Predictability and Aversive Expectancies in Anxiety and Depressive Disorders
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Identifying Words: Anxiety disorders, generalized anxiety disorder, panic disorder, agoraphobia, fear conditioning, context conditioning, startle, psychophysiology

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Total requested accrual: 1271

Healthy volunteers:	663
Patients	608
Sub-study 1: 110 patients	44 healthy volunteers
Sub-study 2: 115 patients	86 healthy volunteers
Sub-study 3: 38 patients	38 healthy volunteers
Sub-study 4: 30 patients	30 healthy volunteers
Sub-study 5: 30 patients	30 healthy volunteers
Sub-study 6: 30 patients	
Sub-study 7: 40 patients	40 healthy volunteers

Project uses ionizing radiation: no

IND/IDE: no

Durable power of attorney: no

Multi-institutional project: no

Data and Safety Monitoring Board: no

Technology Transfer Agreement: no

Samples are being stored: yes

Flesch Kincaid reading level of consents

Control: 7.3

Cognition: 8.4

PRECIS

High-generalized anxiety is a concomitant of many anxiety disorders and is often regarded as a vulnerability marker for these disorders (Barlow, 1988). One characteristic of patients with anxiety disorders and high trait-anxious individuals is inappropriate expectancies of aversive events. The overall aim of the present protocol is to investigate mechanisms that may promote the development of these aversive expectancies using expectancy-based, associative-learning models.

During aversive conditioning in which a phasic explicit-cue (e.g., a light) is repeatedly associated with an aversive unconditioned-stimulus (e.g., a shock), the organism develops fear to the explicit cue as well as to the environmental context in which the experiment took place. We have obtained preliminary evidence suggesting that contextual fear represents aspects of aversive states that are central to anxiety disorders (Grillon, Ameli, Goddard, Woods, & Davis, 1994; Grillon & Morgan, 1999; Grillon, Morgan, Davis, & Southwick, 1998). In this protocol, we seek further evidence for the relevance of contextual fear to mood and anxiety disorders.

One important determinant of contextual fear in both humans and animals is predictability: contextual fear increases when aversive events (e.g., electric shock) are unpredictable, as opposed to when they are predictable. The present protocol will examine the role of predictability of aversive states and of conditioning on threat appraisal in individuals with mood and anxiety disorders.

A second aim is to examine the interaction between experimentally-induced anxiety and cognitive processes, more specifically working memory, in mood and anxiety disorders.

Contents

LIST OF ABBREVIATIONS:

N: Neutral
P: Predictable aversive event
U: Unpredictable aversive event

WM: Working memory
US: Unconditioned stimulus

GAD: Generalized anxiety disorder
SAD: Social anxiety disorder
MDD: Major depressive disorder
BP: Bipolar disorder

SCID: Structured Clinical Interview for DSM-IV
DSM: Diagnostic and Statistical Manual of Mental Disorders

1. INTRODUCTION

Fear and anxiety are normal adaptive responses to threat. Anxiety is considered pathological when it is either excessive or inappropriate to the context. Thus, pathological anxiety could result from overactive structures involved in responses to threat, abnormal relational learning (conditioning), or from inefficient affective information processing that prevents an organism from effectively implementing goal-directed behaviors. The present protocol builds on our past results to study the impact of induced anxiety (e.g., during anticipation of shock) on physiological and subjective measures on fear and anxiety and on task performance in patients with mood and anxiety disorders.

1- Past studies

One past objective was to better understand aversive conditioning processes in anxiety disorders. We have reported 1) increased stimulus generalization in panic disorder (Lissek, et al., 2010), 2) relational learning deficits in panic disorder (Grillon, Lissek, Pine, McDowell, & Levenson, 2007), and 3) heightened conditioning with socially relevant stimuli in social anxiety disorder (Lissek, et al., 2008).

Another objective was to study the processes that separate fear and anxiety. While fear is a phasic response associated with an identifiable source that is thought to predict occurrence of an aversive stimulus, anxiety is a more persistent feeling of apprehensive anticipation of future danger (Barlow, 1988). We have demonstrated that the characteristics of these two forms of aversive responses – cued fear and generalized anxiety - could be modeled by administering predictable and unpredictable aversive events (shocks), respectively (Grillon, 2002; Grillon & Davis, 1997). Fear is operationally defined as an increase in startle amplitude during a threat cue that predicts an aversive event (fear-potentiated startle). Anxiety is operationally defined as an increased in startle amplitude during long periods of unpredictable aversive events (anxiety-potentiated startle). One of the past objectives of this protocol was to examine far-potentiated startle and anxiety-potentiated startle in anxiety disorders. We have found that patients with panic disorder and PTSD, but not generalized anxiety disorder (GAD), showed elevated anxiety-potentiated startle (Grillon, et al., 2008; Grillon, et al., 2009). Given that the underlying neural mediators of fear and anxiety are partially distinct (amygdala versus bed nucleus of the stria terminalis) (Davis, Walker, Miles, & Grillon, 2010), these results provide clues to potential pathophysiological mechanisms and targets for treatment.

2- Current studies

We are currently pursuing this line of research to address new questions. As indicated above we found increased anxiety-potentiated startle in PTSD and panic disorder but not GAD. Failure to obtain increased anxiety-potentiated startle in GAD may have been due to the use of mildly aversive stimuli (e.g., airblasts). We are currently examining whether more potent aversive stimuli such as shocks will lead to elevated anxiety-potentiated startle in GAD. We are also examining the specificity of the heightened anxiety-potentiated startle in patients with various types of anxiety disorders (e.g., social anxiety disorder). We are also testing patients with major depression (MDD) and bipolar disorder (BP) of contradictory finding regarding fear-potentiated startle and more generally emotional responses to

aversive stimuli in MDD. While MDD has been associated with a blunting of emotional responses not only to positive stimuli but also to negative stimuli, and reduced fear-potentiated startle in studies that use mildly evocative emotional stimuli (McTeague, et al., 2009) we have reported that depressive mood increased anxiety-potentiated startle (Robinson, Overstreet, Letkiewicz, & Grillon in press) during shock threat. This protocol will clarify whether MDD is associated with emotional blunting or with increased fear and/or anxiety.

Anxiety and Cognition

Our past research has mainly focused on response expression. Our new objective is to examine the interaction between induced anxiety, goal-directed behaviors, and emotional pathology. Anxiety can be adaptive, but it can be debilitating when it interferes with our daily life and our goals. High levels of anxiety have been associated with poor performance and processing inefficiencies on cognitive tasks (Eysenck & Calvo, 1992) suggesting that cognitive changes may be a key component of clinical anxiety. Yerkes and Dodson proposed that the detriment in performance due to high emotional arousal (e.g., anxiety) can described by a U-shaped function, where performance increases as arousal increases to an optimal level and then as arousal levels continue to increase, performance begins to decrease (Yerkes & Dodson, 1908). In support of this proposal, high trait anxious individuals (i.e., those with an above-optimal level of emotional arousal) have been shown to experience disruption in executive processes (Eysenck & Calvo, 1992) and in the ability to perform at work, which can lead to career dissatisfaction and job loss. However, the mechanisms by which performance is affected (e.g., attentional narrowing, executive processing deficiencies, perceptual focus), and the degree to which performance is reliably hindered or facilitated by anxiety is not clear. The current study will allow us to address this gap in our knowledge. Inarguably, understanding the link between emotion and cognition in patient groups with emotional pathology is of particular importance to the development of successful therapeutic interventions.

