PROTOCOL SUBMISSION TEMPLATE

1. Title Page

Protocol Title: fMRI Investigation of Explicit Cue and Contextual Fear

Protocol Number: 02-M-0321

Date of This Submission/Version: May 01, 2023

Principal Investigator

Maryland Pao, MD	Branch/Institute OCD, NIMH	Bldg/Rm 10/6-5340	Phone 301-435-5770	E-mail paom@mail.nih.gov

Total requested accrual
Total accrual ceiling: 1952
(726) Patients
(1226) Healthy Volunteers

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

IND/IDE ☐No ☒Yes *(attach FDA documentation)*
Drug/Device/#:
Acoustic startle
Shock device
7T fMRI
Sponsor: _____

Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☒No ☐ Yes

Data and Safety Monitoring Board ☒No ☐ Yes

Technology Transfer Agreement ☐ No ☒ Yes

Confidential Disclosure Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

Table of Contents (number pages)

3. Précis:

This protocol examines the neurobiology of fear and anxiety using various approaches. During fear 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. Experimental evidence suggests that cued fear and contextual fear model different aspects of anxiety. Studies in patients indicated that contextual fear may model an aspect that is especially relevant to anxiety disorders (Grillon et al., 1994, 1998a,b; 1999). However, the neural basis for the expression of contextual fear has not previously been elucidated in human imaging studies.

One important determinant of contextual fear is predictability: contextual fear increases when a threat (e.g., electric shock) is unpredictable, as opposed to when the threat is predictable. The aim of this study is to compare the neural substrates underlying fear evoked by predictable versus unpredictable shocks. Animal studies have indicated that conditioned responses to predictably cued threat and to less explicit threat are separate processes mediated by distinct brain structures. Psychophysiological data suggest that the proposed procedure can differentiate between these two responses. Hence, we anticipate that this procedure will allow us to compare brain correlates of these responses in humans.

Another objective is to study effects of threat of shock on processing and learning of threat cues in the amygdala, the visual and auditory systems, and motivation/reward systems. This will be investigated by means of event-related magneto-encephalography (MEG) and fMRI measurements using various paradigms.

Finally, a last project will examine how pharmacologic manipulation of gamma-aminobutyric acid (GABA) levels with the benzodiazepine alprazolam affects the relationship between GABA concentration (quantified with magnetic resonance spectroscopy, MRS), visual- and auditory-induced gamma oscillations (measured with MEG), and fMRI BOLD response. This project was completed in 2014.

1. Introduction and Background:

1.1 Fear versus anxiety: different underlying brain mechanisms?

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) identifies several anxiety disorders, suggesting heterogeneity of symptoms and etiology among them. In people suffering from explicit cued fear (e.g., specific phobia), fear is associated with an identifiable stimulus that is thought to predict the occurrence of an aversive event. By contrast, sustained anxiety occurs when people have learned that aversive events are both inevitable and unpredictable, in other words, that harm will come without warning (Kandel, 1983; Barlow 2000; Grillon, 2002). The purpose of the fast and intense fear-response is to mobilize resources to enable appropriate flight/fight action. This response is adaptive in the face of an identifiable threat, on which the action can be based. In contrast, anxiety is a feeling of apprehension that is more akin to worrying. It is more sustained and is not generally associated with a clear event. The physiological symptoms of fear and anxiety are rather similar. Hence, the animal experimental literature has often taken these responses as similar besides their intensity and duration. For ease of design, up to date most human imaging studies have focused on cue-specific fear (e.g., fear conditioning, Labar, 1998; Büchel, 1998). The present protocol aims to dissociate brain mechanisms involved in fear and anxiety responses. The characteristics of fear on the one hand, and anxiety on the other hand, can be modeled by responses that are evoked experimentally by either an explicit cue or a context, respectively. Whereas experimentally evoked responses to specific cues capture aspects of (pathological) fear, responses evoked by experimental contexts are un-signaled and, thus, capture some essential characteristics of chronic anxiety. This protocol seeks to understand the neural basis of these different responses.

We have reported data consistent with the hypothesis that patients with anxiety disorders and high trait anxious subjects are excessively sensitive to contextual fear (Grillon et al. 1994; 1998a; 1998b; 1999). For example, veterans with posttraumatic stress disorder (PTSD) exhibit unconditioned enhanced startle responses in environmental contexts where aversive stimuli are expected, compared to experimental contexts where no such stimuli are expected. Moreover, differences in the means of evocation of the response (specific stimulus only in simple phobia) between anxiety disorders suggest differences in the networks involved. Moreover, sensitivity to pharmacological treatment differs across disorders (benzodiazepines are not really successful in the treatment of simple phobia). Additionally, fear-potentiated startle evoked by a discrete stimulus (such as the discrete cues in the proposed fMRI study) has been shown to be insensitive to benzodiazepine treatment in 4 different experiments across two different laboratories (Baas et al., 2002).

Animal research has provided evidence for differentiation between two defense systems. A fast acting, phasic system associated with fear responds to cued threat. A more sustained system associated with anxiety responds to contextual threats that are not tied to a specific cue (Blanchard et al., 1993; Davis, 1999; Lang et al., 2000). Furthermore, these studies have revealed that whereas cued fear is predominantly modulated by the

amygdala (LeDoux, 2000; Davis, 1992), the amygdala, hippocampus (Kim and Fanselow, 1992; Philips and LeDoux, 1992), and bed nucleus of the stria terminalis (BNST, Walker and Davis, 1997) may be involved in contextual fear, though the hippocampus may be primarily involved in processing of contextual cues rather than in generating the fear response. These findings suggest that cued fear and contextual fear are separable processes that are mediated by distinct brain structures. Numerous human imaging studies have demonstrated the activation of the amygdala during cue specific fear responses (e.g., Labar et al., 1998; Büchel et al., 1998). In contrast, sustained states of anxiety in healthy humans have not been extensively explored. Therefore, the main objective of this protocol is to examine the brain mechanisms associated with a sustained state of fear or anxiety in humans.

1.2. Rationale: Unpredictability, contextual fear, and sustained anxiety

Explicit cue fear can be readily evoked using threat or classical conditioning. Contextual fear can be observed in experiments that elicit fear as a response to the experimental context (e.g., the cage, the experimental room) in which the experiment was conducted. Contextual fear is partly determined by the predictability of the aversive event (Seligman and Biknik, 1977; Odling-Smee, 1975). Contextual fear increases with increased shock unpredictability (Odling-Smee, 1975). Unpredictability of aversive events may cause any part of the context in which they may occur to be regarded as unsafe. In contrast, when a discrete cue predicts the occurrence of the shocks, the context becomes a safety signal, reducing contextual fear. In this case, the specific cue will evoke the fear response and the context will become inhibitory (Seligman, 1977; Ameli et al, 2001; Grillon, 2002). Studying the neural systems involved in modulation of defense systems during predictable and unpredictable shock is important for understanding the interplay between fear activation and fear inhibition mechanisms. This is highly relevant to understanding clinical anxiety and fear, since generalization of fears to situations that do not justify them is characteristic of most anxiety and trauma-related disorders.

