Title Page
Protocol Title: Effects of arousal and stress on classical conditioning
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Total requested accrual
0 Patients
1575 Volunteers

Project Uses Ionizing Radiation: x☐ No ☐ Yes (attach *RSC/RDSC documentation)*
☐ Medically-indicated only
☐ Research-related only
☐ Both

IND/IDE ☐ No X☐Yes *(attach FDA documentation)*
Drug/Device/#:
Startle Device
Acoustic Startle
Sponsor: N/A

Durable Power of Attorney x☐ No ☐ Yes
Multi-institutional Project x☐ No ☐ Yes
Institution_____ FWA #_____
Date of IRB approval_____ *(attach IRB documentation)*

Data and Safety Monitoring Board x☐ No ☐ Yes

Technology Transfer Agreement x☐ No ☐ Yes
Agreement type and number _____Expiration Date_____

Confidential Disclosure Agreement x☐ No ☐ Yes

Samples are being stored x☐ No ☐ Yes

Flesch-Kincaid reading level of consent forms:

Shock Stress 7.8
Sural Nerve Stimulation 8.3

PRECIS

Objective: Fear and anxiety are adaptive responses to different types of threats. Fear is a short-duration response evoked by explicit threat cues and anxiety a more sustained state of apprehension evoked by unpredictable threat. This protocol studied fear using Pavlovian fear conditioning in two studies, Studies 1 and 3. Study 2 focused on anxiety. Studies 1 and 3 will be discontinued to focus uniquely on the study of anxiety. Specifically, we will examine the interactions between anxiety induced experimentally using verbal threat and cognitive processes. We will seek to 1) characterize the effect of anxiety on key cognitive processes including working memory and attention control and 2) examine the extent to which performance of cognitive tasks distract from anxiety.

Study population: This more than minimal-risk protocol will test medically and psychiatrically healthy volunteers aged 18-50. Pregnant or nursing women will be excluded.

Method: Fear and anxiety will be measured using the startle reflex to brief and loud sounds. Fear conditioning will be assessed using shock as unconditioned stimulus. Cognitive performance will be examined during periods of unpredictable shock anticipation.

Outcome measures: The study will include cognitive performance and measure of aversive states, primarily the startle reflex.

Contents

Abbreviations

CS	conditioned stimuli
US	unconditioned stimuli
CR	conditioned response
CTXacq	acquired contextual stimuli
CTXext	extinction contextual stimuli
CTXnov	novel contextual stimuli
WM	working memory
SART	Sustained attention to response task

1. INTRODUCTION

The overall objective of this protocol since its inception has been to enhance our understanding of fear and anxiety, and associative learning using experimental approaches from two perspectives: fear conditioning and unpredictability. Specifically, we have argued that fear and anxiety can be differentiated by contrasting defensive responses to short-duration conditioned stimuli (CS) that predict unconditioned aversive stimuli (US, e.g., shock) and long-duration context associated with unpredictable US, respectively. The specific objectives and summary of findings of studies that have been completed are presented in the Appendix. Tasks that were planned but never implemented are also described in the Appendix. We will no longer study fear and will now focus our research on anxiety by examining its interaction with cognition.

Despite increasing knowledge of affective components as well as neural and psychopharmacological mechanisms of anxiety, very little is known about the role of cognitive processes and how the affective and cognitive components of anxiety interact. Cognitive processes could be involved in the etiology and/or maintenance of anxiety. It is clear that the maintenance of anxiety depends on cognitive factors such as attention, attention control, orienting, and working memory (Clark and Squire 1999; Carrillo, Gabrieli et al. 2000; Sarter, Bruno et al. 2003). In addition, prominent symptoms of anxiety are cognitive. Anxious people complain of difficulty concentrating because they are highly distractible. It is therefore possible that poor cognitive functioning contributes to interference from intrusive anxious thoughts (Eysenck, Derakshan et al. 2007). Context, such as physical exertion can influence anxiety and its effects on cognitive performance (Chang, et al., 2012).The present protocol builds on our past results to study the impact of induced anxiety (e.g., during anticipation of unpredictable shock) on physiological and subjective measures of anxiety and task performance in healthy volunteers.

This protocol was initially separated into three objectives: 1) Fear conditioning, negative valence, and executive functions (Study 1), 2) Interaction between cognition and anxiety (Study 2), and 3) mechanisms of extinction and retrieval of fear memories (Study 3). The protocol studied fear using Pavlovian fear conditioning in two studies, Studies 1 and 3. These two studies will be discontinued to focus uniquely on the study of anxiety (Study 2). With the removal of Study 1 and Study 3 leaving only Study 2, we have dropped the label of Study 2. The protocol will only contain information on the current study (previously labeled Study 2). The completed or canceled studies are described below, including Study 2 sub-studies, along with a brief summary of outcomes.

Study 1
Study 1 is completed. The goals were reached and the study has been stopped.

Brief Description: Study 1examined affective and cognitive processes that affect fear conditioning. Study 1 included three separate sessions, each lasting approximately three hours. All three sessions were to be completed within a period of 6 months.

Outcome: Panic disorder is characterized by sudden, repeated, and unexpected attacks of intense fear and overwhelming anxiety about when another attack may strike. We have reported that patients with panic disorder and healthy individuals with a history of panic attacks show a hypersensitivity to unpredictable threats, suggesting a possible link between panic and sustained anxiety. The main objective of this study was to examine the degree to which CO2-induced symptoms of panic related to fear evoked by predictable shock or anxiety evoked by unpredictable shock, as well as activity in the neural systems that mediate and regulate these affective states. Psychological and physiological symptoms of panic were assessed during an 8-min 7.5% CO2 challenge task. Psychological, physiological, and neural symptoms of fear and anxiety were measured during 2 sessions (1 psychophysiology and 1 fMRI) where subjects experienced several blocks of no threat (N), predictable shock (P), and unpredictable shock (U) (NPU threat task) (Balderson et al, 2017). We used a principle component analysis to characterize panic susceptibility (PS), and found that PS significantly predicted dlPFC activity to the unpredictable cue during the NPU threat task. When examining the weighted beta coefficients from this analysis, we observed that self-reported fear/anxiety during the CO2 challenge negatively loaded onto dlPFC activity during the NPU task. Consistent with this observation, dlPFC activity during the unpredictable cue was also negatively correlated with anxiety during the NPU sessions. Together, these results suggest that panic symptoms and anxiety are regulated by the same prefrontal cognitive control system.