We will focus on the interaction between anxiety and working memory (WM). WM is the ability to maintain relevant information in mind and to keep irrelevant information out of mind. As such, WM will enable us to examine two of the main symptoms of anxiety, inability to focus and increased distractibility. Anxiety may be especially detrimental to WM by decreasing one's ability to keep irrelevant anxious thoughts out of mind, hence compromising efficient information processing. It has been suggested that high-load cognitive tasks may be more susceptible to anxiety-related disruption (Eysenck, Derakshan, Santos, & Calvo, 2007). This proposal, known as the processing efficiency theory (Eysenck & Calvo, 1992), is based on the claim that the deleterious effects of anxiety on cognition are greatest when task demands are high because the executive processing resources that subserve working memory are also engaged by worrisome thoughts. In contrast to Eysenck et al., (2007)'s cognitive theory, Bishop has argued that anxiety has a greater impact on perceptual tasks that place a lower demand on processing resources, while tasks that place a higher demand on resources reduce the deleterious impact of anxiety (Bishop, 2008). In order to lend clarity to these competing views, we will investigate the extent to which induced-anxiety interferes with WM as memory load increases (using n-back and digit span tasks described below). Further, we will investigate the impact of anxiety on spatial versus verbal WM, using a verbal and spatial n-back task and the Corsi test (another visuospatial test of WM). Previous research suggests that spatial WM tasks may be more susceptible to anxiety-related disruption when anxiety is

induced in healthy subjects (Shackman et al. 2006; Vytal et al., 2013). However, it is unclear whether or not pathological anxiety results in a similar pattern of disruption. By addressing this question, we will be better informed about the nature of anxiety-related disruption and be closer to delineating the mechanisms of such disruption.

While the detrimental effect of anxiety on cognition is well-known, less is known about how task performance affects anxiety. However, it is clear that being engaged in a WM task is an effective way of reducing anxiety. One of the aims of this protocol will be to examine whether patients with mood and anxiety disorders can similarly use WM engagement to reduce anxiety.

In contrast to WM where anxiety appears to be only detrimental, there is evidence to suggest that the anxiety can both impair and facilitate performance on sustained attention tasks (e.g., Go-NoGo task). It is unknown whether anxiety acts a distractor, impairing response inhibition to infrequent "NoGo" trials (resulting in commission errors), or whether anxiety improves the ability to withhold these prepotent responses. Some research suggests that commission errors are the result of "mind wandering" (Smallwood et al., 2004), which may be increased when subjects are anxious. Other research suggests that anxiety can increase inhibition of motor responses (prepulse inhibition; Grillon and Davis, 2007), which would lead to better performance when subjects are anxious. Here we hope to clarify whether or not pathological anxiety impairs or facilitates performance on non-emotional sustained attention tasks.

2. STUDY OBJECTIVES

a. Objective 1: To examine fear-potentiated startle and anxiety-potentiated startle in mood and anxiety disorders. To what extent does heightened anxiety-potentiated startle cut across distinct anxiety disorders? Are fear-potentiated startle and anxiety-potentiated startle also elevated in mood disorders?
b. Objective 2: To examine the interaction between induced-anxiety and WM as cognitive load increases. Does anxiety affect WM performance and is this effect dependent on cognitive load? Can patients with mood or anxiety disorder reduce their anxiety by being engaged in a WM task?

3. SUBJECTS

a. **Description of Study Populations**
   - Participants will be 18 to 50 years old and will be diagnosed with one of the following: generalized anxiety disorder, panic disorder, SAD, specific phobias, major depression, and bipolar depression according to DSM-IV. Patients on scheduled/regular psychotropic medications, other than lithium or Depakote, will stay on their medication. Although patients will not be taken off medications for the purposes of this study, included patients will not be currently taking psychiatric medications or any other medications that may interfere with study results (see exclusion criteria for other characteristics of patient sample). The only

exception is that bipolar depressed patients may be taking mood stabilizers, either lithium carbonate or Depakote. Subjects taking PRN medication that is allowed according to the medication table below, must be both willing and safely able to hold the medication for 5 half-lives. Additionally, healthy controls who are age and sex matched to patients will be studied.

- The accrual ceiling is 608 patients with mood or anxiety disorders and 663 healthy volunteers. This accrual ceiling includes a drop out/withdrawal/non-analyzable data rate of 15% for sub-studies 1 (11 patients, 44 controls) and 2 (115 patients, 86 controls) and 25% for sub-study 3 (38 patients, 38 controls).
- NIH, but not NIMH, employees may participate. NIH employee participation is guided by intramural institute policy.

b. **Inclusion criteria for both patients and healthy controls**

- All subjects must be able to give written informed consent prior to participation in this study.
- PATIENTS ONLY: May have DSM-IV-TR diagnoses of an anxiety disorder (GAD; SAD; Panic disorder; specific phobia) or mood disorder (MDD; BP).
- PATIENTS ONLY: May be taking the mood stabilizers, Depakote or Lithium Carbonate.
- Speaks English fluently
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c. **Exclusion criteria for healthy subjects**

- Female subjects who are currently pregnant
- Subjects who meet DSM-IV criteria for current alcohol or substance abuse
- Subjects with a history of alcohol or substance dependence within 6 months prior to screening
- Current Axis I psychiatric disorders as identified with the Structured Clinical Interview for DSM-IV-TR axis disorders, non-patient edition (SCID-np). Past history of any psychotic disorder or bipolar disorder.
- IQ < 80
- Medical illnesses (such as diabetes or hypertension) or neurological illnesses (such as carpal tunnel syndrome for shocks to be delivered on affected arm; organic brain impairment; seizure disorder) likely to interfere with the study.
- Subjects who are on a medication that may interfere with the study (see Table below).
- Employee of NIMH or an immediate family member who is a NIMH employee.

d. **Exclusion criteria for patients**

- Patients who would be unable to comply with study procedures or assessments;
- Female patients who are currently pregnant;
- Patients who meet DSM-IV criteria for current alcohol or substance abuse

- Subjects with a history of alcohol or substance dependence within 6 months prior to screening;
- Patients who are on a medication (other than mood stabilizers lithium carbonate or Depakote) that may interfere with the study (see Table below).
- Medical illnesses (such as diabetes or hypertension) or neurological illnesses (such as carpal tunnel syndrome; organic brain impairment; seizure disorder) likely to interfere with the study.
- Patients will be excluded if they have a current or past history of, delirium, dementia, amnestic disorder, any of the pervasive developmental disorders; or cognitive impairment.
- Current Axis I psychiatric disorders as identified with the Structured Clinical Interview for DSM-IV-TR axis disorders, non-patient edition (SCID) with the exception of the mood and anxiety disorders. Past history of any psychotic disorder or bipolar disorder.
- IQ < 80
- Employee of NIMH or an immediate family member who is a NIMH employee.

e. Additional exclusion criteria for the active avoidance task
   - Color blindness

Table of drug exclusions for both healthy volunteers and patient volunteers (Y=acceptable; N=exclusion). Excluded medications are those that are known to cross the blood brain barrier and/or affect physiological responses (ex: beta blockers like Inderal, antihistamines like Benadryl or systemic corticosteroids like cortisone).

Drug Class	**Episodic Use *(p.r.n.)***	***Chronic Use***	***Restrictions***
Analgesics	Y	N	Non-narcotic analgesics only Ex. Advil (Ibuprofen), Tylenol (Acetaminophen)
Antacids	Y	Y	e.g.., Zantac (Ranitidine) except episodic (p.r.n.) use, is acceptable if subject has not taken medication for 5 half-lives prior to study
Antianginal Agents	N	N	
Antiarrhythmics	N	N	
Antiasthma Agents	Y	Y	Systemic corticosteroids are not allowed e.g., Cortisone
Antibiotics	Y	N	
Anticonvulsants	N	Y	For bipolar subjects – only Depakote or lithium permitted
Systemic Antifungal Agents	N	N	Acceptable for episodic (p.r.n.) use if subject has not taken the medication for 5 half-lives prior to sudy visit
Antihypertensive	N	Y	Except for Beta-Blockers, e.g., Inderall (Propranolol) or Lopressor/Toprol (metoprolol)

Antihistamines -Sedating	Y	N	Sedating: e.g., Benadryl (Diphenhydramine), Sudafed PE (diphenhydramine), Alka-Setzer and Aller-Chor (chlorpheniramine) as long as the subject has not taken in the last 5 half-lives prior to the study visit
Antihyperlipidimics	N	Y	e.g., Zocor (simvastatin,)
Anti-inflammatory Drugs	Y	Y	Systematic corticosteroids are NOT allowed, e.g., Cortisone
Antivirals	N	N	Except for treatment of HSV with agents without CNS activity e.g., Zovirax (acyclovir), Cytovene(ganciclovir), Famvir (famciclovir), Valtrex (valacyclovir)
Cough/Cold Preparations	Y	N	Non-sedating antihistamines are allowed (e.g.Zyrtec-D (cetirizine), Claritin-D or Alavert-D (loratadine) and Allegra (fexofenadine) Dextromethorphan preps N/N Guanfacine Y/Y Pseudoephedrine N/N e.g., Nyquil, Dayquil or Mucinex preparations, that include sedating antihistamines, are acceptable if subject has not taken the medication for 5 half-lives prior to study visit
Hormones	N	Y	Only thyroid hormone replacement, oral contraceptives, and estrogen replacement therapy are allowed.
Muscle Relaxants Psychotropic Medication	N Y	N N	e.g., Flexeril (Cyclobenzaprine) Acceptable for episodic (p.r.n.) use if subject has not taken the medication for 5 half-lives prior to study visit.