The present set of experiments will enable us to study the processes associated with phasic cue fear, sustained contextual fear, and inhibitory fear mechanisms. We have shown significant behavioral differentiation between contexts that induce high and low contextual fear by modifying shock predictability. In this procedure, subjects are instructed that in one condition a transient visual stimulus predicts possible shock administration (cue fear), while in another condition a similar visual stimulus (e.g., a blue square) is still presented, but is no longer predictive of the shock. Recent data from this procedure using the startle reflex as a measure of fear showed that giving shocks predictably or unpredictably increased contextual fear compared to a control condition (no shock administered). In addition, contextual fear was smaller in the predictable compared to the unpredictable condition. Furthermore, replicating our previous studies, startle was increased during the presence of the discrete cue in the predictable condition (replicating Grillon 1991, 1993, etc, see section C preliminary results). These results indicate that in this experimental design cued fear and sustained anxiety can be assessed separately. In this study we will explore to what extent the brain systems activated by predictable and unpredictable threat are different.

1.3. Human brain imaging studies

Brain imaging studies of the fear or anxiety systems in healthy controls have mostly used conditioned responding to explicit cues (as our specific aim 1). Human studies have yielded ample confirmation of the involvement of the amygdala in emotion in general and in fear and anxiety in particular. Patients with lesions of the amygdala and surrounding areas show impaired fear conditioning. (LaBar et al, 1995). Fear-conditioning (Labar et al., 1998; Büchel et al., 1998), emotional face processing (Morris et al., 1998), instructed threat (Phelps et al., 1999), and processing of threat-related words (Isenberg et al., 1999) all involve the amygdala.

Compared to explicit cue fear, few studies have been conducted on contextual fear or anxiety in humans. In one imaging study that explicitly studied contextual fear conditioning (Armony and Dolan, 2001), a behavioral and a psychophysiological measure failed to confirm a differentiation between conditioned responding (CS+ versus CS-) in a safe (CS+ not reinforced) and a conditioned (CS+ reinforced) context. There was an increase in skin conductance responding to both the CS+ and CS- in the reinforced context suggesting a general increase in arousal. The results showed differences in activation of auditory and parietal cortices, but not in areas implicated in anxiety processing such as the limbic system or areas intimately connected with limbic structures. This pattern of findings is consistent with general arousal or attention, rather than with anxiety.

The crucial comparison in the present design will be to compare background activity (i.e., activity in absence of the transient visual cues) in predictable, unpredictable, and neutral conditions. Our preliminary results suggest that structures involved in contextual fear will be more active in the unpredictable compared to the predictable condition, and in the predictable condition compared to the neutral condition. Hypotheses that can be derived from animal literature are that hippocampus, the amygdala, and related subcortical structures (e.g., extended amygdala, such as the BNST) will be involved in contextual fear. In addition, orbitofrontal areas of the prefrontal cortex (PFC), as well as parts of the anterior cingulate gyrus (medial PFC), are activated in states of anxiety (e.g., Benkelfat et al., 1995; Rauch et al., 1997), and have been implicated in the regulation of the limbic system (Simpson et al., 2001; Drevets, 2000; Davidson and Irwin, 1999; Hariri et al., 2000; Garcia et al., 1999). Therefore, areas of the prefrontal cortex may be involved in both the generation of anxiety, as well as its inhibition. We hypothesize that areas involved in generation of sustained anxiety are activated in the unpredictable condition relative to the predictable and neutral conditions. In addition, inhibitory processes may mediate the decreased contextual fear when threat is predictable. Areas involved in inhibition will be activated during the absence of the specific cue in the predictable condition in comparison with the neutral and unpredictable conditions. Several areas of the prefrontal cortex are likely to be involved in either of these processes: the orbital, medial, and dorsolateral PFC, as well as the anterior insula (see Charney and Drevets, 2002, for a review). In sustained anxiety we predict specifically that the anterior insula, the posterior orbital cortex, and the dorsomedial prefrontal cortex will be activated (specific aim 2a).

The anterior insula is activated in several kinds of anxiety-induction tasks, such as threat of shock as a background to a motor learning task (Chua et al., 1999; PET), and as common area of activation symptom provocation in three different anxiety disorders (Rauch et al., 1997; PET). The orbital prefrontal cortex has been associated with the generation of anxiety, as again in the symptom provocation study (right medial orbital cortex; Rauch et al., 1997), and in threat of shock (Chua et al., 1999, left orbitofrontal cortex; Drevets et al., 1994, posterior orbital cortex).

In the medial PFC / ACC several studies indicate activation with the generation of anxiety. Benkelfat et al. (1995) reported that with CCK4-induced anxiety CBF in the left ACC is increased. Chua et al. (1999) reported activation of the left anterior cingulate gyrus with threat of shock as a background to a motor learning task, if state anxiety scores were used as a covariate (PET). On the other hand, Simpson et al. (2001) reported increased blood flow in two areas of the medial inferior prefrontal cortex (subgenual prefrontal cortex and anterior medial prefrontal cortex) while anticipating shock relative to a baseline non-anxious condition, only in those subjects that reported the highest levels of anxiety. In contrast, subjects with lower levels of anxiety actually showed *decreased* activation in these two areas.

These results suggest that activation in the medial prefrontal area can correlate with generated anxiety positively. However, various support for an inverse relationship between mPFC activation and anxiety has been reported. Lesions of the prelimbic cortex in rats, corresponding to the human dorsomedial PFC, lead to increased freezing to a conditioned tone (Morgan & LeDoux, 1995). Also, an inverse relationship between amygdala activation and firing rate of medial prefrontal neurons has been reported (Garcia et al., 1999).

When human subjects anticipate aversive shocks, the dorsomedial anterior cingulate cortex activation is increased with respect to resting or teeth clenching control, but the degree of activation within each condition is inversely correlated with anxiety ratings (Drevets, 1994) This would suggest that this region is activated to *modulate* emotional responses (Drevets, 1999). Indeed, a recent other study showed a similar inverse relationship between activation of a specific region of the right lateral orbital cortex and the amygdala (Hariri et al., 2000). In addition, research on depression has suggested that in that disorder abnormalities in the ventral anterior cingulate regions (subgenual and pregenual anterior cingulate cortex) may result in abnormal modulation of emotional processing in limbic structures (for a review, see Drevets, 2000). The present protocol allows evaluation of inhibitory influences on the limbic structures in the absence of specific cues in the predictable shock condition. We intend to explore the role of (parts of) the medial prefrontal cortex in the inhibition of anxiety. The prediction is that parts of the medial PFC will be activated in the predictable condition (specific aim 2b). Given evidence mentioned above, parts of the dorsomedial / ACC region may (also) be expected to be also activated in a condition that *generates* anxiety. Therefore, to test specific aim 2b we will evaluate the predictable condition with respect to the neutral baseline condition. In both of the predictable and neutral conditions we expect relatively low contextual anxiety, which in the predictable condition may be due to active inhibitory

processes. In addition, we will also explore the differential involvement of medial prefrontal cortex in the predictable and unpredictable conditions against each other.