Resultant Publications

Balderston NL, Liu L, Roberson-Nay R, Ernst M, Grillon C. 2017. The relationship between dlPFC activity during unpredictable threat and CO2-induced panic symptoms. *Transl Psychiatry* 7: 1266

Study 2b: Effect of anxiety on response inhibition.

Study 2b. has been completed and the goals were reached.

Brief Description: This study examined the effect of threat of shock on response inhibition in a stop signal task. During stop-signal tasks, the go signal was presented on all trials; on a minority of trials after the go signal and just as the subject is about to respond, a stop signal appeared (usually a sound), indicating that one should not press the button on that trial.

Outcome*:* Inhibition is a key theme in psychiatric research, including anxiety disorders. However, inhibition encompasses many processes. Studies show that anxiety impairs cognitive inhibition, the inhibition of thoughts, emotions and perception. In a first study, we explored the effect of anxiety on behavioral inhibition, operationally defined as the inhibition of pre-potent motor responses. We hypothesized that anxiety promote a cautious behavioral stance. As a result, anxiety should improve response inhibition. This hypothesis was confirmed in several studies that examine the effect of anxiety induced by the threat of shock on response inhibition in a go/nogo experiment with frequent go (91%) and infrequent nogo (9%). We showed that induced anxiety increased the rate of correct nogo responses without affecting go responses (Grillon et al, 2013, 2017; Robinson et al 2013). These study reveal that while anxiety affects negatively many cognitive and behavioral processes, it also has some beneficial effects. We also examined individual differences in the effect of anxiety on response inhibition (Grillon et al, 2016).

We showed that subjects who most benefited from anxiety were characterized by low attention control. We thus confirmed that anxiety increases inhibitory control of prepotent responses—a mechanism which is adaptive under threat—and show that this effect is greater in those who rely more upon such prepotent responding, i.e., those with low attentional control.

Resultant Publications

Grillon C, Robinson O, Krimsky M, O"Connell, Alvarez G, Ernst M. 2017. Anxiety-mediated facilitation of behavioral inhibition: threat processing and defensive reactivity during a go/nogo task. *Emotion* 17: 259-66

Grillon C, Robinson O, Marthur A, Ernst M. 2016. Effect of attention control on sustained attention during induced-anxiety. *Cognition & Emotion* 30: 700-12

Robinson OJ, Krimsky M, Grillon C. 2013. The impact of induced anxiety on response inhibition. *Front Hum Neurosci* 7:69

Study 2g: Exercise, anxiety and cognition:

Study 2g. has been completed and the goals were obtained.

Brief Description: This study aimed to understand the influence of acute physical exercise on the interaction between anxiety and cognition. The study had a within-subjects design and took place over three outpatient study visits, one exercise screening visit and two study visits, each separated by five to eight days. During one visit, the participant will pedal lightly on the stationary bicycle for 30 minutes. During the other visit, the participant will pedal on the exercise bicycle at a moderate intensity, ensured by levels of resistance and defined by target heart rate of resting heart rate plus 60-70% of her heart rate reserve. Then, after completion of the exercise, participants will perform an N-back task under threat of shock and safe conditions to measure working memory (Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013). After 15 minutes (10 min break, 5 min startle habituation), participants will undergo the NPU task under threat of shock and safe conditions to measure physiological response to fear and anxiety (Schmitz & Grillon, 2012).

Outcome: Exercise has been proposed as a first line of treatment for anxiety disorders. We examined potential mechanisms that may be responsible for the anxiolytic effect of anxiety. A first study investigated whether exercise affected fear and anxiety differentially. Results showed that biking at 60-70% of heart rate reserve for 30 min reduced anxiety evoked by unpredictable shock but not fear evoked by predictable shock (Lago et al, in press). These results suggest that exercise therapeutic effect may involve specific modulation of the anxiety and not the fear system. A second study examined whether exercise anxiolytic effect was mediated by improved cognitive control. Two observations motivated this study. First, engagement of attention control during increased working memory (WM) load can decrease anxiety. Second, exercise can improve attention control. Therefore, we hypothesized that exercise would boost the anxiolytic effects of increased WM load via its strengthening of attention control. Anxiety was induced by threat of shock and was quantified with anxiety-potentiated startle (APS) (Lago et al, in press). Subjects were tested after two types of activity, exercise (biking at 60-70% of heart rate reserve) and control-activity (biking at 10-20% of heart rate reserve). After each activity, participants completed a WM task (n-back) at low- and

high-load during safe and threat. Results were not consistent with the hypothesis: exercise vs. control-activity increased APS in high-load (p=.03). However, this increased APS was not accompanied with threat-induced impairment in WM performance (p=.37). Facilitation of both task-relevant stimulus processing and task-irrelevant threat processing, concurrent with prevention of threat interference on cognition, suggests that exercise increases cognitive ability.

Resultatnt Publications

Lago T, Hsiung A, Leitner B, Duckworth C, Chen K, et al. in press. Acute exercise decreases anxiety to unpredictable threat but not fear to predictable threat in humans. *Depress Anxiety*

Lago TR, Hsiung A, Leutner BL, Duckworth CJ, Balderston NL, et al. in press. Exercise modulates the interaction between cognition and anxiety in humans. *Cognition & Emotion*

Study 2h. Stop Signal Task:

Study 2h. has been stopped and will not be continued.

Brief Description: Participants performed paradigms which tested whether threat impacts the initiation and the inhibition of behavioral responses. Participants were presented with stimuli, and asked to either initiate a response (i.e. "go") or inhibit their response (i.e. "stop"), based on what stimuli is presented.