4. STUDY DESIGN AND METHODS

a. Study Overview

- **Sub-study 1** examines fear-potentiated startle and anxiety-potentiated startle in individuals with mood and anxiety disorders. It requires a single testing session lasting up to 4 hours.
- **Sub-study 2** examines the interactions between anxiety and working memory in mood and anxiety disorders. It requires a single testing session lasting up to 4 hours.
- **Sub-study 3** will examine the effect of CBT on emotional reactivity and on the ability to focus on task-demand during threat. This study is restricted to patients with anxiety disorders and will not include patients with mood disorder. We do not include mood disorder patients because 1) we do not run CBT treatment for mood disorders and 2) individuals with mood disorders are recruited for treatment studies by Dr. Zarate's group. The study will require an initial pre-treatment testing phase requiring 1 or 2 visits lasting up to 4 hours each, an 8-session

CBT, and a post-treatment testing phase requiring 1 or 2 visits lasting up to 4 hours each. This study is stopped.

- **Sub-study 4** examines the effects of threat on cognitive processes through the use of an emotion recognition task. It requires a single testing session lasting up to 2 hours.
- **Sub-study 5** examines emotional attention conflict using a modified Stroop task under threat and safe conditions. It requires a single testing session lasting up to 2 hours.
- **Sub-study 6** examines the influence of acute physical exercise on the interaction between anxiety and cognition. It requires 3 outpatient sessions lasting up to 5 hours each. This study is stopped.
- **Sub-study 7** examines whether threat impacts the initiation and inhibition of behavioral responses. It requires a single outpatient testing session lasting up to 3 hours.
- All the studies will be conducted in one of Dr. Ernst's laboratories.
- All subjects will be screened face to face under the Screening Protocol 01-M-0254. A series of clinical assessments and treatment services (as delineated in Sections H and I) have been implemented for patients with an anxiety and mood disorder.
- Following study completion, patients will receive psychiatric treatment within the adult anxiety treatment team for a 1-2 month period before being referred to long-term psychiatric care outside of NIH in the DC Metro area.

b. Recruitment

- Healthy subjects and individuals with either high trait anxiety or an anxiety disorder or major depression will be recruited.
- Healthy subjects (will be recruited through the lists from NIH office of Public Relations and Public Liaison (PRPL). Individuals with either high trait anxiety or an anxiety disorder or major depression will be recruited through mechanisms developed by PRPL.
- Patients with anxiety disorders are recruited directly into this protocol. .
- Mechanisms include advertisements placed through university newspapers, the city paper, and local gazettes, Web Links, and public service announcements, and advertisements in Montgomery County and DC Metro buses. These advertisements are or will be approved by the IRB prior to use. 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. Recruitment methods for healthy subjects and patients will include advertisements placed in university newspapers, city newspapers or magazines, local gazettes, web links, listservs, public service announcements,

Instagram, and posting of flyers in local eateries or small businesses or sites such as public libraries. 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. Moreover, recruitment efforts will include advertisements in Montgomery County buses. We also will utilize websites, such as college papers and local media. 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. One such site will be the Club PCR site used by the research assistants at NIH. 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. Ads may be placed on the CC Twitter, Facebook pages, 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. Mood disorder patients are not directly recruited for this protocol; rather; these patients will be referred by Carlos Zarate's group (NIMH). Patients from Dr. Zarate's group will already have been cleared under the screening protocol (01-M-0254). A physician in Dr. Zarate's group will approach subjects who meet criteria for this protocol and give them information about the study. If they choose to participate, we will follow with the consent process. Consenting practice will comply with the NIH Clinical Center MAS Policy pertaining to consent of non-English speaking speakers. Additionally, study information will be distributed to local chapters of such self-help organizations as Anxiety Disorders Association of America, Freedom from Fear, and the National Alliance for the Mentally Ill. Last, speakers from Drs. Zarate or Pine groups may distribute flyers at venues where they have discussed NIH research protocols. All such advertisements and flyers have been submitted to the IRB and received IRB approval. Any new advertisements or changes to existing advertisements will be submitted to the IRB for approval prior to publication.

- Healthy volunteers may also be identified or screened through the NIMH protocol #17-M-0181 titled "Recruitment and Characterization of Research Volunteers for NIMH Intramural Studies."
- 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 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.

- 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 target all potential volunteers within that age range (18-50 years old) and location (within 50 miles) and will not specifically target anxiety volunteers. 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.
- Potential subjects will undergo an initial pre-screening by phone. (See Appendix A) This is done prior to consent procedures to determine potential eligibility. During the phone screen, the following will be described: a) purpose and goals of the study, b) the experimental procedures involved, c) the time commitment required, d) compensation, and e) inclusion/exclusion criteria. It will be emphasized to participants that unpleasant shocks and air puffs will be administered during the course of the study. If individuals are interested in participating in the study they will be given an appointment to come to NIH for a first visit. In addition to receiving information via telephone, potential participants will receive complete information about this study by mail including actual copies of the consent form.
- Mood Disorder Patients will be referred by Carlos Zarate's group (NIMH/). We will be recruiting patients from Dr. Zarate's group, who already have been cleared under the screening protocol (01-M-0254).
    - A physician in Dr. Zarate's group will approach subjects who meet criteria for protocol 03-M-0093 and give them information about the study so that they may decide whether or not to participate.
    - Study information will also be distributed to local chapters of the Anxiety Disorders Association of America, Freedom from Fear, and the National Alliance for the Mentally Ill. All such advertisements have been submitted to the IRB by Susanna Sung and recently received IRB approval. Any new advertisements or changes to existing advertisements will be submitted to the IRB for approval prior to publication.

- NIH Employees, staff and family members will not be directly recruited by or through their supervisors or coworkers to participate in this study.

c. Screening

- Upon arrival for their face to face screening visit, participants will have the opportunity to fully review the 0093 protocol consent form and to ask questions. If they express interest in participating in the study, they will then be screened under the MAP Screening Protocol 01-M-0254 with the following measures to evaluate if they are indeed eligible for the study:

- Pregnancy test (urine test) for women of child bearing age
- Urine drug screen (amphetamines, benzodiazepines, cocaine/metabolites, opiates, morphine, codeine, THC)
- Wechsler Abbreviated Scale of Intelligence (WASI) (Weschler, 1999)
- Subject demographic information
- Vital signs (sitting blood pressure and pulse), height, weight
- Medical history and physical examination
- Structured Clinical Interview for DSM-IV (patients only)
- Structured Clinical Interview for DSM-IV-TR non-patients edition (non-patients only)
- Concomitant medication and pharmacotherapy history
- Inclusion/exclusion criteria

- Following the medical and psychiatric screening assessment under protocol 01-M-0254, English-speaking participants will be asked to fill out the following questionnaires:
  - State-Trait Anxiety Inventory (STAI) (Spielberger, 1983)
  - NEO-Personality Inventory (Costa & McCrae, 1992)
  - Anxiety Sensitivity Index (Peterson & Reiss, 1992)
  - Beck Anxiety Inventory (Beck & Steer, 1987)
  - Beck Depression Inventory (BDI) (Beck & Steer, 1987)
  - Fear Questionnaire (Marks & Mathews, 1979)
  - Penn State Worry Questionnaire (Meyer, Miller, Metzger, & Borkovec, 1990)
  - Positive and Negative Affectivity Scale PANAS (Watson, Clark, & Tellegen, 1988)

- Mood and anxiety disorders are associated with deficits in attention control. As an exploratory aim, we will examine whether measures of memory and sustained attention relate to any experimental measures. English-speaking participants will be tested on the following cognitive tests:

- Digit Span: In this test of memory capacity, subjects are presented with a series of digits (e.g., 2, 4, 9, 7) and must repeat them back;
- Corsi test: In this visuo-spatial short-term memory test, subjects are shown 9 blocks on a monitor. The subjects observe a sequence of blocks that lit up. Their task is to repeat the sequence. The task begins with a small number of blocks and then gradually involves more blocks;
- Go-NoGo Task; in this test of sustained attention and impulsivity, subjects are shown different letters one at a time. They have to press a button to each letter except infrequent x.