1.4. Processing of threat-related stimuli

MEG (magnetoencephalography) monitors the activity of the brain by detecting the magnetic fields generated by neurons, both with a time resolution in the order of milliseconds. MEG is a relatively new technique. The advantage over EEG is that tracing back the sources of magnetic activity is much more reliable than determining the sources of electric activity. Moreover, Dr. Coppola and his staff are working on ways of detecting amygdala activity with MEG. We will conduct investigations of visual and auditory processing and learning of threat information using MEG and fMRI.

1.5. Relationship between GABA concentration, gamma activity, and hemodynamic response

Recent studies suggest that oscillations in the gamma frequency range (30–100 Hz) are well correlated temporally, spatially, and functionally with hemodynamic changes in cortex (Niessing et al., 2005). For example, a recent study (Muthukumaraswamy, Singh, Swettenham, & Jones, 2010) reported that 1) endogenous resting concentration of GABA was positively correlated with the frequency of stimulus-induced gamma oscillations in the occipital cortex and 2) the magnitude of the BOLD response was strongly negatively correlated with both GABA concentration (R=-.64) and gamma oscillation frequency (R=-.88). These results indicate a close relationship between gamma activity and the BOLD response, and further suggest that the level of neural inhibition (i.e., GABA concentration) in the brain contributes to the variability of gamma oscillations and BOLD signal. They also provide important clues as to the role of inhibition/excitation on the BOLD signal and gamma oscillations, as well as possible mechanisms of action of classical anxiolytics such as benzodiapezines. One issue with the above study (Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009) is the correlational nature of the results. We propose to obtain more direct evidence of the influence GABA on BOLD activity and gamma oscillations by modulating experimentally GABA levels in healthy participants. More specifically, we will increase GABA activity with administration of the benzodiazepine alprazolam and we will reduce GABA activity with increasing subjects' levels of anxiety using a threat of shock procedure. Using MRS, we recently showed 18% reduction in GABA activity in healthy subjects anticipating shocks (Hasler, van der Veen, Grillon, Drevets, & Shen, 2010).

As an initial study, we determined the influence of the benzodiapezine alprazolam on gamma oscillations under threat of shock and safe conditions. This involved using MEG only. This study has been completed and results have been published (Cornwell et al., 2017). We do not plan to pursue this study in the fMRI scanner or using MRS techniques.

1.6. Preliminary Results

In the proposed experiment we will use a verbal threat and conditioning through experience to manipulate contextual fear. The procedure was piloted using psychophysiological measures in 15 healthy subjects. The procedure consists of 3

conditions, neutral (N), predictable shocks (P), and unpredictable shocks (P), each lasting 2 minutes. Subjects were informed of the current condition by means of a computer monitor that displayed the following information: “no shock” during the neutral condition, and “shock only during cue” during the predictable and “shock at any time” during the unpredictable shock conditions. During each condition a visual cue (a colored geometric shape) was presented twice, with 8 s duration. As instructed, these cues were relevant only during the predictable condition. In this condition, the cue signaled the possibility of receiving a shock. In the unpredictable condition, shock could be given at any time and in the neutral condition no shock could be administered. Acoustic startle stimuli were delivered in the presence and in the absence of the cues. Figure 1 presents the results, which show that startle was potentiated both by the explicit threat cue and by the threatening context. Indeed, 1) startle was significantly potentiated by the cue only in the predictable shock condition (FPS to specific cue) and 2) there was a significant linear increase in baseline startle in the absence of cues from the N to the P to the U condition (FPS to context). Additional pair-wise comparisons showed significant differences in no-cue startle magnitudes between the N and P conditions and between the P and U conditions. The finding that P elicited contextual fear, compared to N is consistent with our previous studies (Grillon et al., 1993). The finding that U resulted in more contextual fear than P is consistent with both the safety signal hypothesis (19) and conditioning theories (20), which predict that the presence of a signal for shock reduces fear to the context.

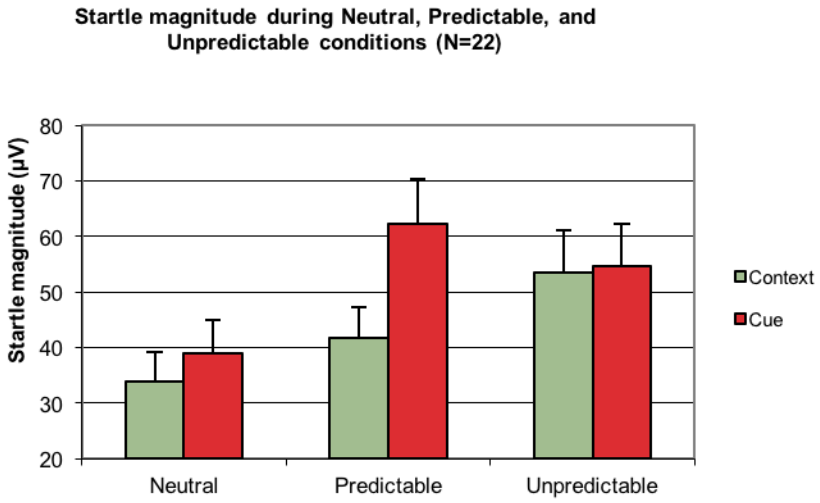


Figure 1.
Preliminary startle data: Three shock conditions are no shock (neutral), predictable shock, and unpredictable shock. The cues predict shock administration in the predictable condition only. Error bars denote SEM.

Although this procedure was designed to differentially affect two response systems assumed to be associated with cued fear ('fear') and contextual fear ('anxiety'), an alternative explanation of these startle results is possible. The startle results may reflect quantitative differences in shock expectation in the presence and absence of the threat cue during predictable versus unpredictable conditions. Because startle is a unidimensional

measure (magnitude), we cannot make a distinction between these accounts based on startle results alone. There are different strategies possible to approach this issue: either study these responses in various conditions that are thought to affect the two responses differently (e.g., with drugs or patient groups), or study the underlying neural mechanisms directly. In the present proposal the latter approach will be taken. Although we expect qualitative differentiation in neural structures activated by predictable and unpredictable shocks, it is also possible that the same structures will be activated in these conditions, but to a different degree.

The threat of shock procedure provides a powerful tool to investigate brain correlates of the responses that are characteristic of fear and anxiety, because both responses can be measured within one experimental session using the same timing and sensory stimulation parameters. The instructed threat of shock procedure has been studied extensively in our and other laboratories. It yields highly robust fear-potentiated startle, as well as robust effects on autonomic and subjective measures (Grillon et al., 1991, 1993, 1998; Baas et al., 2002). The effect of instructed threat is highly reliable in that it is observed in virtually every subject tested. For these reasons, this procedure is highly suitable to study expression of fear and anxiety in an fMRI scanner. Unlike skin conductance and heart rate measurements, startle requires (auditory) probes to be evoked. This circumstance precludes concurrent monitoring of startle potentiation in the fMRI or MEG scanner. However, results from previous experiments have shown that the instructed threat procedure yields reliably results during a second session (Baas et al., 2002). Reliable correlations have been demonstrated between amygdala activation and psychophysiological measurements in a separate session using skin conductance as the dependent variable (LaBar et al., 1998).

