engineer in the Non-invasive Neuromodulation Unit in the Experimental Therapeutics and Pathophysiology Branch, NIMH. He has experience at FDA reviewing safety and effectiveness data of neuromodulation technologies, and research experience on the physiological effects of TMS. He will be an associate investigator for the study and will assist in study trials and data analysis. He will not be consenting subjects.

Valeria Martinez-Kaigi, M.S. is a predoctoral fellow with the Experimental Therapeutics & Pathophysiology Branch (ETPB) and Section on the Neurobiology and Treatment of Mood Disorders. She has experience working with TMS and with patients in a research setting. She will help with recruitment, administrative issues, , and management of the NNU resources. She will not obtain informed consent.

Morgan Roberts, Ph.D. is a research coordinator in the Section of Neurobiology of Fear and Anxiety in the National Institutes of Mental Health. She earned her M.S. in counseling psychology from the University of Kentucky and is currently a doctoral candidate at the George Washington University. She has completed the fMRI safety training course. Her management of the IRTAs work will be supervised by Dr. Ernst. Her

role will be to help with recruitment, administrative issues, and management of the SNFA resources. She will not be able to obtain consent.

Salvatore Torrisi, Ph.D. is a postdoctoral Fellow in the Section on the Neurobiology of Fear & Anxiety. Dr. Torrisi received an MFA in Computer Music Composition from California Institute of the Arts, and an MA in Applied Linguistics and a Ph.D. in neuroscience from the University of California, Los Angeles (UCLA). His Ph.D. at UCLA was conducted under the guidance of Drs. Lori Altshuler and Susan Bookheimer in the Mood Disorders Research Program from 2008-2013, where he studied fMRI-based brain connectivity during emotion regulation in Bipolar Disorder. Dr. Torrisi will be involved in subject testing and data analysis on this protocol. He will not be able to obtain informed consent.

Adam Gorka, Ph.D. is a postdoctoral fellow in the Section on the Neurobiology of Fear & Anxiety. He completed an undergraduate degree in psychology at the University of Pittsburgh. He then completed his Ph.D under the supervision of Dr. Ahmad Hariri at Duke University. His doctoral work focused on the neural and personality correlates of avoidance behavior using psychophysiology and fMRI. Dr. Gorka will be involved in subject testing and data analysis. He will not be able to obtain informed consent.

Tiffany Lago, M.D. is a PGY4 psychiatry resident/ clinical fellow in the Section on the Neurobiology of Fear & Anxiety. She completed an undergraduate degree in psychology at Barrett, the Honors College at Arizona State University. Her honors thesis was on the effects of dam stress on rat pups' emotionality and hippocampal functioning in adulthood under the mentorship of Cheryl Conrad, Ph.D. and Linda Leucken, Ph.D. She then joined the Behavioral Endocrinology Branch of NIMH as an IRTA with Peter Schmidt, M.D., where she studied the effects of ovarian steroids and affective state on spatial memory. She attended medical school at Indiana University School of Medicine and completed her PGY1- PGY3 years of residency at Boston Medical Center. Dr. Lago will be involved in subject testing and data analysis for this protocol. Dr. Lago is qualified to obtain informed consent.

Marilla Geraci, RN, is a credentialed RN, and senior clinical research nurse. Ms. Geraci has provided nursing support for many NIMH intramural studies in anxiety disorders, and has been included as a coauthor on publications. She began working with SNFA studies in 2004. She will provide nursing coverage and assess the subjects during testing. Ms. Geraci will obtain informed consent.

Patrick Korb, RN, is a Clinical Research Nurse. Mr. Korb will be providing clinical service for Dr. Grillon and Dr. Ernst. He will provide nursing coverage and assess the subjects during testing. Mr. Korb is qualified to obtain informed consent.

Emily Page, RN, FNP-BC - Nurse Practitioner, Molecular Imaging Branch, NIMH/NIH; Emily has been a nurse practitioner for 5 years and a nurse for 8 years prior to that. She is a credentialed clinician at the NIH Clinical Center and has patient care credentials including prescriptive authority from the NIH Clinical Center. She previously worked on

PET imaging protocols with the Innis lab. She has experience working with patients with Alzheimer's disease, Frontotemporal Dementia, TBI, MDD, and healthy volunteers. She will be an active medical investigator by providing screening for medical and psychiatric disorders, implementing treatment when appropriate, and providing referrals as necessary. She will provide clinical coverage. She will be able to obtain consent.

Yumi Yi C.N.R.P. is a nurse practitioner in the ETPB. She is board certified by the American Nurses Credentialing Center as an Adult Psychiatric Nurse Practitioner. She has extensive experience providing comprehensive primary and mental health care services. She will provide clinical care for the TMS study visit. She is able to obtain informed consent.

Lorie Shora, **RN, MSN, FNP/CHP**, is a nurse practitioner in the ETPB. She is Board certified by the American Nurses Credentialing Center as a Family Nurse Practitioner and is ACLS certified. She has extensive experience providing comprehensive primary and mental health care services. Ms. Shore will provide clinical care for the TMS study visit. Ms. Shora is authorized to obtain informed consent.

Zhi-de Deng, Ph.D. is a postdoctoral fellow in the NNU of the ETPB. He is expert in modeling noninvasive neurostimulation, in neurostimulation devices, and in the analysis of brain imaging and electrophysiological data. Dr. Deng will assist with transcranial magnetic stimulation procedures and research/data analysis. He will not obtain informed consent.