- Some participants may have participated in the NIMH healthy volunteer protocol #17-M-0181. For those participants, identifiable data may be shared between protocols 17-M-0181, 01-M-0254 and this protocol 03-M-0093. The information from 17-M-0181 may be used for screening for this protocol as long as: it has been within a year for the demographic information, medical history and physical examination, SCID, WASI, and questionnaires. The urine pregnancy and drug screen will need to be within two weeks. The urine pregnancy is also repeated prior to any study procedures on the study visit day under this protocol.

- Additional laboratory examinations (i.e. blood testing or EKG) may be completed if clinically indicated and if consent is obtained for those procedures.

- If the screening protocol (01-M-0254) finds them to be eligible, they will undergo the informed consent process for 03-M-0093.

- Following informed consent of the 03-M-0093 protocol, participants will complete the studies described below. Female participants will first have a urine pregnancy test. At the completion of each visit, patients will be clinically assessed for adverse reactions to experimental procedures and psychiatric intervention will follow when clinically indicated.

- **Patients with an anxiety disorder** will undergo an initial psychiatric evaluation with Dr. Ernst as well as an additional structured clinical interview with a credentialed staff clinician to assess the patient's suitability for the study as a part of the screening protocol (01-M-0254), The staff clinician will have clinical experience with either inpatient or outpatient psychiatric populations. All clinical work with anxiety patients will be supervised by Dr. Ernst.

d. Study Procedures

Prior to the study visit, the study clinician will review the subject medication list. If the subject takes a PRN medication (such as Claritin PRN for seasonal allergies) the clinician will assess how often the subject takes the PRN medication and determine the half-life of the medication. Study visits will be planned when the subject is not taking the PRN medication on a regular basis. The clinician will notify subject when study visit is planned and how long medication needs to be held prior to the study visit. If the subject needs to take the PRN medication such that 5 half-lives cannot pass before the study visit then the study visit will be rescheduled.

- **Sub-study 1: fear-potentiated startle and anxiety-potentiated startle:** Sub-study 1 will test 110 patients and 44 healthy controls (See 10. STATISTICAL ANALYSIS). This experiment examines fear and anxiety responses during anticipation of predictable and unpredictable aversive events (shocks or airpuffs). There are three different conditions, neutral (N), unpredictable aversive events (U), and predictable aversive events (P). Each of these conditions will be signaled by a written text displayed on a computer monitor (e.g., shock only when blue square present). In each condition, an additional cue (e.g., blue square) will be presented. The cues will be different for P, N, and U. They will be meaningless in the U and N conditions. However, in the P condition they will signal a possible shock/airpuff. The cues will be presented for 8 sec. The N, P, and U conditions will last approximately 2 minutes each. The cues will be presented twice per condition. These conditions will be presented in two blocks with the following predetermined orders: 1) P-N-U-N-U-N-P (FPS1) and 2) U-N-P-N-P-N-U (FPS2). The two blocks will be counterbalanced across subjects. In the P condition, 25-35% of the cues will be reinforced with an aversive event. Variation in fear and anxiety will be evaluated with the startle reflex evoked by loud sounds (see below).

    - **Saliva:** Up to 10 saliva samples per subject may be obtained to measure levels of cortisol and DHEA-S. Samples will be collected just after screening during study visit 1, after baseline heart-rate assessments during study visits 1 and 2, before and after the verbal-threat task of study visit 1, before and after the aversive-conditioning procedure and before and after the retention of aversive-conditioning procedure of study visit 2. Although cortisol samples would ideally be collected at the same time of day for all subjects, doing so would significantly slow data collection. Because cortisol analyses are of secondary importance to the current protocol and because time of collection can be statistically controlled during analyses, saliva will be collected during morning or afternoon visits, the time at which the samples are collected will be documented, and the variance associated with time of day will be covaried out of cortisol analyses. Of

note, each participant will be scheduled consistently in morning or afternoon time-slots to reduce the within-subject variability in the time saliva samples are collected.

- **Startle response**: The startle reflex will be elicited with a 102 dB white noise (40-ms duration) delivered via headphone. The eyeblink component of the startle reflex will be recorded binaurally with two AgCl 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, pulse, skin conductance activity (responses and spontaneous fluctuation), and respiration rate 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 will 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 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.
- **Unconditioned stimulus (US)**: The US will be an electric shock or a strong jet of air directed to the throat (airpuff). We have shown that anticipation of these stimuli produces a robust and reliable potentiation of startle. We have decided not to use a US of shock for the verbal-threat task in order to limit the number of shocks given to participants during the course of this study.
- **Electric shock:** Electric shocks are among the most efficient ways to induce anxiety in the laboratory. The shocks will be delivered through two disk electrodes placed on one of the forearms, wrists, or hands. The shock will have intensity up to 4.0 mA and duration up to 500 ms. The PI has used shocks in various experiments in over 600 subjects in the last 10 years at Yale University. The shock is generally described by subjects as rather anxiogenic and unpleasant. The mean rating of aversiveness on a scale of 1 (not at all painful) to 10 (extremely painful) is about 5. Subjects will be given sample shocks before shocks are used in experimental procedures to give participants the opportunity to avoid experimental tasks involving electric shocks if they wish. Our experience is that over 95% of subjects who received the shock chose to participate in the experiment. The Yale IRB committee has not received any complaints resulting from these experiments.

  Jet of air: The system that produces the jet of air consists of a compressed air cylinder, a regulator, a solenoid valve controlled by an AC switch, and 4-mm internal diameter polyethylene tubing. The jet of air will have duration up to 200 ms and intensity up to 80 psi (measured at the level of the regulator). The tubing will be fixed on the subjects' neck via a special collar. It will be directed to the throat at the level of the larynx.