Outcome: This study was an extension of our response inhibition study with go/nogo task (Study 2b). Results did not show clear outcome.

Study 3:

Study 3 has been stopped and will not be continued.

Brief Description: This study examined the effects of memory retrieval on extinction. It examined whether exposure to contextual stimuli present during acquisition or extinction can serve as implicit reminders of acquisition or extinction, respectively, and can reactivate the memory of the CS+-shock or CS+-no shock association. This study required 4 sessions –two at the Clinical Center and two sessions through an on-line program off campus.

Outcome*:* In the lab, postdocs are given the freedom to develop their own research in their way for independence. After the postdoc who proposed the study left, we did not pursue the substudy because 1) the substudy objectives were not a high enough priority compared to other competing studies and 2) other postdocs were not interested in working of this project.

The choice is to place this study on hold until a new postdoc implements it or to remove it from the protocol. I selected to remove the study. It will not be continued.

2. STUDY OBJECTIVES

Interaction between cognition and anxiety

It is well-established that anxiety interferes with behavior because it distracts from ongoing goals (Vytal, Cornwell et al. 2012). In fact, one of the main complaints of anxious individuals is their inability to remain focused due to distracting anxious thoughts. However, some individuals (e.g., soldiers) can perform very well under stress, down-regulating their fear and anxiety. In addition, most people can also be distracted temporarily from their anxiety while being involved in a task (e.g., watching TV or working). Objective 2 will examine the interactions between experimentally-induced anxiety and performance on cognitive tasks.

A leading theoretical account of emotion/anxiety interaction assumes that 1) worry is the component of state anxiety responsible for the effect of anxiety on performance on cognitive tasks and 2) that cognitive interference occurs especially at the level of working memory (WM) (Eysenck, Derakshan et al. 2007). The interaction between threat of shock and WM tasks depends on WM load. We have demonstrated that 1) threat of shock interferes to a greater extent with low load WM tasks than high load WM tasks (see Fig 2 below) and 2) anxiety is reduced under high WM load compared to low WM load (see Fig 4 below) (Vytal, Cornwell et al. 2012). These results suggest that when WM is fully occupied by a task, there is little WM left to allocate to anxious thoughts. However, it is unclear whether these effects are due specifically to WM per se or to more general attention control mechanisms as we have also evidence of performance impairment during shock threat in tasks that requires little WM.

Executive functioning is involved in goal directed behaviors. It is also probably involved in implicit and explicit emotion regulation, when emotion (e.g., anxiety) competes with our goals. There are different components of executive functioning including working memory, inhibition, and conflict. How each of these components interacts with anxiety will be investigated in this study.

This objective examines the role of components of executive functioning and context in the interaction between cognition and anxiety. The two key questions are 1) to which degrees various cognitive processes (e.g., working memory, inhibition) are affected (impaired/facilitated) by anxiety induced by shock anticipation, and 2) to which degree various cognitive tasks down-regulate experimental anxiety.

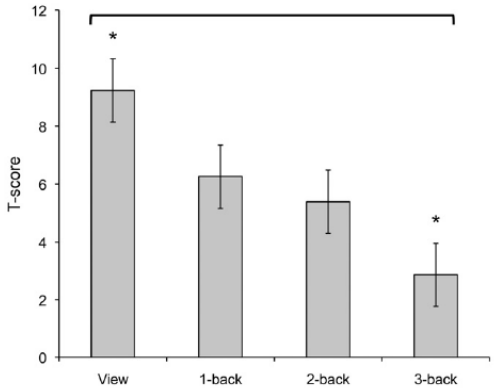

Figure 4. Mean startle magnitude difference between threat and safe. Anxiety-potentiated startle magnitude during View (no task) was significantly higher, and during 3-back significantly lower, than anxiety-potentiated startle at all other levels of cognitive load. Anxiety-potentiated startle during 1-back and 2-back did not differ. Anxiety-potentiated startle during all conditions was different from zero. Error bars represent the within-subjects standard error for the repeated measures general linear model (GLM) comparing startle during threat versus safe across load. *$p < .05$.

3. SUBJECTS

a. Description of study populations

The participants will be medically and psychiatrically healthy volunteers (males and females) ages 18-50. The accrual ceiling is 1575 subjects. NIH, but not NIMH, employees may participate. NIH employee participation is guided by intramural institute policy.

b. Inclusion criteria

- Males and females
- Age 18 – 50 years

c. Exclusion criteria

- Non-English speaking
- Pregnancy
- Any current ongoing medical illness
- Current Axis I disorders
- Past significant psychiatric disorders (e.g., psychotic disorders) according to DSM-IV
- Current alcohol or substance abuse according to DSM-IV criteria
- History of alcohol or substance dependence based on DSM-IV criteria within 6 months prior to screening
- Current psychotropic medication use
- Current or past organic central nervous system disorders, including but not limited to seizure disorder or neurological symptoms of the wrist and arms (e.g., carpal tunnel syndrome). The latter exclusion is for shock studies only.
- Positive urine toxicology screen
- Employees of NIMH or an immediate family member of a NIMH employee.

4. STUDY DESIGN AND METHODS

a) Study overview

This protocol examines the interaction between cognition and induced anxiety. This study consists of the following sub-studies. Subjects can enroll in one or more sub-studies. They will sign a consent form for each sub-study. These sub-studies require 1 outpatient visit to the Clinical Center for up to 4 hours or up to two visits if it includes a telehealth consenting visit.