Susan Goo, MSN, is a Senior Clinical Research Nurse for the NIH Clinical Center Nursing Department. Her education includes a bachelor's degree in Psychology from the University of California, Irvine and a Master's Degree in Nursing from California State University, Fullerton. Over the course of her 33 year nursing career she has worked in a variety of settings, primarily in psychiatric nursing. Currently, she is the primary nurse for the National Center for Complementary and Integrative Medicine (NCCIH), Pain and Integrative Neuroscience (PAIN) group, where she provides nursing assessments, administers intravenous drugs, and monitors effects of medications and procedures. She is the Clinical Educator for the Outpatient Behavioral Health Clinic. Her current certifications include: Curriculum in Good Clinical Practice (GCP), CPR Basic Life Support, and Nonviolent Crisis Intervention. She will be providing clinical service for Dr. Grillon and Dr. Ernst. She will provide nursing coverage and assess the subjects during testing. She will be fully trained and approved by a senior staff member that she is able to demonstrate understanding of the study procedures and risks prior to obtaining informed consent. She has taken the required "Elements of Informed Consent" training. Ms. Goo is qualified to obtain informed consent.

Martina Lavrisha, RN, MSN MPH is a Clinical Research Nurse at NIH since 2009 and on OP4 since 2016. She will coordinate the nursing services for Drs. Grillon & Ernst, in addition to completing nursing assessments and care for the SNFA studies. She will be fully trained and approved by a senior staff member that she is able to demonstrate understanding of the study procedures and risks prior to obtaining informed consent. She

has taken the required "Elements of Informed Consent" training. Ms. Lavrisha is qualified to obtain informed consent.

Eudora Jones, CRNP, is a licensed Nurse Practitioner within the Noninvasive Neuromodulation Unit (NNU). Dr. Jones is board certified by the American Nurses Credentialing Center (ANCC) as a Psychiatric-Mental Health Nurse Practitioner. Dr. Jones is also Advanced Cardiac Life Support (ACLS) cerified. Dr. Jones has numerous years of experience providing psychiatric care in both inpatient and outpatient settings. Dr. Jones may prescribe medication, perform psychological evaluations, conduct physical examinations, and participate in assigned research protocols. She is qualified to obtain informed consent.

12. Anticipated Benefit

There is no direct benefit to the participants but is likely to yield generalizable knowledge about the underlying brain mechanism of fear and anxiety.

13. Classification of Risk (for the study as a whole)

a. For adults classify risk as ONE of the following:

More than minimal risk.

b. For adults without consent capacity (if applicable) classify as ONE of the following:

Not applicable.

c. For children classify as ONE of the following:

Not applicable.

d. Overall risk and benefit consideration

The risks are reasonable in relation to anticipated benefit.

14. Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in section 11.b above, will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

b. Consent procedures

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.

c. Consent documents

A healthy volunteer consent is submitted with this protocol. The consent form contains all required elements.

15. Data and Safety Monitoring

a. Data and safety monitoring

Data and safety will be monitored by the Principal Investigator. This study will be monitored by an independent safety monitor. Dr. Pedro Martinez will serve as the independent monitor for this study.

Independent Safety Monitor

Name, Degree	Branch/Institute	Bldg/Rm	Phone	E-mail
Dr. Pedro Martinez	NIMH//BEB/SBE	10-CRC 2-5330	301.402.0615	martinep@mail.nih.gov

b. Data and safety monitoring plan

The PI will prepare a report on data and safety parameters for the Independent Monitor annually. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

In the event of unanticipated problems or serious side effects, the Principal Investigator and/ or the ISM may decide to stop or suspend the study. The Combined Neuroscience IRB safety subcommittee may also decide to stop or suspend the study for safety reasons. Safety events that would warrant suspension or stopping of the study include repeated serious adverse events related to the study procedcures.

The study will be suspended if there is any UP or SAE until the MAI and IRB review the event and approve continuation.

16. Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the PI and research team and the NIMH Office of Regulatory Compliance (ORO).

b. Quality assurance plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the CNS IRB. This study will undergo audits at least once every three years and for cause.

17. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, 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 PI in consultation with the CD.

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems, and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

a. For Drugs and Biologics

Not applicable.

b. For Devices:

Not applicable.

18. Alternatives to Participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

19. Privacy

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

20. Confidentiality

a. For research data and investigator medical records

We will actively protect confidentiality of the subjects and the data in each step. Information will be stored using a confidential case number, and no identifiers (name, address, etc.) will be used that could allow direct linking of database information to individual subjects.

Data will be kept in password-protected computers. Only study investigators will have access to the data with personal identifying information. Results will be published as group data without the use of characteristics that would identify individual subjects.

The PI will train study staff regarding obtaining and handling potentially sensitive and private information about NIH employees and staff through staff discussions and written branch/section procedures. Information about sensitive information (e.g. drug and alcohol use, specific medical diagnoses) will be in the participant's NIH medical record.

b. For stored samples

Not applicable.

c. Special precautions (e.g. certificate of confidentiality; attach documentation if certificate used)

Data will be stored using codes that we assign. Data will be kept in password-protected computers. Only study investigators will have access to the data.

21. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts-of-interest to report.

c. Role of a commercial company or sponsor

Not applicable.

22. Technology Transfer

No technology transfer agreement is in place for this protocol.

23. Research and Travel Compensation

Volunteers will be compensated for participation based on NIH standards. Travel compensation is not provided. If subjects do not complete the study they will be paid half of the compensation. NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

VOLUNTEER PAYMENT SCHEDULE

Procedure	Duration	Amount
Outpatient Visit for MRI	3 hrs	$170
Outpatient Visit for TMS session	3 hrs	$170

24. References

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25. Attachments/ Appendices

- Eligibility checklist

- Recruiting advertisements
- PRPL Questions

26. Consent Forms

- Healthy volunteer consent
- Pilot consent form
- Offline consent form