- **Sub-study 2: Interaction between anxiety and working memory:** Sub-study 2 will test 115 patients and 46 healthy controls. We will also need approximately 40 healthy controls to pilot various versions of the tasks for a total of 86 controls. (See 10. STATISTICAL ANALYSIS). These subjects will perform memory tasks under one of two anxiogenic conditions: 1) shock threat and 2) a social stressor (either a speech stressor or social observation). The speech stressor will serve as a more ecologically-valid stressor than the shock threat.
  - **Memory tasks**: 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). They will also be asked to remember lists of these stimuli after a short delay.
  - Shock threat experiment: This experiment will be conducted in a single session. Subjects will perform a working memory task during alternating periods of shock threat and safety. Startle stimuli will be administered occasionally in the safe and threat conditions to assess changes in anxiety level.
  - S**ocial stressor experiment:** This experiment will be conducted in two parts, one, where subjects will be observed and given negative verbal feedback, and another where subjects will anticipate giving a speech. The impact of these social stressors will be measured by performance on the memory tasks described above. The observation stressor will be comprised of a single session where subjects will engage in memory task under observation by two investigators who will remain in the periphery. Subjects will be told that investigators may come into the room to periodically monitor performance. Half of the task runs will not be observed. When the investigators are present, they will give negative feedback throughout the run (e.g., "Focus your attention on the task," "Please improve your performance"). This session will last around 90 minutes.
  - The speech stressor portion will be comprised of two sessions, one in an anxiogenic context (speech stressor) and the other in an emotionally-neutral context (no speech stressor; subject are just asked to read a text). Subjects will be shown a short video depicting other "participants" (actors) giving a brief speech in the presence of three judges. The video will be used to familiarize the participants with the task, as well as to induce anticipatory anxiety. Subjects will be told that they will be giving a similar speech on a different topic in an hour or so. They will be told that they will have a short time to prepare after they have completed a series of tasks. After the video, subjects will be presented with a series of startle probes and asked to complete the cognitive tasks while they periodically receive startle probes to index their anxiety across the experiment. After completing the cognitive tasks, subjects will be given 1 minute to prepare for a 3-minute speech that they will give in front of a few investigators (at least 2 IRTAs). They will be presented with another series of startle probes and after they will give a short speech. Finally they will

receive another series of probes, and complete a final series of questionnaires. On the neutral context day, subjects will watch a neutral video clip that has no relation to the study/speech stressor. They will complete the same cognitive tasks and receive startle probes throughout. They will not be required to give a speech at the end of the experiment.

- **Sub-study 4: Emotional Expression Multimorph task:** Sub-study 4 will test 30 patients and 30 healthy controls. The task will require one session lasting up to 2 hours. Stimuli will be pictures of faces exhibiting anger, disgust, fear, happiness, sadness and surprise (Ekman, 1993).. Subjects will indicate which emotion the face displays. Latency and accurate response will be used as measures of facial emotion recognition. The task may be slightly modified (e.g., timing, number of morphed faces, number of emotions) to improve sensitivity of the task to our specific goal. The cognition consent will be used for this study.

- **Sub-study 5: Effect of anxiety on Stroop task:** We have studied the effect of anxiety on the Stroop task in healthy adult volunteers (01-M-0185). Sub-study 5 will test 30 patients and 30 healthy controls. The task will require one session lasting up to 2 hours. Stroop tests are used to study emotional distraction. In the affective Stroop task, participants say if stimuli are congruent or incongruent. A visual image of either positive, negative, or neutral emotional content taken from the IAPS (Lang and Greenwald, 1988) is presented between the stimuli. Participants experience two types of trials: task and view. Task trials (b and c in figure below) involve the participant responding via button press with respect to the number of numbers in an array that is temporally bracketed by positive, negative or neutral images. View trials (a in figure below) involve no participant response and only the display of positive, negative or neutral images. Considerable work with this task has shown that task performance relies on the recruitment of regions implicated in top-down attention (dorsomedial and lateral frontal and parietal cortices); (K. S. Blair et al., 2007). Recruitment of these regions is associated with a diminished amygdala response to positive or negative images (K. S. Blair et al., 2007). Patients with anxiety disorders show a reduced ability to recruit dorsomedial prefrontal cortex during task trials and increased amygdala responses to emotional distracters (K. S. Blair, Vythilingam, et al., 2012; K. S. Blair et al., 2013).This task will be conducted during alternating periods of shock threat and safety. Some parameters of the task task (e.g. timing parameters of the task, organization of the safe vs threat epochs, and nature of the congruent/incongruent stimuli) may be modified as it fits our protocol goals, but won't change the basic design. The cognition consent will be used for this study.

- ***Sub-study 7. Active Avoidance/Stop Signal Tasks (Patients N = 40):***

Participants will perform paradigms which test whether threat impacts the initiation and the inhibition of behavioral responses. Participants will be presented with stimuli, and will either initiate a response (i.e. "go") or will inhibit their response (i.e. "stop"), based on what stimuli is presented. Participants will perform the stop-signal paradigm, the active avoidance of signaled threat paradigm, or a modified version of one of these two tasks.

Participants will perform the stop-signal paradigm during a safe condition and a threat condition that involves the delivery of aversive electrical stimuli. Participants will be presented with an arrow which points either left or right, and will be instructed to press a button with their index finger or middle finger based on which direction the arrow is pointing. During stop trials, a colored circle will appear behind the arrow and participants will be instructed to refrain from making any response. Independent stair-case procedures (Leotti & Wager, 2010) will be used to track performance and will determine the amount of time required to accurately initiate responses during "go" trials and accurately inhibit responses during "stop" trials. During the threat condition, one group of subjects will receive aversive electrical stimuli during the commission of performance errors, while another group of subjects will receive an identical amount of electrical stimuli regardless of performance. Stair-case procedures from the safe and threat conditions will be compared to determine whether processing speed of "go" and "stop" performance is impacted by threat.

Participants will perform a modified version of the active avoidance of signaled threat paradigm (Gorka, LaBar, & Hariri, 2016). Participants will hold down a computer key and will view images. The images will differ based on their color (low, medium, and high), and participants will always view two images presented one after the other. Participants will be instructed to lift their finger when seeing the medium-medium color combination (i.e. "go" trials), and to refrain from lifting their finger during all other combinations (i.e. "stop" trials). Participants will perform alternating blocks of the safe and threat condition, and each block will consist of a series of "go" and "stop" trials.

Participants will be instructed that following the completion of each safe block, they will be informed how many mistakes they made during the preceding block and will receive zero shocks regardless of their performance. Participants will further be instructed that following the completion of each threat block, they will be informed how many mistakes they made during the preceding block and will receive a single electric shock. We will use independent stair-case procedures to titrate task difficulty so that all participants are ~50% accurate during go trials. Additionally, the range of mistakes which result in a single electrical stimulus will be sufficiently wide that all participants will receive a single shock following each threat block. Participants will be told that the task becomes more difficult over time and that it is impossible to make zero mistakes and to avoid the shock completely, but that the duration of the shock is dependent on their performance and that it is possible to shorten the duration of the electrical stimulus by committing fewer mistakes. The duration of the electrical stimulus which participants receive following each threat block will vary as a function of the participant's performance in the preceding block, such that larger numbers of performance errors result in an electrical stimulus of longer duration. The shock will not last longer than 200msec. Participants will receive electrical stimuli immediately following the end of each threat block.

This research will allow us to determine whether threat preferentially impacts different types of performance (i.e. response initiation vs. response inhibition) and whether linking aversive outcomes to performance impacts the effect of threat on these behavioral phenomena.

This study will require one session in the outpatient clinic at NIH that may last up to 3 hours.

- **Measures Common to all sub-studies:**

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.

- **Assessments**

Adverse events will be rated weekly. The primary efficacy measure will be the Clinical Global Impressions Scale (CGI) Improvement rating (Guy, 1976). The CGI is a well-documented outcome measure in studies investigating the efficacy of treatments for psychiatric disorders including social anxiety disorder (Liebowitz, et al., 1992), and panic disorder (Barlow, Gorman, Shear, & Woods, 2000). A second aspect of the CGI, the CGI Severity index, will also be administered. Clinician ratings of anxiety symptoms will be completed weekly. Anxiety symptoms will be rated with the Hamilton Rating Scale for Anxiety [HAM-A] (Hamilton, 1959).

Research Questionnaires used in each study: In addition to using the trait portion of the STAI to classify healthy controls as high or low in trait anxiety, the state portion of the STAI will be administered prior to every experimental task (i.e., test of orienting response to innocuous stimuli, dot probe, verbal threat, aversive conditioning, eyeblink conditioning) to assess subjective measures of fear and anxiety that may influence outcome measures from experimental tasks. The remaining research measures (NEO, BDI, BAI, cognitive tests, etc.) are given for exploratory purposes.

For the purposes of screening, we use a standard set of questionnaires to assess mood and anxiety for subjects including SCID, BDI-II, IDSR, HAM-D, HAM-A, and MADRS.