The proposed procedure was designed and tested with acoustic startle to tap into the two response systems proposed (i.e., fear versus anxiety). However, with startle data it is not possible to conclude on a qualitative distinction between brain responses associated with explicit cue fear and contextual fear. An alternative hypothesis is that predictable and unpredictable shock activate the same neural structures, but to a different degree.

1.7 Interaction between cognitive performance and emotional pathology (anxiety & depression)

High levels of anxiety and depression have been associated with poor performance and processing inefficiencies on cognitive (Derakshan & Eysenck, 2010; Hembree, 1988), and motivation/reward processes (Scott-Parker et al., 2012; Der-Avakian and Markou, 2012; Schechner et al., 2013) suggesting that cognitive and motivational changes may be a central component of clinical anxiety. Yerkes and Dodson (1908) 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. 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 (Fales, Becerril, Luking, & Barch, 2010; Eysenck, Derakshan, Santos, & Calvo, 2007) and in the ability to perform at work, which can lead to career dissatisfaction and job loss (Waghorn, Chant, White, & Whiteford, 2005). 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 use of fMRI to study anxiety patients (target populations and procedures as described in protocol 0093) will allow us to discern the neural substrates of such processes and in turn provide us with evidence of the types of processes that support the interaction between cognitive performance and emotional psychopathology.

Further, in addition to demonstrating that anxiety affects cognition, cognitive processes have been shown to effectively decrease fear in anxious individuals (e.g., Cognitive Behavioral Therapy [CBT]-like strategies; Kalisch et al., 2005). Identifying the neural correlates that support successful mitigation of anxiety (via cognitive loading) will provide important information for therapeutic interventions. Selectively targeting these cognitive mechanisms will provide an important avenue of intervention in addition to the ubiquitous use of intervention techniques that modulate emotional response (e.g., benzodiazepines).

Similar to individuals with anxiety disorders, individuals with major depressive disorder (MDD) exhibit impaired executive functioning, and have particular trouble inhibiting negative information (Lissnyder, Koster, Derakshan, & Raedt, 2010). However, unlike anxious individuals, depressed individuals have lower tonic levels of emotional arousal, and therefore their task performance may be impacted by mechanisms different from those affected by clinical anxiety. Depressed individuals tend to exhibit decreased activity in dorsolateral PFC and increased activity in subgenual ACC and amygdala in comparison to healthy controls (Mayberg et al., 1999), whereas anxious individuals tend to exhibit decreased activity in VmPFC and increased activity in amygdala and insula (Stein, Simmons, Feinstein, & Paulus, 2007). By investigating the differences in cognitive processing under stress between anxious and depressed individuals, we will be better equipped to treat their similar behavioral deficits via different therapeutic vehicles.

The effects of anxiety on reward processing have also been documented, particularly in adolescents (see Schechner et al., 2013). In addition, harm avoidance and intolerance to uncertainty have been documented in patients with anxiety disorder as well as fearful temperament (e.g., Helfinstein et al., 2012; Krain et al., 2008), raising the question of the potentially differential effect of fear vs. anxiety on reward/motivational systems and decision-making.

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. In order to target this interaction, we will induce variable levels of stress in subjects and monitor their brain reactivity and behavioral

performance on several different tasks. We will use a threat paradigm based on one that we used to collect pilot behavioral data from patients outside the scanner. The paradigm will consists of periods where subjects are under the threat of receiving a shock, and period where they are safe from shock. During these conditions, subjects will engage in a series of different cognitive tasks (e.g., 1) working memory verbal n-back: identify a letter that was presented one, two, or three trials ago; 2) working memory spatial n-back: identify a location where a stimulus appeared one, two, or three trials ago; 3) long-term memory task where subjects will be asked to remember lists of words and pictures), or engage in no task at all (resting state). By parametrically modulating the cognitive load (i.e., difficulty) and emotional content (i.e happy or fearful) of the stimuli, we will be able to more precisely identify the point at which cognitive disruption occurs along with the corresponding neural correlates. In addition, we will be able to estimate the optimal point of cognitive facilitation and determine whether or not this differs between patient groups. We will also use reward/decision-making tasks to probe the interaction between fear/anxiety systems and motivation/reward systems.

This approach to studying stress has been successfully established outside of the scanner environment, thus providing an effective tool with which to study the changes in brain connectivity and event-related responses associated with stress, and the interaction between stress and cognitive performance. We have reliably shown that stress (induced by a threat-of-shock paradigm) is reduced when subjects are fully engaged in difficult cognitive tasks, and that performance on easier, and potentially more susceptible cognitive tasks, decreases as stress increases (Vytal et al., in prep). This protocol can uniquely and discretely identify three essential transition points in the relationship between stress and cognition: 1) where anxiety overpowers performance, and 2) where the level of attentional and perceptual engagement overcomes anxiety and 3) where anxiety facilitates certain types of cognitive processing (such as threat stimuli; Robinson et al, In press; Robinson et al, In submission). By studying stress across several different levels of task difficulty and stimuli valence (parametric modulation) we will have a direct window into the nuanced interactions between emotion and cognition in two different populations with affective disorders. Both types of mood disorders are marked by impaired cognitive processing and changes in emotional reactivity to affective stimuli(increased in patients with anxiety disorders, and decreased in patients with MDD e.g. Robinson et al, In submission). This approach will allow us to study the changes in neural mechanisms associated with changes in stress and cognitive performance in such populations, thus providing important information about how these mechanisms differ from healthy controls. At the neural level, we will be able to better elucidate the differences in these complex cognition and emotion interactions between patients with mood pathology and healthy controls. Ideally, this study will increase our understanding of when and how patient populations with different types of emotional pathology experience emotional interference that negatively affects their behavioral performance. Further, we will be able to establish how their behavioral and neural patterns differ from individuals with a healthy response to stress.

1.8 Rationale: Hippocampal dysfunction in posttraumatic stress disorder (PTSD)

Hippocampal dysfunction is implicated in the symptom expression of PTSD. We will be conducting a series of experiments to better understand this vulnerability: (a.) behavioral pattern separation (memory) task (BPS), (b.) whole brain resting state task, and (c.) the Morris water maze (spatial memory) task (MWM).

(a.) It has long been established that individuals with PTSD tend to overgeneralize attributes of fearful stimuli to non-fearful stimuli.(Lissek et al., 2010) While the hippocampus has been implicated in this vulnerability, there is little mechanistic understanding of the contributing neural systems that support overgeneralization. To address this knowledge gap, this study aims to examine the effect of experimentally-induced contextual anxiety on stimulus generalization using the behavioral pattern separation (BPS) task (Stark, Yassa, Lacy, & Stark, 2013). Ideally this study will increase our understanding of the contexts (safe vs. threat of unpredictable shock) for which stimulus generalization may occur, as well as the neural systems that may relate to stimulus generalization in participants with PTSD (vs. no-PTSD).