Study A: Working memory and anxiety
Study C: Effect of anxiety on sustained attention to response task (SART)
Study D: Effect of anxiety on Stroop task (closed for accrual; data analysis only)
Study E: Pilot studies
Study F: Pilot study of sural nerve stimulation

b) Recruitment

Subjects will be recruited via advertisements in the local media (such as the free Express or Examiner; newsmagazines such as Washington Post Local Living sections and the like); postings on college campuses and on NIH campus; in coffee houses and fast food places with permission of managers; local libraries; as well as in Montgomery County and DC metro and buses. We also will utilize websites, such as college papers (e.g. Maryland Diamondback, American University Student Media online publications, etc) and local media (e.g. the Gazette and Washington City paper). The web ads will direct readers to the NIH Patient Info website. We also will advertise on approved listservs as provided by Public Relations and Public Liaison office (e.g. NIMH Outreach Partnership Updates) or local groups (e.g. Community Service Announcements – City of Gaithersburg, Montgomery County Providers, Howard County Providers, Frederick Providers Council, etc) . IRB-approved ads will be posted on these listservs with the permission of the moderator and IRB-required statement on how the receiver was identified. One such site will be the Club PCR site used by the research assistants at NIH. The ads will direct the participant to PRPL whose staff will conduct initial screening and pass along potentially appropriate callers to the PI or his staff. We may also post the advertisements on college recruiting websites for research studies, such as SONA. SONA is a software platform to post research studies. We will use IRB approved language for the posts and request that participants reach out to our group to express interest. No PII will be shared on the platform. Advertisements may also be placed on coffee sleeves and handed out with coffee orders at coffee shops. These advertisements will be IRB-approved prior to use. IRB-approved text ads will be sent from Instagram, Twitter and Facebook Accounts such as NIH/CC and NIMH-Extramural. Twitter and other web language will be sent to local publications and groups, such as Family magazine, in hopes they will send it out to use on their own page. Notecards and/or flyers may be posted in places such as grocery stores, coffee shops, community centers, and bookstores, or placed in advocacy group offices, in doctor's office waiting rooms, libraries, and retail establishments 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 accord with their policy. They may be given directly to those requesting study information. Postcards may be sent using commercially-available mailing lists via direct mail. The postcards will identify the source of the mailing list. ResearchMatch may be used to recruit participants for this protocol. We may also use the Survey tool in REDCap (Research Electronic Data Capture) to assist with recruitment, pre-screen potential participants, and evaluate our recruitment strategies. REDCap is a secure web application for building and managing online surveys and databases. REDCap Survey will not contain PHI and contains workflows that support the collection of de-identified data. Interested persons may be directed to REDCap from a link in our IRB approved advertisements. In our secure confirmation email to potential healthy participants, we would like to have the option to include the link to offer to allow participants to pass along as a WOM recruitment strategy with the disclaimer that they should

not forward the confirmation email. Potential participants will be given an automated survey when they click on the link. The survey would ask the potential participant our non-PII prescreening questions that we currently ask via a phone prescreen. The subject will be provided with a numeric code automatically by the database and will also be provided the phone number for our study staff and will be asked to call our research team to continue screening. The potential participant will be instructed to provide their unique numeric code during this phone screen. The unique code will allow the staff to connect the person to the non-PII information that has already been entered by the potential subject. This allows us to speed up the prescreening process as we will have access to their answers. We will be the only ones with access to the responses. We will also use REDCap to develop an anonymous survey to evaluate our screening efforts.

Ads may be placed on the CC Twitter, Facebook page, and newsletters. IRB approved ads may be place on website such as advocacy groups, university student sites, and newspaper sites. In addition ads will be place on Craigslist under the "Volunteer" category. The email address will be hidden from public view to prevent spam. IRB-approved Tabletop tent cards will be displayed on tables in public areas such as the NIH cafeterias, with the approval of the venue owner/manager.

We may use paid advertising on social media sites, such as Facebook, Instagram, and Twitter, to recruit potential volunteers whom are within our age range and located near the NIH (within 50 miles). We will not use behavioral targeting for any advertising campaign. We will only use advertisements and videos that have been previously IRB approved. Accounts used for Twitter, Facebook, and Instagram are the NIH sponsored accounts.

We may also identify potential healthy volunteers through the NIMH protocol titled "Recruitment and Characterization of Research Volunteers for NIMH Intramural Studies." There is no direct solicitation of employees/staff by supervisors nor co-workers.

Audio advertisements will be played during radio or podcasts, Potential radio stations may be public radio (i.e., WAMU) or other mainstream radio stations (i.e., 97.1 Fresh FM). Potential podcasts may be ones like Ted Radio Hour, Moth, Criminal, Diane Rehm, etc. An animated short video advertisement may also be posted on the NIMH YouTube channel. This video may also be posted on official NIH, NIMH, and NIH Clinical Center social media accounts such as Facebook, Instagram, and Twitter It may be sent via sent via electronic listservs such as NIH postbac listserv, community listservs, advocacy and provider listservs, and running/ health listservs.

Email addresses provided on the advertisements may be changed to the NIH email of other staff on this protocol following any staff changes or changes in the individual responsible for referrals.

During an initial telephone interview, the nature and duration of the various studies will be explained. Potential participants will then be screened for inclusion with screening protocol 01-M-0254, and subjects who qualify will be invited to participate in the study. A copy of the study consent form will be mailed to the subjects. This will give them an opportunity to review the consent form and ask questions.

c) Screening

Consent procedures are conducted prior to any research activities. The screening for all three studies is done under the screening protocol 01-M-0254. Screening will include a medical and a psychiatric history will be taken and a medical exam. In addition, the following laboratory tests will be obtained:

- Pregnancy test for females of child bearing potential
- Urine toxicology screen
- Subject demographic information
- In addition, a CBC, hepatic panel, electrolytes, creatinine, BUN, thyroid panel; and ECG may be done at the discretion of the examining physician or nurse practitioner.

Results of the screening are recorded in CRIS. Subjects are contacted if abnormal results are revealed during screening. A test may be repeated to confirm a result if needed. If the abnormal result is confirmed, a clinician will contact the subject to advise him/her on treatment options outside NIH.