The non-analogue measures from these reports may be completed by the participant 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. Participants may enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. There are no potentially actionable questions to be used by this system and thus the answers will not require real-time monitoring.

e. End of Participation

- Participation in the studies is expected to be completed within six months, though precise timing depends on the needs of the patients. Subjects will be terminated from the protocol when they request to discontinue participation. Moreover, subjects requiring clinical care after the completion of treatment at NIH will transition to community-based clinicians. Clinical care will be provided in the community, outside of the current protocol.
- All subjects are seen on a regular basis by a trained clinician. Any worsening of symptoms will be reviewed by a panel of clinicians. This review will be designed to remove subjects from the

trial who show significant worsening, in an effort to minimize any potential adverse consequences of treatment.

5. STORAGE OF DATA AND SAMPLES

We collect up to 10 saliva samples for each research participant. Saliva samples are stored in Building 49, Rm #B1B56. Saliva samples are discarded once cortisol levels are extracted.
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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.

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

a. Psychiatric Assessment: It is highly unlikely that study participants will become upset about the questions or interview process used in the study. Each measure has been used extensively in hundreds of psychiatric patients and healthy controls without adverse effects. Subjects may refuse study participation at any time, and the standard interviewing and data collection procedures will cease, should any adverse reactions be noted. All subjects completing the interviews and questionnaires will be provided with phone numbers so that they can have any questions answered that they feel have not been satisfactorily addressed. The medical investigators for this study have extensive interview experience, and they will determine if there is a need for clinical intervention; if necessary, arrangements for appropriate clinical services will be made.

b. Electric Shock. The shocks are designed to be moderately painful and to evoke anxiety. Based on the PI's research 20 year experience with experiments involving the administration of electric shocks to both anxiety patients and healthy controls, little to no adverse reactions to the electric shocks are expected among patients and controls. However, 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 are excluded from shock studies that use the wrist for placement of electrodes.

Although no adverse reactions are anticipated, clinical intervention, as delineated under "Emergency Treatments", will be carried out under the supervision of Dr. Ernst in the event that subjects display or report distress during study procedures.

c. Psychophysiological Recording. The psychophysiological measures that will be obtained are non-invasive, requiring the administration of no needles, drug, or dyes. During electrode placement, the possibility of skin irritation from contact with the saline electrode paste exists. However, this is unlikely since the salt concentration of the paste is similar to that of human sweat. The risk is equivalent to that of an EEG recording. Because the PI's past studies in anxiety patients and controls using virtually identical recording procedures have resulted in little discomfort to participants, no adverse reactions to such procedures are expected.

d. Auditory Startle Stimulus. Loud sounds can cause hearing problems, but the auditory stimuli that will be used in the startle studies are safe (i.e., there is no danger of hearing impairment). This is because of the physical characteristics of the startle sound (40-ms duration, 102-dB white noise) are within the safe range. Auditory startling sounds of much higher intensities and longer duration (500 ms) are frequently used in startle studies (Hawk & Cook, 2000; Lipp & Siddle, 1998)(Hawk & Cook, 2000; Lipp & Siddle, 1998). Forty-ms/102dB sounds are well below the recommended limits stated in the documentation of various safety regulations. For example, the Occupational Safety & Health Administration (OSHA) recommends that exposure to impulsive or impact noise should not exceed 140 dB peak SPL. The National Institute on deafness and other Communication disorders (NIDCD) website recommends no more than 15 min unprotected exposure at 100 dB. Finally, we use white noise, which are safer than pure tones.

e. Air Puff. The air puff is very intense and mildly unpleasant, but harmless. There is no known risk event associated with the air-puff

f. Experimental Procedures. A series of procedures designed to minimize risk will be applied to participants in this protocol. Both patients and healthy controls will undergo a sample shock procedure before any experiments involving electric shock are run to assess each individual's reaction to the shock, to adjust the shock to a level that is well tolerated, and to give the participant a chance to withdraw from the study if they so wish. An additional procedure is the observation of participants by way of a non-recording video camera and two-way audio telecom throughout testing. Participants are instructed to signal the experimenter through the video camera or by calling the experimenter's name at any point during the experiment if they wish to end the experiment or if they wish to speak with the experimenter for any reason. Upon completion of testing procedures for a given visit, patients with a psychiatric disorder are evaluated by Dr. Ernst to assess for any adverse reactions to experimental procedures. Hence, any problems that stem from these procedures will also be detected and treated in a timely manner.

g. Delayed Treatment. Although anxiety and mood patients will not be taken off any medication for the purposes of this study, we will only include those patients who are not

currently taking psychotropic medications. The only exception to this is the bipolar depressed patients. These individuals, because of the risks associated with mood destabilization, will be permitted to continue taking either Depakote or lithium carbonate as mood stabilizers. Nonetheless, patients included in this study may be at risk of symptom worsening because of the absence of other psychopharmacologic treatment. If patients choose to enroll in the study despite this risk, their symptoms will be monitored by medical staff. If symptoms worsen and are deemed by our medical staff to be a threat to the patient's welfare, the patient will be discontinued from the study and receive brief treatment by our medical staff while referral efforts to community care are underway.

h. 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 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. The participants will be reminded that they may stop looking at the pictures at any time.

i. Grip force measures: This measure is not expected to increase risks in human subjects.

8. SUBJECT SAFETY MONITORING

- Subject monitoring will be conducted by the Principal investigator, Monique Ernst, MD, Ph.D.
- All patients will be monitored at each visit for their participation for signs of discomfort or desire to discontinue the protocol. Specifically, they are in constant view of the research staff during the experiment. They are instructed to wave at the (non-recording) camera or else to call the researcher if they wish to stop the experiment. The researcher is within earshot of the participant at all times. The participants will also be explicitly told during the consenting procedure as well as after the sample shock procedure that it is their right to discontinue the study at any time.
- **Criteria for Withdrawal:** If a patient experiences clinically significant worsening, displays any suicidal ideation or active plans of suicide, reports adverse reactions to the experiment at any point during the study, appears to be in distress at any point during the procedures, or demonstrates adverse responses by way of the post-experimental assessment of adverse events, the patient will meet with Dr.Ernst or Dr. Zarate, or their clinical staff, who will assess the patient's suitability for continuation in the study and will administer appropriate psychiatric treatment. If the psychiatrist determines that continuation is clinically counter-indicated, the patient will be removed from the protocol but will receive treatment at the NIH under the care of Dr. Ernst or Dr. Zarate until appropriate, long-term psychiatric care is arranged. The reasons for patients' discontinuation from the study will be logged and changes to procedures necessary to prevent future adverse reactions will be made.
- **Role of Medical Doctors:** The medical advisory investigator for this study, Dr. Monique Ernst, will serve as the primary treating psychiatrist for anxiety patients enrolled in this protocol. Dr.

Ernst's role will include serving as the intervening psychiatrist in the event of adverse reactions or symptom worsening as well as delivering short-term treatment (1-2 months, not to exceed 3 months) to anxiety patients upon their completion of the protocol. Additionally, Dr. Ernst will complete the initial psychiatric evaluation (described below) and will be on call for subjects along with other psychiatrists on the child anxiety on-call schedule to allow 24-7 on call coverage for patients in this protocol. In the event that participating patients experience symptom worsening while off-campus, they will call the child anxiety on-call schedule. When Dr. Ernst is the on call-physician, he will provide the psychiatric treatment for this patient. Otherwise, the on-call physician at the time the patient calls will intervene and provide the necessary care. Dr. Zarate and his associates in his program will serve as the primary treating psychiatrists for depressed patients in this protocol. Dr. Zarate or a clinician in his group will be the primary treating physicians. They will intervene in the event of adverse reactions or symptom worsening and will as deliver appropriate treatment.