(b.) Human fMRI studies have long investigated the hippocampus without differentiating between its subfields, even though theoretical models and rodent studies suggest that hippocampal subfields support different and potentially even opposite functions.(Leutgeb, Leutgeb, Moser, & Moser, 2007) Of focus, the dentate gyrus (DG)-cornu ammonis 3 (CA3) subfield circuit of the hippocampus minimizes interference between new information and previously stored similar memories.(Besnard & Sahay, 2016) This process is dependent on two complimentary operations, pattern separation (implicating the DG) and pattern completion (implicating the CA3). This study will use resting state scans during ultra-high field fMRI (7-Tesla) to delineate the different resting state networks of the hippocampal subfields. We aim to define different processes and functions that are presumably carried out by the DG and CA3 subfields, in particular regarding the seemingly opposing functions of pattern separation and pattern completion, as well as specific processes that are related to behavioral, functional, and structural alterations in participants with PTSD (vs. no-PTSD).

(c.) The Morris water maze (MWM) task (Deuker, Doeller, Fell, & Axmacher, 2014) is one of the most widely used tasks to test hippocampal-dependent learning, including acquisition of spatial memory and long-term spatial memory.(Bromley-Brits, Deng, & Song, 2011) The task is relatively simple, which relies on distal cues to navigate from start locations around the perimeter of a virtual reality open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials. The aim of this study is to further investigate the impact of hippocampal-dependent deficits in PTSD (vs. no-PTSD).

1.9 Rationale: Avoidance symptoms in posttraumatic stress disorder (PTSD)

Active avoidance is the commission of an overt action which functions to directly reduce exposure to threat. Although avoidance symptoms are relevant to understanding heterogeneity within PTSD diagnosis (Asmundson, Stapleton, & Taylor, 2004), no

research has empirically investigated whether persons with PTSD exhibit altered behavioral or neural responses during active avoidance paradigms. Previous research has demonstrated that the active avoidance of signaled threat (AAST) paradigm can be used to measure facilitated behavioral performance in response to threat contingencies, and that individual differences in how participants respond during the AAST are linked to stable personality traits (Gorka, LaBar, & Hariri, 2016). To address this knowledge gap, this study aims to investigate whether persons suffering from PTSD exhibit abnormal behavioral responses during performance of the AAST, and to determine how neural circuits underlie between-subject differences in active avoidance behavior.

2. Study Objectives:

The overall objective of this protocol is to investigate the brain correlates of responses to predictable and unpredictable shocks. Several contrasts derived from the design will allow this investigation.

A) Explicit cue fear will be evoked by the presentation of a specific cue that is associated with an electric shock. In order to study explicit cue fear we will examine the BOLD response to the presentation of cues that predict a shock (fMRI study). We predict that the amygdala will be activated in this comparison, along with several cortical areas that are involved in explicit cue fear, in particular the anterior insula (Phelps et al., 2001) and the anterior cingulate (Büchel et al., 1998). In addition, sensory processing of auditory and visual stimuli that predict threat will be facilitated (MEG/fMRI study). This will be reflected in the augmentation of the activation evoked in sensory areas and the amygdala to those stimuli relative to non-threatening stimuli.

B) In contrast, sustained contextual fear or anxiety will be elicited by unpredictable aversive events. To measure this background state, we will compare the BOLD responses during conditions in which subjects anticipate shocks being delivered predictably and unpredictably to conditions where no shocks are anticipated. In all hypotheses concerning the background state of anxiety, BOLD responding during the absence of the explicit cues will be utilized.

1) In order to evaluate activations associated with the sustained anxiety state in the unpredictable condition, the contrasts of unpredictable shock versus neutral and versus predictable shock will be used. Regions that are hypothesized to be activated are the amygdala, and the anterior insula, as well as posterior orbital cortex and dorsomedial prefrontal cortical areas.

2) A second hypothesis with regard to the sustained state of anxiety is that in the condition where shock is predictable, this sustained state is inhibited in comparison to the condition in which the shock is unpredictable. To evaluate this hypothesis, the contrasts predictable shock versus neutral and versus unpredictable shock will be used. We predict that in the predictable shock condition, areas of the lateral orbital cortex, and ventral anterior cingulate cortex are activated in comparison with the unpredictable shock condition, as well as the neutral condition.

C) A third hypothesis is that while anticipation of unpredictable shocks will reduce GABA concentration, leading to low peak frequency of stimulus-induced gamma oscillations in the occipital cortex and increases BOLD response, the benzodiazepine alprazolam will have the opposite effect (increased GABA, leading to high peak gamma frequency and decreased BOLD signal).

D) A fourth hypothesis is that under threat of shock, prefrontal executive control regions will be less successfully engaged by patients with high anxiety or MDD than healthy controls. Further, we predict that both patient groups will recruit the cortical and subcortical limbic regions (e.g., amygdala, bed nucleus of stria terminalis (BNST), and insula) to a greater extent than healthy controls when under threat of shock. In contrast to anxiety patients, MDD patients will additionally recruit subgenual/rostral ACC in response to threat.

E) In the PTSD hippocampus investigation, we hypothesize that the hippocampus and dorsolateral prefrontal cortex (dlPFC) will play a key role in the effect of anxiety on BPS Task performance and that participants with PTSD will exhibit amplified pattern separation deficits. We also hypothesize that pattern separation deficits will be associated with PTSD symptom severity in the dentate gyrus as indicated in the high-field 7-Tesla resting state scan. Lastly, we hypothesize that the PTSD group (vs. no-PTSD) will have increased hippocampal-dependent spatial memory deficits on the Morris water maze (MWM) task.

F). We hypothesize that during the AAST paradigm, participants will exhibit enhanced neural responses to the avoidance cue during the threat-of-shock condition, within areas of the medial prefrontal cortex, amygdala, and striatum. Furthermore, we hypothesize that participants with PTSD will exhibit reduced medial prefrontal cortex responses during threat contexts, and elevated avoidance behavior. Lastly, we hypothesize that individual differences in avoidance behavior will be associated with the magnitude of task elicited neural activity within the medial prefrontal cortex, amygdala, and striatum.

3. Subjects:

a. *Description of study populations*

This study will recruit 1226 healthy volunteers and 726 psychiatric patients with a current diagnosis of generalized anxiety disorder (GAD), panic disorder, social anxiety disorder (SAD), specific phobia, posttraumatic stress disorder (PTSD), or major depression. All participants enrolled in this study must be in good physical health and between 18-50 years old for the duration of the study. This study is open to all persons regardless of sex, race, ethnicity, sexual orientation, or rank.

b. *Inclusion criteria*

All screening procedures described in this section are conducted under screening protocol 01-M-0254. Subjects must meet the following inclusion criteria in order to participate in the study:

1) Male or female volunteers ages 18-50 years old.

2) Judged to be in good physical health on the basis of medical history, a clinical MRI scan, and physical examination. Physical exams will be conducted by a NIMH credentialed physician or nurse. Clinical laboratory tests will be ordered based on his/her discretion.

3) Healthy subjects judged to be in good psychiatric health on the basis of the Structured Clinical Interview for DSM-IV-TR. The SCID will be administered by a credentialed NIMH clinician. The PTSD comparator group (i.e., war-zone exposed healthy controls), must endorse war-zone exposure on the SCID, PTSD module.