- In addition to the medical and psychiatric assessment under protocol the screening protocol 01-M-0254, participants may be asked to fill out the following questionnaires:

    - State-Trait Anxiety Inventory (STAI) (Spielberger 1983)
    - NEO-Personality Inventory (Costa and McCrae 1992)
    - Anxiety Sensitivity Index (Peterson and Reiss 1992)
    - Beck Anxiety Inventory (Beck and Steer 1987)
    - Beck Depression Inventory (BDI) (Beck and Steer 1987)
    - Fear Questionnaire (Marks and Mathews 1979)
    - Penn State Worry Questionnaire (Meyer, Miller et al. 1990)
    - Positive and Negative Affectivity Scale PANAS (Watson, Clark et al. 1988)
    - Multidimensional Personality Questionnaire Harm Avoidance (MPQ-HA) (Tellegen 2002)
    - Mood and Anxiety Symptoms Questionnaire (MASQ) short-Form (Keogh and Reidy 2000)

- Adverse Life Event Checklist (Gray, Litz et al. 2004)
- Attention Control Scale (Derryberry and Reed 2002)

The non-analogue measures from these reports may be completed by the participant on the Clinical Trial Survey System (CTSS) online system. Subjects will have signed the 01-M-0254 screening consent prior to filling out these measures on CTSS. AIs may then collect data from the Clinical Trials Database (CTDB) for the purposes of this study. Participants may enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. Two items will require real time monitoring. Item #9 on the Beck Depression Inventory (BDI) and item #61 on the Mood and Anxiety Symptom Questionnaire-Short Form (MASQ-SF) inquire about suicidality. An associate investigator must check both items before the participant leaves the Clinical Center. If the participant indicates anything higher than a "0" ("I don't have thoughts of killing myself") on the BDI item #9 or a "1" ("not at all") on the MASQ-SF item #61, the associate investigator must notify a staff clinician. The staff clinician must assess for immediate safety and refer the participant to appropriate resources. If the screening protocol (01-M-0254) finds them to be eligible, they will undergo the informed consent process for 01-M-0185.

d) Study procedures

Procedures for the separate studies are outlined below.

Anxiety will be induced with the threat of shock.

- The threat of shock experiment requires a single session. Performance of cognitive tasks will be conducted during alternating periods of shock threat and safety.

Sub-study A: Working memory and anxiety
This study will examine whether greater WM capacity or practice is associated with increased or decreased anxiety during performance of a WM task during threat of shock. On the one hand, increase in WM capacity could be associated with more ability to allocate resources to anxious thoughts, therefore increasing anxiety. On the other hand, better WM reflects better ability to screen out intrusive stimuli and thoughts, thereby decreasing anxiety. As for task practice, we hypothesize that practice will make more resources available to allocate to anxious thoughts.

Subjects will be asked to remember verbal and nonverbal stimuli. These stimuli consist of words, pictures, letters or spatial locations in series of stimuli. Participants will be instructed to remember one, two, or three stimuli back from the current stimulus on the screen (n-back task). There will be 2 groups. One group will practice the N-back task

minimally (e.g., 5 min) and the other group will have more extensive training (e.g., 20 min).

Sub-study C: Effect of anxiety on sustained attention to response task (SART)
SART are sustained attention tasks during which a button-press response is made to frequent targets and responses are withheld to rare non targets. SART have been used extensively to study inattention, "off-task thinking", task-unrelated thoughts, and 'mind wandering" (Robertson, Manly et al. 1997). An advantage of this task is that it enables us to identify periods when the mind wanders. When the mind wanders, subjects make errors of commitment (they button-press to non-targets). We will use SART to attempt to identify periods of anxious thoughts.

Subjects will have to respond to frequent targets (e.g., digits 1, 2, 3…) and to withhold response to rare non targets (e.g., 3). We will examine the association between response speed and anxiety as assessed with startle. Mind wandering in SART is associated with faster RT. We will examine whether faster RT is associated with larger potentiated startle. We will also probe subjects' thoughts (on-task, off-task, anxious thoughts) after each startle stimulus to assess whether anxious thoughts predict startle potentiation.

Sub-study D: Effect of anxiety on Stroop task
We recently wrote a review on the effect of threat of shock on cognitive performance. Results suggest that threat of shock impaired performance on the emotional Stroop but tended to facilitate performance on the classic Stroop. However, results of the classic Stroop studies were not straightforward. This study will examine the effect of the threat of shock on classic and emotional Stroop in a within-subject design.

Stroop tests are used to examine conflict of emotional distraction. In the classic Stroop test, the name of a color is printed in a color that conflicts or does not conflict with the word (e.g., the word red printed in blue). In the emotional Stroop, the words are not colored words but emotional words (e.g., the word death printed in blue). The subject's task is to name the color of the word.

Sub-study E: Pilot studies
We will pilot experiments that examine the effect of threat of shock on various cognitive processes. The objectives are 1) to assess the feasibility of implementing cognitive tasks conducted under threat of shock while startle stimuli are occasionally administered, 2) improve upon study design, and 3) attempt to predict appropriate sample sizes for neuroimaging, psychopharmacological, and clinical studies carried out in our other protocols (03-M-0093, 02-M-0321).

The design of the experiment will be similar to the threat of shock experiments in other Study 2 studies. Subjects will perform cognitive tasks during alternating safe and threat periods. Occasional startle stimuli will be delivered to assess anxiety. We will focus on 4 cognitive domains: 1) sensory/perceptual processes, 2) attention/control, 3) working

memory, and 4) complex executive functions (e.g., planning, decision making). The following tasks will be piloted:

- **Sensory/Perceptual processing**: We will examine the effect of threat of shock on the ability to remain focused on goal-relevant stimuli in the presence of potentially interfering distractors. We will use a response-competition paradigm during which subjects make speeded responses indicating whether a central target is one of two pre-specified targets while attempting to ignore peripheral distractors (Lavie 2005). Slower responses in the presence of an incongruent distractor will be compared with a congruent distractor. Perceptual load is manipulated by varying the number of items that are similar to the target.
  Conflict tasks: Tasks such as the Flanker task will be used. This task requires subjects to attend to a centrally presented stimulus and ignore the flanking stimuli surrounding it. When the flanking stimuli are mapped to the opposite response from the center stimulus (incompatible trials), subjects respond more slowly because of the need to exercise top-down control. We are interested in this task because it examines top-down control without relying on working memory.
  Executive function/cognitive flexibility: We will examine threat of shock on complex executive functions testing including decision making (e.g., Iowa Gambling task), planning ability (e.g., Tower of London), set-shifting (e.g., Wisconsin Card Sorting test), and solving tasks (e.g., anagrams).
  Working memory: We have found that threat of shock reduced performance on n-back WM tasks. Because of the nature of N-back tasks, it is unclear whether reduced performance is due to anxiety interference with memory or other processes such as perception, encoding, or retrieval. We will investigate whether we can dissociate the effect of anxiety on these processes using Sternberg WM tasks.