- **Healthy Controls:** Throughout experimental procedures, psychiatrically healthy subjects will be monitored by both a non-recording video camera and by a two-way audio telecom. Procedures will be stopped for any subject who asks to stop any procedure at any point. Subjects will be asked if they wish to continue with other phases of the study. Procedures will also be stopped for any subject who exhibits signs of distress during any phase of the study. At this point, subjects will be interviewed to confirm that they are in fact in distress and psychiatric intervention will follow when necessary. Subjects will be asked if they wish to continue with other phases of the study.
- **Psychiatric Patients**: Mood patients accepted into this protocol will have been thoroughly screened and assessed for inclusion/exclusion criteria by clinicians of the Experimental Therapeutics & Pathophysiology Branch of NIMH. For English speaking bipolar depressed patients, Dr. Zarate and his team will be responsible for screening evaluations, the experiments and clinical assessments during each visit.
    - Monitoring during the experimental sessions is conducted by a research assistant under the supervision of a physician from this branch. Like healthy controls, patients will be monitored via non-recording camera and two-way audio telecom throughout experimental procedures.

    - All patients with anxiety psychiatric disorders will be provided with Dr. Ernst's pager number as well as other phone numbers for emergency psychiatric coverage, 24-hours/day. Patients with mood disorders will be provided with Dr. Zarate's or one of his physician's pager number as well as other phone numbers for emergency psychiatric coverage, 24-hours/day.

    - Patients will be monitored by way of multiple clinical assessments. The clinical assessments by the psychiatrist addresses not only the primary (mood) diagnosis, but also any allowed comorbid conditions with which the patient suffers. Symptom severity of both the primary and comorbid conditions is monitored and interventions provided by medical staff as deemed medically necessary

Below is a table delineating all scheduled clinical assessments for patients during each visit.

Schedule of Clinical Assessments for Patients*

Session**	Time	Type of Assessment	Clinical Staff
1	upon arrival	Initial psychiatric evaluation	Dr. Pine
	upon arrival	SCID	credential staff clinician
	upon arrival	Assessment of symptom severity(C-MARS)	credential staff clinician
	end of session	Assessment of adverse reactions	Dr. Pine
2	upon arrival	Assessment of symptom severity(C-MARS)	credential staff clinician
	end of session	Assessment of adverse reactions	Dr. Pine
3	upon arrival	Assessment of symptom severity(C-MARS)	credential staff clinician
	end of session	Post-exp. psychiatric assessment	Dr. Pine

* Only includes scheduled assessments and does not include assessments/interventions to be administered in the case of adverse events.
**1 week interval between visits.

- **Initial Psychiatric Evaluation**: At the outset of screening, patients will meet with Dr. Ernst or Dr. Zarate for an initial psychiatric assessment. During this evaluation, the psychiatrists will assess the patients' suitability for this study based on the patient's psychiatric/medical history as well as the patient's current symptom profile. Patients participating in studies 1 and 2 will be informed that the study is not designed to treat their psychiatric disorder/s and that their condition may worsen while participating in this study. Additionally, patients will be reminded that psychopharmacologic and psychotherapeutic treatment options for anxiety and mood disorders are available in clinical practice outside of the NIH and delaying such treatment may have adverse effects on their mental health.

- **Study Phase:** If, during the course of the study, a subject's condition deteriorates to the point where emergency treatment is needed, the subject will be removed from the protocol. At this point or any other point where subjects are removed from the study for non-emergent reasons, clinical care will be provided by staff, under the direction of Drs. Ernst or Zarate. This care will continue until the case has been successfully stabilized, through the use of standard clinical practice in the treatment of anxiety and mood disorders. If patients require in-patient care, they will be hospitalized in one of the NIMH inpatient units.

- **Clinical Care After Treatment:** Following the completion of the study, psychiatric patient participants in the anxiety protocol will receive short term psychiatric treatment lasting 1-2 months (not to exceed 3 months) under the care of Drs. Ernst and the adult anxiety program staff. The total amount of time that out-of-study care will be provided is expected to vary, given the diverse clinical needs of patients. Ideally, the team will provide care for between one and two months in most cases. However, efforts to transition care to the community will intensify after two months and the study team will make every effort to ensure that no more than 3 months of treatment are provided. Similarly, the depressed patients will receive care as outlined above under the direction of Dr. Zarate and the mood disorders program staff. The time line of care as detailed above will be followed for these patients as well.

9. OUTCOME MEASURES

a. Primary outcome measures: Primarily, we are interested in exploring the affective, cognitive and behavioral of our study's dependent measures. The primary outcome measures will be the startle reflex and performance on cognitive tasks.
b. Secondary outcome measures: These measures include psychophysiological measures other than startle (e.g., skin conductance) and state and trait questionnaires.

10. STASTISTICAL ANALYSIS

The overall target N for the protocol is 663 healthy controls and 608 patients. Participants who drop out will be replaced. Participants may participate in more than one sub-study.

- Sub-study 1
    - **Analysis of data/study outcomes:** Startle amplitude during the verbal threat procedure will be averaged over cue and no cue (ITI) periods within conditions. There will be separate analyses for anxiety disorders and mood disorders. The data will be submitted to a Group (GAD, SAD, panic disorder, control or MDD, bipolar, control) x Condition (P, N, U) x Cue (cue, ITI) ANOVA. If this interaction is significant, within-group simple-effects comparing startle amplitudes during the ITI of predictable versus unpredictable conditions will be compared for all groups separately. Larger startle magnitudes in the unpredictable versus predictable condition suggest heightened sensitivity to unpredictability. Between group contrasts will also be computed to compare difference scores (i.e., differences between startle magnitudes during unpredictable and predictable conditions) across groups. In our pilot and published studies, we obtained the lowest effect size (f=0.30) for contextual fear (anxiety-potentiated startle).

- **Power analysis:** We set power at .80 and experiment-wise, two-tailed alpha at 0.05. Based on these parameters, we will need 18 subjects per group. Assuming a 15 % unusable data rate due to lack of startle response or equipment failure, approximately 22 subjects per group will be recruited for a total of 110 patients and 44 healthy controls.

- Sub-study 2
  - **Analysis of data/study outcomes:** Startle amplitude will be averaged over conditions (safe, threat) and WM load (View, 1, 2, 3). There will be separate analyses for anxiety disorders and mood disorders. The data will be submitted to a Group (GAD, SAD, panic, controls or MDD, bipolar, controls) x condition (safe, threat) x WM load (1-back, 2,-back, 3-back).
  - **Power analysis:** Based on our published study in healthy controls (Vytal et al, in press), where we found a 2-way interaction between condition and WM load with an effect size of η^2=0.20, we estimate that we will need 20 subjects per group. Given a rate of 15% of unusable data, approximately 23 subjects per group will be recruited for a total of approximately 115 patients and 46 healthy controls. We will also need approximately 40 healthy controls to pilot various versions of the tasks for a total of 86 controls.

- **Sub-study 3**
  - **Analysis of data/study outcomes:** patients will be tested twice, before and after the 8-week treatment, while controls will be also tested twice, 8 weeks apart. Startle amplitude and performance will be averaged over conditions (safe, threat) and WM load (view, 1, 2, 3 for startle and 1, 2, 3 for performance). These data will be submitted to omnibus Group (patients, controls) x condition (safe, threat) x WM load (view, 1-back, 2-back, 3-back for startle and 1-back, 2-back, 3-back for performance) x Time (first session, second session) ANOVAs. However, the analysis will focus on the 3-back condition, where greater group differences are expected. Changes in fear-potentiated startle and differential performance (threat minus safe) in the 3-back condition from session 1 to session 2 will be compared between the two groups. Secondary measures will be subjected to multiple-regression analyses. For all such analyses, questionnaire scores will serve as predictor variables and different experimental outcomes will serve as dependent variables. Analyses will regress questionnaire scores dependent measures of interest: 1) anxiety-potentiated scores, 2) WM scores, and 3) attentional-bias scores. In so doing, we will be able to assess the combined and unique contributions of questionnaire scores to the variability in dependent measures.
  - **Power analysis: We estimate that we will need 30** subjects per group. Given a rate of 25% of unusable data or non-completers, approximately 38 controls and 38 patients will be recruited.