4) Able to understand procedures and agree to participate in the study by giving written informed consent.

5) This protocol (02-M-0321) will include patients with a primary diagnosis of generalized anxiety disorder, panic disorder, SAD, PTSD (from war-zone exposure), specific phobia, and major depression according to DSM-IV.

6) Abstain from drinking caffeinated beverage including coffee, tea and caffeinated soft drinks and from smoking for at least 1 hour prior to testing. They will also be instructed not to drink alcohol on the night prior to testing and on the day of testing.

8) Speaks English fluently

c. ***Exclusion criteria***

Subjects will be excluded from the study if they meet the following exclusion criteria:

1) Clinically significant organic disease, e.g., cardiovascular disease.

2) Clinically significant abnormalities in physical examination.

3) Any medical condition that increases risk for fMRI (e.g. pacemaker, metallic foreign body in eye).

4) History of any disease, which in the investigators' opinion may confound the results of the study, including, but not limited to, history of organic mental disorders, seizure, or mental retardation.

5) Have a current diagnosis of alcohol or substance abuse ACCORDING TO DSM IV CRITERIA

6) Have a lifetime diagnosis of alcohol or substance dependence ACCORDING TO DSM IV CRITERIA.

7) Unless subject is enrolled as a patient, subjects should not have current Axis I psychiatric disorders as identified with the Structured Clinical Interview for DSM-IV, non-patient edition (SCID/NP).

8) If a healthy volunteer, past bipolar depression and any history of psychosis or delusional disorders.

9) If a healthy volunteer, first degree relative with history of psychotic disorder such as schizophrenia or bipolar disorder.

10) Healthy participants may not be on psychotropic medications.

11) Pregnancy, i.e., a positive β-HCG urine test conducted prior to each experiment session.

12) Current or past history of cubital tunnel syndrome or carpal tunnel syndrome for shock studies that use the wrist for placement of electrodes. Cubital tunnel and carpal tunnel syndrome are exclusionary only for diagnosis on same arm as electrodes and are not exclusionary for studies that place shocks on ankles or feet.

13) Reynauds syndrome for the cold pressor test experiment

14) Color blindness (for the active avoidance task only)

Additional exclusion criteria for patients

- Patients who would be unable to comply with study procedures or assessments
- Patients will be excluded if they have a current or past history of any psychotic disorder, bipolar disorder, delirium, dementia, amnestic disorder, cognitive disorder not otherwise specified, any of the pervasive developmental disorders, or mental retardation

In terms of the rationale for the inclusion of healthy participants with past (but not current) mood or anxiety, there are no data, to our knowledge, suggesting that patients with past but not current mood or anxiety disorders are dissociable from healthy controls in terms of phasic reactivity to anxiogenic stimuli and thus past mood or past anxiety disorders are not criteria for ineligibility.

d. An eligibility checklist for this protocol is provided as an attachment.

4. Study Design and Methods:

i. Study overview

This protocol consists of three scanning sessions. The first two are fMRI tasks with durations of 2.5 hours each (healthy participants and patients) and the third is an MEG task lasting 2.0 hours (healthy participants ONLY). Proceeding any of these testing sessions, participants will come to the NIH for a screening session (see *Screening Methods* below under **Section 7.a.iii.**). Qualifying participants will complete one of these three tasks (depending on subject preference and recruitment needs of each task) and will then be offered to participate in a second and perhaps 3rd task with the limitation that they will have at least a two week break between studies including electric shocks.

A collaborative agreement exists with Dr. Carlos Zarate's group to recruit, screen and test all the participants with Major Depressive Disorder under this protocol.

ii. Recruitment

English speaking healthy volunteers ages 18-50 will be recruited via advertisements in the local media. Individuals with an anxiety disorder or major depression will be recruited with assistance from PRPL.

Recruitment strategies will include advertisements placed through university newspapers, the city paper, and local gazettes, Web Links, DC and Montgomery buses and metrorails, and public service announcements. 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 such as those provided by OPR, advocacy groups, campus groups, and Club PCR used by the research assistants at NIH. 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. Additional study information will be distributed to local chapters of the Anxiety Disorders Association of America, Freedom from Fear, and the National Alliance for the Mentally Ill.

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 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.

Healthy volunteers may also be identified through the NIMH protocol #17-M-0181 titled "Recruitment and Characterization of Research Volunteers for NIMH Intramural Studies. Healthy volunteers screened through protocol #17-M-0181 may be given information and recruitment materials for this protocol. We may receive a list of potential participants that were identified as healthy volunteers under protocol #17-M-0181 and have agreed to be contacted by other NIMH groups.

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. The written advertisements will be used in color as submitted, or may be printed in black and white. The color of the ads may vary. Color changes will not be used to change the emphasis of an ad. The size of the ads may vary, but all parts of the ads, including fonts and pictures, will be changed proportionately to the rest of that ad. Disproportionate changes in size will not be used to change the emphasis of an ad. Email addresses 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.

Recruitment efforts will be made to match the number of healthy controls to the number of patients in each experiment, except in studies that enroll only healthy controls (e.g., MEG) or in studies that include multiple healthy control groups (e.g., Active Avoidance task). Participant groups will include both men and women and recruited in a 1:1 ratio to the best of our recruitment capacity. Additionally, we will make all efforts to recruit healthy controls who are age, sex, race, and IQ matched (as assessed by the Wechsler Abbreviated Scale of Intelligence™ (WASI™) to our patient groups.

All potential participants will undergo a telephone prescreen questionnaire either under this protocol (see attached pre-screen questionnaires for healthy participants and patient participants) or under the screening protocol 01-M-0254 if recruited by Dr. Zarate's lab. Potential patient participants will only be pre-screened by clinician level investigators (RNs, MSWs, PAs, MDs, MSs) whereas potential healthy volunteer participants may be pre-screened by any investigator or research contact, including IRTAS. During the pre-screening, the experiment will be

described. In particular, subjects will be informed that unpleasant stimuli (shocks) will be administered. They will also be told that the procedure involves fMRI, MEG, or MRS scanning. All questions asked during the pre-screen are based on inclusion and exclusion criteria (e.g., "What is your age?" or “Do you wear braces, have a permanent retainer, or have any metal implants?”). After the initial telephone prescreening, individuals who appear to qualify for inclusion will be invited to complete a more comprehensive outpatient screening at the NIH Clinical Center through screening protocol (01-M-0254)

1. A PDF of the employed IRB-approved advertisement is attached.

2. Telephone pre-screen questions for this protocol are attached.

iii. Screening methods

The outpatient screening for participants in this protocol will take place via the mood and anxiety disorders screening protocol, 01-M-0254, and subjects will be asked to provide informed consent for 01-M-0254 on the day of screening. Screening procedures may consist of the following criteria:

- Pregnancy test (urine beta HCG test) for women of child bearing age
- Blood draw for genetic analysis (3 EDTA [purple top] tubes)
- 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)
- Risk for self-harm screen
- Life Events Checklist 5 – Extended Version (LEC-5)
- The Clinician Administered PTSD Scale (CAPS)
- Self administered Questionnaires: These questionnaires including BDI-II, IDSR, HAM-D, HAM-A, MADRS PSWQ and POMS..