Sub-study F: Pilot studies of sural nerve stimulation

In Protocol 01-M-0185, the shocks are delivered via electrodes located on the forearm or fingers. In order to obtain an objective measure of unpleasantness, we will pilot a new technique using up to 30 subjects in a session lasting up to 2 hours.

This pilot experiment will be conducted under the supervision of the PI, who used this technique when he was a graduate student.

The objective of this pilot study is to learn to implement this new technique. The shock will be administered on the sural nerve in the retromalleolar space and recording of the response from the biceps femoris muscle (see *Electric shock: sural nerve* below). We will learn how to locate the best placement of the stimulating electrodes to stimulate the sural nerve and of the recording electrodes to record the R3 reflex. We will also need to determine the best parameters of stimulation. Finally, we will examine how to organize the equipment in experiments that investigate the interaction between anxiety and cognition. Hence, in a subgroup of subjects, we will pilot delivering sural nerve stimulation while they perform a working memory or vigilance task.

Procedures common to all studies

Startle response
The startle reflex will be elicited with a 102 dB white noise (40-ms duration) delivered binaurally via headphones. The eyeblink component of the startle reflex will be recorded binaurally with two electrodes placed under each eye. Eyeblink responses will be scored in the 20-100 ms window following the onset of the startle stimulus.

Autonomic measures
Heart rate, skin conductance activity (event-related responses and spontaneous fluctuations), and finger pulse volume (FPV) will be recorded during testing to evaluate changes in autonomic arousal. The heart rate will be monitored with two disposable electrodes, one on each wrist. A computer algorithm will detect the R-wave in each cardiac cycle and calculate the number of whole and fractional heart beats for 500 ms periods in each condition. The skin conductance will be measured using two Ag-AgCl electrodes filled with a .05M NaCl electrolyte. Electrodes will be placed on the distal phalanges of the index and second fingers of the left hand. FPV will be measured with a finger plesthymograph.

Grip force measures
The strength of hand compression (i.e. grip force) will be recorded during testing to evaluate behavioral indices of motivation and performance. Participants will hold a hand clench dynamometer which will assess hand compression in units of kilogram-force (kgf). The magnitude of grip force will serve as a within-subject measure of motivation. Additionally, patterns of dynamic grip force behavior will serve as measures of fine motor skill.

Procedures to induce anxiety

Electric shock: forearm or fingers
Electric shocks are one of the most efficient ways to induce anxiety in the laboratory. The shocks will be delivered through two disk electrodes located on the forearm or on two fingers.

Test shocks

At the beginning of the study a shock workup procedure is conducted to determine the setting for the overall procedure for each subject. The level of shock is initially set at 3.5 μAmp (the low range). A shock is administered and the subject is asked to rate it on a scale from 1 (not at all painful) to 5 (extremely painful). We then increase the level of shock slightly until the subject identifies the sensation at a rating level of 4. No more than three test shocks are given and the level is selected based on the subjective ratings provided by the participant. No subject will ever receive a shock rated more than 4 for the duration of their study. In addition, subjects are reminded that they have the opportunity to withdraw from the study at anytime if they wish.

Participant rating of the experience:

At the end of the study subjects are asked to retrospectively rate the shock experiment on a scale from 1 (not at all painful) to 10 (extremely painful). This provides us with an assessment of the overall unpleasantness of the experience during the entire shock experiment.

Electric shock: sural nerve
The shock will be administered on the sural nerve in the retromalleolar space and recording of the response from the biceps femoris muscle. The aim is to have an objective measure of unpleasantness. The R3 reflex will be evoked using a method similar to that described in Rhudy et al (2005). Briefly, electric stimulation of the sural nerve will be four to six rectangular wave pulses of 1- to 2-ms duration every 2 to 5 ms. A trial will begin with 0 mA and increased in 1.0-1.5-mA steps until a R3 reflex is detected. Stimulus intensity will then be decreased in 0.75 mA steps until a reflex is no longer detected. This up–down staircase procedure will be repeated two more times, but with 0.5-mA steps until the R3 threshold is determined. Once the R3 threshold is determined, the intensity of stimulation during experiments in which we used shock to evoke anxiety will be set at 1.2 times the threshold intensity. At this intensity, subjects usually report a slightly painful pin-prick sensation (Rhudy et al 2005).

e. End of Participation

The participant is discharged following each experiment. Subjects will be terminated from the protocol if they request to discontinue participation.

5. MANAGEMENT OF DATA AND SAMPLES

a. Storage

No samples will be collected or stored. The samples that were previously stored at the Neuroendocrine Core Facility (NCF) have been discarded.

Questionnaires are labeled with a subject number. The master list and computerized data are maintained in password protected computers. Only study personnel will be given a password and have access to the data. Data will be stored without identifiers, and only study personnel with have access to the code key.

Additional information regarding the web site: The servers are located on the main NIH campus, building 10, room 4A11A. The room is locked and accessible only by selected NIMH IRP Computer Support staff. NIMH IRP computer support will collect userID, day/time website is accessed, and the subjects' responses to the questions. The subjects' names will not be used. Two servers will be utilized: one server will be used to host the web application; the second server will be used to store the data. The web application will require userID and password for access. The site itself will be SSL encrypted (https). The MS SQL database that contains the data will be on a separate

server, isolating the data should the web server become compromised. The data stored on the MS SQL database will be encrypted and protected. Before deployment to the general public, the ISSO and IRT will perform vulnerabilities scan on the site. All security vulnerabilities will be corrected before the site goes 'live'.

b. Data and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) Policy. Samples are not collected or stored. Data may 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 from this protocol may be open-access or restricted access.