11. HUMAN SUBJECT PROTECTIONS

a. Subject selection

i. Statement of equitability:

- The race distribution of the samples studied in this project will be similar to that of the greater Washington DC metropolitan area, and will include representation from all minorities excepting American Indians, who we have much less opportunity to study. Because the NIMH is located in a suburban area in which African-Americans may be under-represented, if at any point the race composition of subjects recruited during the proposed imaging study fails to reflect the race distribution of the larger geographic area, we will intentionally emphasize recruitment of subjects from more urban areas through collaborations being formed with Howard University. While we have not found significant race effects on the clinical or psychobiological parameters assessed, we will continue to perform secondary analyses to explore potential effects of race on these domains.
- We exclude non-English speakers since not all the instruments and test we use are translated and validated in Spanish or other languages.
- We expect ***to enter about equal number of fem***ales and males in the study. Pregnant females will be excluded because the effects of shock are unknown on a developing fetus. The upper limit for age range of 50 years is set to achieve greater homogeneity in our sample and thereby reduce the variability of the startle reflex, which tend to decrease with increased in age. This is necessary to increase the signal to noise ratio and the sensitivity of our study to detect differences between conditions.

ii. Rationale for selection if not equitable

N/A

b. Justification for inclusion/ exclusion of children

- In order to elicit anxiety responses similar to those occurring in anxiety disorders it is important that out stressor is highly unpleasant. We thus chose to use electric shock as the stressor. Though shock stressors are well tolerated by adults (and used frequently in the literature to assess stress reactions in healthy and disordered individuals), such methods may be inappropriate for children. We will not enter children under age 18 because of ethical concerns about exposing them to threat of shock. Moreover, concerns regarding the legal inability to provide informed consent before age 18 (and the consequent dependence on parental decision) preclude inclusion of subjects under age 18.

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

We exclude patients with cognitive impairment as they would be unable to self-administer the necessary test instruments. Subjects with other Axis I disorders other than mood and anxiety

disorders because of the confounding effects of other psychiatric disorders. Pregnant women are excluded because the effects of MRI and shocks on the fetus are not known.

d. Justification of sensitive procedures (use of placebo, medication withdrawal, provocative testing)

- 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 and to detect patients control differences.

e. Safeguards for vulnerable populations e.g. DPA, pregnancy testing, contraception use, ethics consult, HSPU involvement

- We assess pregnancy via urine test within 24 hours of any testing and exclude all participants who are pregnant.

- Protections for NIH employees, staff and family members 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 NIH employees, staff and family members through staff discussions and written branch/section procedures. Prior to enrollment, potential participants will be informed that sensitive information (e.g. drug and alcohol use, specific medical diagnoses) will be in the participant's NIH medical record.

12. ANTICIPATED BENEFIT

- The experimental procedures involved in this study provide no direct benefit but are likely to yield generalizable knowledge about the psychophysiology of anxiety in health and disorder. Results from this study may help others with similar diagnoses in the future by facilitating the development of more effective assessment and intervention strategies for anxiety disorders.

14. CONSENT DOCUMENTS AND PROCESS

a. Designation of those obtaining consent

- Study investigators designated as able to obtain consent listed in KSP form, will obtain informed consent. Consent for NIH employees, staff and family members will not be obtained by coworkers.

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.
- Prior to commencement of the experiments, all participants will have been fully informed and will be consented under protocol 03-M-0093. There will be two separate consents, one for controls, and one for anxiety volunteers.

- 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.
- Informed consent for the study will be obtained in-person or via Telehealth prior to any tests. The consent forms include a checklist that includes all procedures outlined in the protocol. Prior to consenting, all procedures subjects will experience will be marked so that the participant understands exactly what they will be doing. If a subject returns to do a different task within the same protocol, they will be re-consented with appropriate procedures marked
- During the consent process, participants will be reminded that their participation is voluntary and that they may discontinue their participation at any time without jeopardizing their continuing medical care at this institution, or losing benefits they would otherwise be entitled to. Patients will be informed that they will be provided with treatment recommendations, short-term treatment, and referrals to other treatment providers should they decide to withdraw from the study. All subjects will also be informed that Drs. Ernst or Zarate, or other psychiatrists acting for them, will have the right to withdraw them from the study at any time for clinical reasons or if they have failed to follow instructions. Importantly, patients will be informed that upon completion of the study they will be provided with continued short-term treatment for their psychiatric disorder at no cost at the NIH for up to two months after which they will be referred to an appropriate psychiatric team for long-term treatment.

- All subjects who will participate in studies 1 and 2 (that do not involve CBT) will be informed that no immediate personal medical or psychiatric benefits will be derived from participation. It will be explained that these experiments are designed to gain information that will lead to better understandings of the associations between brain function and anxiety arousal that will potentially benefit individuals with anxiety disorders in the future, but that the results will not be helpful for indicating current treatment, diagnosis, or prognosis.
- Subjects will be informed of all potential risks of participation during the consenting process. Of note, patients with anxiety and/or mood disorders will be told that the study is not designed to treat their psychiatric disorder/s and that their condition may worsen while participating in this study.
- Additionally, patients participating in studies 1 and 2 will be reminded that psychopharmacologic and psychotherapeutic treatment options for anxiety and mood disorders are available in clinical practice outside of the NIH and delaying such treatment may have adverse effects on their mental health. During the consent process, anxiety patients will be told that Dr. Ernst will be assigned to them as their primary psychiatrist should they participate in the study and that they will be given Dr. Ernst's pager number as well as other methods for contacting NIMH psychiatrists 24 hours a day, 7 days a week. The same procedure applies to depressed patients, who will be provided with Dr. Zarate's contact information.

c. Consent documents

- All consent forms include the required elements
- Consent documents submitted with this protocol include: Control, Patient, and Cognition

15. DATA AND SAFETY MONITORING

a. Data and Safety monitor:
This protocol will be monitored by an independent safety monitor, Dr. Pedro Martinez.

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:
The study will be stopped or suspended for any potentially related serious adverse event. The Principal Investigator, Independent Monitor, and IRB will determine if changes are needed for the research to continue or if it will be closed. Any changes required as conditions for resuming the

research must be submitted as an amendment and IRB-approved before the changes can be implemented.

16. QUALITY ASSURANCE

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

- 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. ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING

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

18. ALTERNATIVES TO PARTICIPATION

- There are antianxiety medications that may be prescribed by family doctors as well as psychiatrists that could alleviate the anxiety symptoms experienced. Some of these treatments are benzodiazepines (alprazolam, e.g.) and some SSRIs (lexapro, e.g.). Cognitive behavioral therapy (CBT) is also readily available by therapists in the community as a treatment of choice for anxiety symptoms. Those who agree to participate in this research will be advised of these alternatives to research participation.

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.

Every necessary step will be taken to prevent identification of study participants or violations of confidentiality of the data. 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. Information will be stored using a confidential code and data will be treated only as groups. All

data entered into a database will appear only in coded form. Members of the research team will have access to these coded data. Only staff directly involved in the care of each subject will have access to clinical documents that contain identifying information. This will include the study PI, the study psychiatrist, clinical staff, and research assistants. This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about NIH employees, staff and family members through staff discussions and written branch/section procedures.

b. For stored samples
Saliva samples are labeled with subject number only. No identifying information appears on the tube. Cross-referenced records are stored separately.

c. Special precautions
N/A

21. CONFLICT OF INTEREST/ TECHNOLOGY TRANSFER

a. Distribution of NIH Guidelines
   - NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflicts of interest
   - There are no conflicts-of-interest to report.

c. Role of a commercial company or sponsor
   - N/A

22. TECHNOLOGY TRANSFER

N/A

23. RESEARCH AND TRAVEL COMPENSATION

- Which populations will be paid and which will not: All volunteers will be compensated for time and research-related inconveniences.
- Amount of compensation: Subjects will be given compensation for their participation in the study based on NIH standards for time devoted to research projects based on the following schedule. All participants will be compensated $150 for completing the study. Subjects may receive an extra $20 for the optional Telehealth consenting visit. Payment will be sent after each visit. The total compensation for a study visit will be $150 or $170 for a study visit and Telehealth consenting visit.
- Travel compensation: No travel compensation will be given to participants in this protocol.

- 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.
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