- Concomitant medication and pharmacotherapy history
- Relevant inclusion/exclusion criteria (see above)

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 02-M-0321. 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.

Participants meeting medical and psychiatric eligibility criteria will be invited back for additional testing visits at which time they will undergo informed consent for this protocol (02-M-0321).

iv. Study design

Threat of Shock

The design is similar to that employed psychophysiologically in protocols 03-M-0093 and 01-M-0185, with some changes to adapt it for fMRI measurement. Figure 2 clarifies the experimental design.

Design Overview

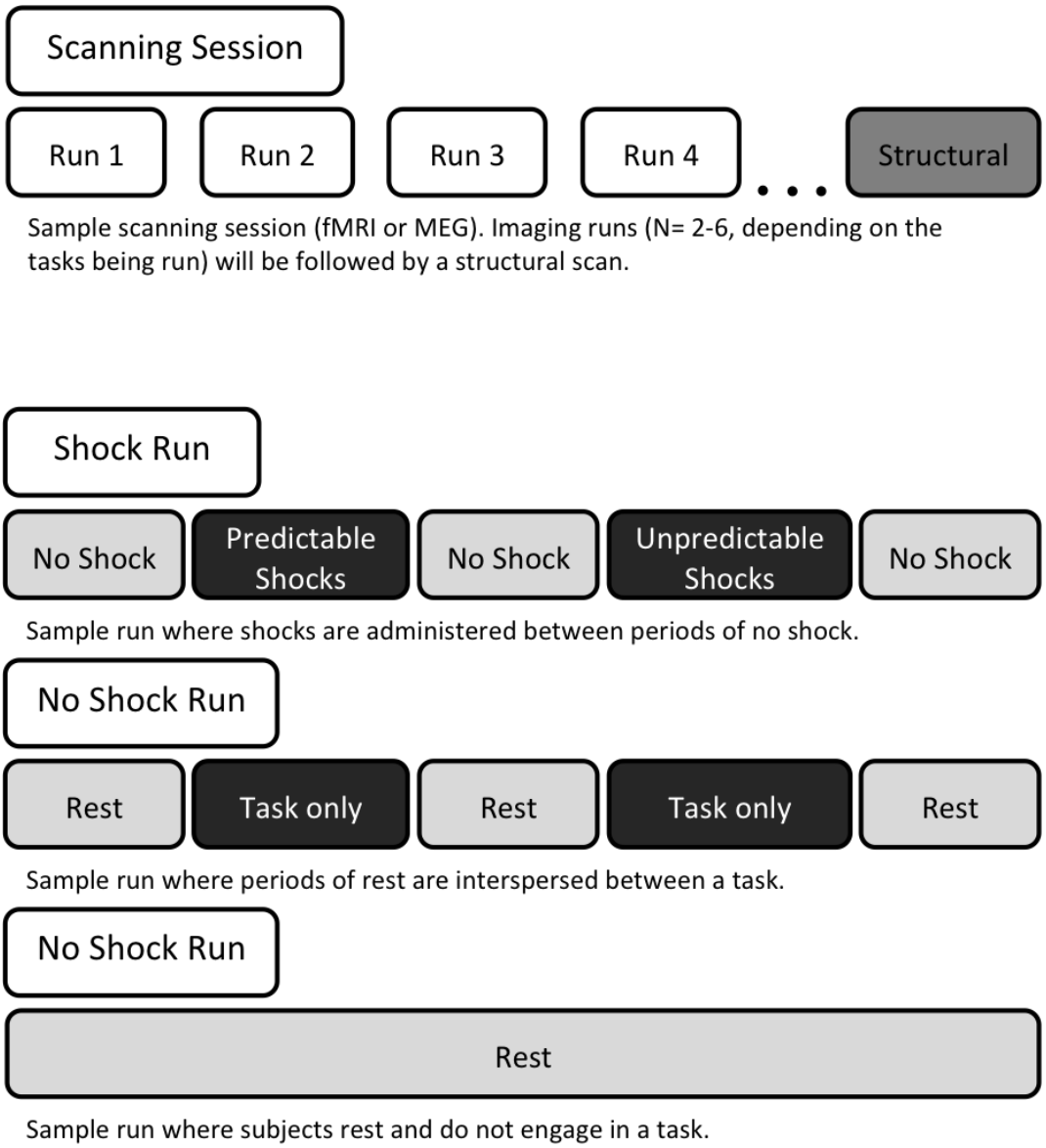


Figure 2.

Experimental design: upper portion gives the design of the entire experiment. Lower part depicts an example of each run type (order of conditions balanced within and between subjects). Shock blocks can be concurrent with tasks described below. When subjects are participating in a no-shock run, the tasks will replace the shock blocks as indicated above. Subjects do not engage in any specific task during resting state runs.

Each condition will have an identical duration of two minutes, and be identical with respect to number of stimulus cues presented. Virtual reality environments (e.g., restaurant scene, airport scene, pool scene) may be used as cues to allow for a richer and clearer differentiation of predictable, unpredictable and neutral conditions. The predictable condition will be defined as the context in which shocks can only occur during an explicit stimulus cue. Subjects will be informed that they will not receive a shock during any other time during this condition. The stimulus cue will appear for approximately 1-8 seconds. The unpredictable condition will be defined as the context in which shocks can be delivered without warning at any time. Furthermore, shocks will be administered to reinforce these instructions with the same number during predictable and unpredictable conditions. Occasional reinforcement is necessary to prevent the instructions from losing credibility. Finally, the neutral condition will be defined as the context in which participants are completely safe from receiving shocks.