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 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.

Data collected prior to Amendment TT will not be shared.

6. ADDITIONAL CONSIDERATIONS

a. Research with investigational drugs or devices

Acoustic startle and shock device used in this protocol are considered non-significant risk (NSR) devices and will only be used within published guidelines. The auditory startle device is manufactured by Biopac and the shock device is manufactured by Digitimer.

Auditory startle does not meet criteria for a Significant Risk device as outlined Under 21 CFR 812.3(m), as an investigational device that:
1. Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: Auditory startle is not an implantable device.
2. Is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: Auditory startle is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

3. Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: Auditory startle, as used under this protocol is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.
4. Otherwise presents a potential for serious risk to the health, safety or welfare of a subject
Response: Auditory startle has been in use numerous for decades and have been cleared by the FDA. Safety guidelines have been developed and updated allowing its dissemination to a wide range of clinical and non-clinical settings. The FDA has generally waived pre-IDE inquiries for auditory startle studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for auditory startle within these limitations.

The shock device (electrical stimulator) does not meet criteria for a Significant Risk device as outlined Under 21 CFR 812.3(m), as an investigational device that:

1. Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device is not an implantable device.

2. Is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

3. Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device, as used under this protocol is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

4. Otherwise presents a potential for serious risk to the health, safety or welfare of a subject
Response: The shock device has been in use numerous for decades and have been cleared by the FDA. Safety guidelines have been developed and updated allowing its dissemination to a wide range of clinical and non-clinical settings. The FDA has generally waived pre-IDE inquiries for shock studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for shock device within these limitations.

7. RISKS AND DISCOMFORTS

The following studies are considered minimal risk studies:

a. Psychophysiological recording: The psychophysiological measures that will be obtained are non-invasive, do not involve the administration of needles, drug, 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.

b. Auditory startle stimulus: The auditory stimuli that will be used in the startle studies are white noise with a duration of 40 ms and an intensity of 102 dB. Auditory startling sounds of much higher intensities are frequently used in startle studies (Hawk and Cook 2000). Sounds of higher intensities and longer durations are also widely used in aversive conditioning in human subjects, where they serve as US (Hodes, Cook et al. 1985; Sandin and Chorot 1989). The short duration (40 ms) of these sounds makes them harmless (i.e., there is no danger of hearing impairment). In addition, white noises are safer than pure tones. The PI has been involved in various studies and collaborations involving over 1000 of subjects with no adverse reactions.

c. Electric shock: In the main studies, the shocks will be delivered through two disk electrodes located on the forearm or on two fingers. The shock is generally described by subjects as anxiogenic and moderately painful. At the end of testing the mean participant rating of aversiveness on a scale of 1 (not at all painful) 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. For instance, 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.

Study 2f pilots a new method of administering the shock. The shocks are anxiogenic and are usually described as inducing a slightly painful pin-prick sensation (Rhudy et al 2005). Typical experiments include over 20 such stimuli (Rhudy et al 2005). Our shock studies usually include less than 10 shocks and most frequently about 5/6 shocks. Subjects with “cubital tunnel syndrome” do not need to be excluded given than the shocks are not delivered on the arm or wrist.

d. Emotional pictures: The emotional pictures will be selected from a set of standardized stimuli that are frequently used in psychophysiological and brain imaging studies of emotions (Lang, Ohman et al. 1988). Because the intensity of these pictures might make some people uncomfortable, their contents will be fully described to the subjects before participation in the study. In addition, a debriefing will be conducted to assess whether subjects are distressed or negatively affected by the pictures and in need

of psychological supports by a clinician. A clinician will then talk to them and will follow up with a call to see if further supportive contact is needed. If so, then a study clinician will meet with the participant and assess for necessary intervention.

7.2 Assessment of Potential Risks and Benefits

This is a more than minimal risk protocol in healthy volunteers. These experiments may enhance our understanding of how stress and anxiety affect associative learning, that is, the way we learn about our environment. We have minimized risks by delivering electric shosks at a level that is judged by the participant as uncomfortable and tolerable. Subjects may stop the experiment at any time if they find the discomfort to be too great. The risks are reasonable in relation to the anticipated benefit.

8. SUBJECT SAFETY MONITORING

An Associate Investigator is in an adjacent room to the participant during all phases of the procedures. There is a non-recording camera that allows AI to monitor the subject. The subject is able to wave if there is a problem or simply call out to the AI as the rooms are connected with intercom. Participants are told they may end any procedure at any time by waving to AI or calling out. The AI can end experiments if the subject is unable to follow instructions. The medically responsible physician or members of his/her staff are available by phone or pager for any medical problems that arise.

9. OUTCOME MEASURES

Primary outcome measures: The primary outcome measures will be the startle reflex, and performance on cognitive tasks.

Secondary outcome measures: These measures include psychophysiological measures other than startle (e.g., skin conductance), psychological questionnaires (Spielberger state and trait anxiety), and performance on standard cognitive tests (e.g., working memory capacity).

10. STATISTICAL ANALYSIS

The accrual ceiling for this protocol is 1575 healthy controls. The overall current target N for the protocol is 148 healthy controls.

Sub-study A (previously Study 2a) 36 subjects
Sub-study C (previously Study 2c) 34 subjects
Sub-study E (previously Study 2e) 48 subjects
Sub-study F (previously Study 2f) 30 subjects

Study 2a: Working memory and anxiety

Analysis of the data: Startle and performance data will be analyzed with ANOVAs with repeated measures using condition (threat, safe) x Load (low, high).
Power analysis: Based on pilot and published works in our lab, we expect an small/moderate effect size for startle potentiation (Cohen's *d*=0.3). A priori calculations indicate that the testing of 30 participants at an alpha of 0.05 would provide power of 0.80 to detect a significant 2 (Condition) x 2 (Load) interaction on startle response. Assuming a 20% attrition rate, we will collect (about) 36 subjects.

Study 2c: Effect of anxiety on sustained attention to response task (SART)
Analysis of the data: The main analysis is to compare startle magnitude when subjects focus on the task and when their mind wander with ANOVAs with repeated measures using condition (threat, safe) x thought (on task, off task).
Power analysis: Based on pilot works in our lab, we expect an small/moderate effect size for startle potentiation (Cohen's d=0.4). A priori calculations indicate that the testing of 28 participants at an alpha of 0.05 would provide power of 0.80 to detect a significant 2 (Condition) x 2 (Load) interaction on startle response. Assuming a 20% attrition rate, we will collect (about) 34 subjects.

Study 2d: Effect of anxiety on Stroop task
Study is 2d is is closed for accrual. We are currently analyzing the data.

Study 2e: Pilot studies
These pilot experiments are designed to determine the parameter of stimulation of study that will be used in our neuroimaging, psychopharmacological, and clinical studies conducted in our other protocols (03-M-0093, 02-M-0321, 02-M-0263). We use tasks in 4 domains, 1) sensory processing, 2) conflict, 3) executive function, and 4) working memory. We will use 12 subjects per domain for a total of 48 subjects.

Study 2f: Pilot study of sural nerve stimulation
This pilot study investigates the location of the stimulating and recording electrodes and different types of stimulus delivery. We have requested 30 subjects.

11. HUMAN SUBJECTS PROTECTION

a. Subject selection

i. Both genders will be equally represented. Ethnic representation will reflect the population from which the sample is recruited.

ii. As people get older the probability that they startle decreases. Because startle is our main independent measure it is important to test subjects who are likely to startle in order to collect analyzable data. Based on our experience, we made the decision that 50 would be the upper age limit to ensure usable data and thus justify the minimal risk.

b. Justification for inclusion/ exclusion of children
Children will be excluded because of the aversive nature of the stimuli used in this protocol.

c. Justification for inclusion of other vulnerable subjects, e.g. cognitively impaired, pregnant, mentally ill
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.

d. Justification for exclusion of decisionally impaired adults:
All subjects must be able to provide their own consent. 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.

e. Justification of sensitive procedures (use of placebo, medication withdrawal, provocative testing)
Not applicable.

f. Safeguards for vulnerable populations
Female participants will undergo urine pregnancy tests prior to experiments. 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.

12. CONSENT DOCUMENTS AND PROCESS

a. Designation of those obtaining consent: Study investigators or Associate Investigators identified in the KSP form will obtain informed consent. NIH employees will not be consented by a coworker.

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.

Telehealth Consent Procedures: Under certain circumstances, there may be a need to obtain telephone or telehealth consent for this study using NIH-approved platforms. Under these circumstances, the individual will be sent a copy of the consent form. After the document is reviewed, an AI authorized to obtain consent will contact the individual and review the procedures and risks. The participant and the investigator will sign their respective copies, and the former will return their copy to the investigator. One copy each of the signed consent form will be placed in the Medical Record, retained by the investigator, and returned to the participant or parent/guardian. Proper documentation will be made in CRIS.

c. Consent documents

There are two consent forms for this protocol, each containing all required elements, and designated as follows:
Shock
Sural nerve stimulation

13. DATA AND SAFETY MONITORING

a. Data and Safety monitor: Data and safety monitoring will be conducted by the P.I. who will review the results for each subject.

b. Data and Safety monitoring plan: All participants are monitored while they are onsite. Paper data are stored in locked cabinets within locked closets/rooms. Electronic data are encrypted and cannot be accessed without obtaining a password. All data are reviewed as they are obtained. The PI will review data and safety parameters at least annually. The PI will document the data and safety review in the research records and at the time of continuing review.

14. STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/ WITHDRAWAL

14.1 Discontinuation of Study Intervention

Discontinuation from the task will mean discontinuation from the study, and remaining study procedures will be stopped. Only clinical evaluations of AEs and follow up calls may occur following discontinuation. Any new clinically relevant finding will be reported as an adverse event (AE).

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Completion of study intervention
- If any significant worsening of symptoms or active suicidal ideation, clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Concomitant medication use
- Investigator discretion
- Positive pregnancy test

14.2 Participant Discontinuation/Withdrawal from the Study

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

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

- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study
- Death
- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded in CRIS.

14.3 Lost to Follow-up

Not applicable.

15. QUALITY ASSURANCE

a. Monitor

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

b. 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.

16. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Classification of an Adverse Event

Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

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

Relationship to Study Intervention

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

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

Expectedness

The LIP and/or the Medical Associate Investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.
All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.
Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.
At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Adverse Event Reporting

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

Serious Adverse Event Reporting

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

Events of Special Interest

Not applicable.

Reporting of Pregnancy

Not applicable.

17. PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to National Institutes of Mental Health Clinical Director. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

17.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

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, …) will be used that could allow direct linking of database information to individual subjects. The master code list will be stored separately from the data. Electronic data are stored on password protected computers. Research staff will be trained to respect the privacy and confidentiality of NIH employees/staff, especially for sensitive, private information (e.g. illegal drug or alcohol use) may be obtained during participation.

b. For previously stored samples

Specimens are no longer being stored. We have properly destroyed previously stored samples.

c. Special precautions

Hard copy data are stored with a double lock method (in a locked cabinet in a locked room). Only study investigators have access to the data.

21. CONFLICT OF INTEREST/ TECHNOLOGY TRANSFER

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

22. TECHNOLOGY TRANSFER

No technology transfer agreement is in place for this protocol.

23. RESEARCH AND TRAVEL COMPENSATION

Volunteers will be compensated for time and research-related inconveniences as shown in table below. If subjects do not complete the study they will be paid half of the compensation. Travel is not compensated. Payments are mailed after each NIH study session. 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
Protocol: Effects of arousal and stress on classical conditioning

Procedure (shock threat – 1 day)	Duration	Amount
Psychophysiology / shock	3-4 hrs	$120

Additional optional telehealth consenting visit	30 minutes	$20
TOTAL	4 hrs	$120 or $140

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