Study Procedures and Groups

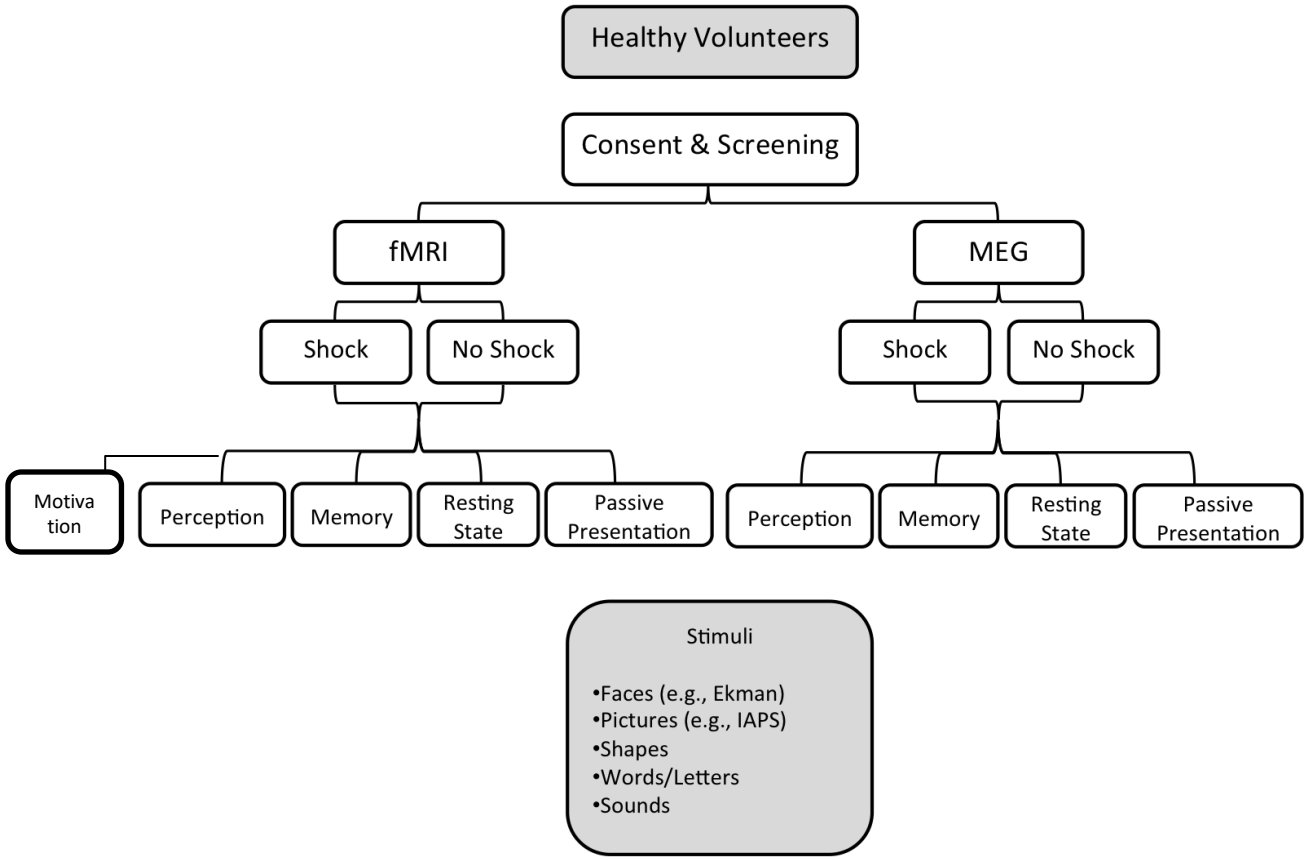


Figure 3. Healthy Controls: Full procedure diagram outlining all experimental procedures. Note, not all subjects will participate in all procedures. In fact, most subjects will participate in only one or two tasks during a scanning session. Healthy subjects will undergo fMRI and/or MEG (depending on whether or not they consent to both) and they will be scanned during runs of either shock or no shock. Each of the bottom level categories represents one type of experiment such that a single run will fit into a single experiment category. Six different experiments are described below (Motivation/reward, Motivation/Monetary Incentive Delay, Perception, Memory, Resting State, Passive Presentation, and ValSal); all experiments follow the general threat procedure described above.

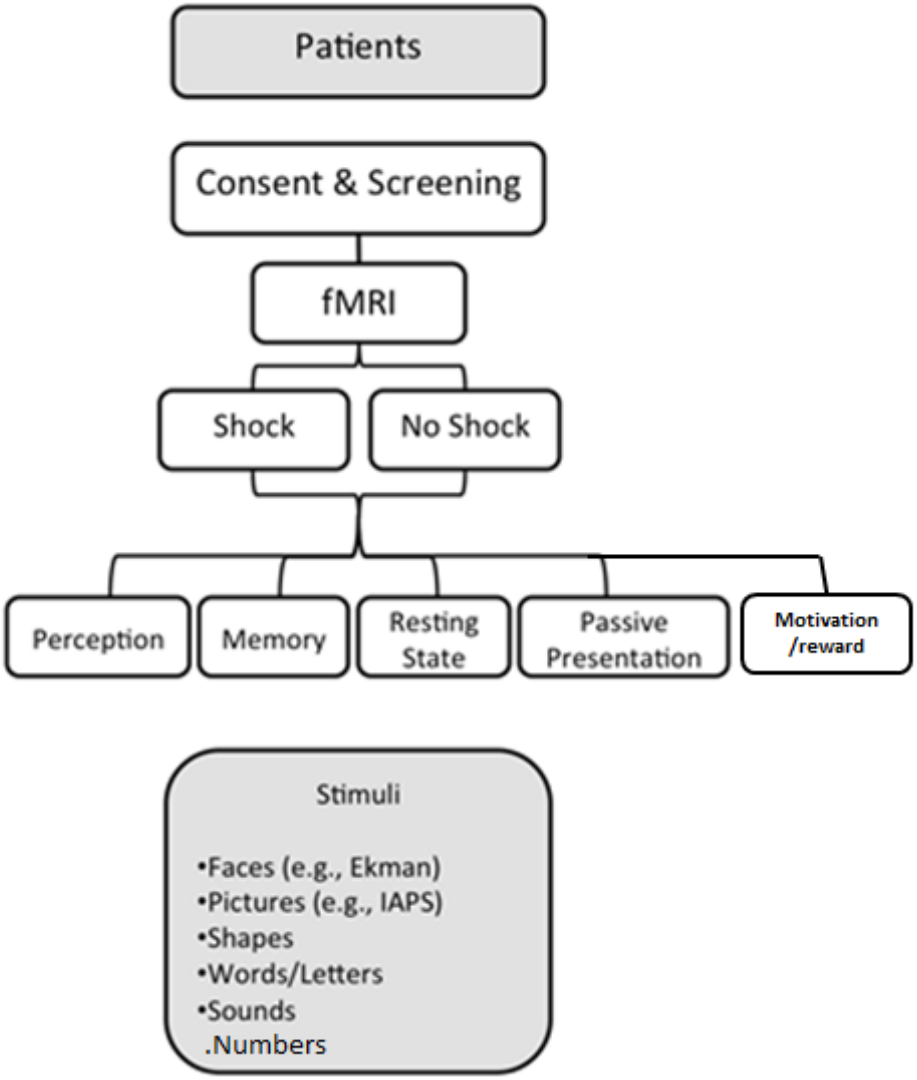


Figure 4. Patients: Full procedure diagram outlining all experimental procedures. Note, not all subjects will participate in all procedures. In fact, most subjects will participate in only one or two tasks during a scanning session. Patients will undergo fMRI ONLY and they will be scanned during runs of either shock or no

shock. Each of the bottom level categories represents one type of experiment such that a single run will fit into a single experiment category. Five different experiments are described below (Motivation/reward, Perception, Memory, Resting State, Passive Viewing, and ValSal); all experiments follow the general threat procedure described above.

Description of studies (subordinate categories: Perception, Memory, Resting State, Passive Presentation, Loss Aversion Task, ValSal Task)

Perception (Healthy N=40; Patient N=20 from each patient group)
Subjects will be asked to identify the expression on faces (e.g. neutral, happy, fearful, sad) or identify the emotion of a word (e.g. 'safe','fear' or '+$'). They will be asked to respond during these stimuli themselves and during neutral pictures (i.e. boxes, stars, circles, scenes) which are paired with (i.e. appear just before) these stimuli.

Time perception (Healthy N=50)
Participants will be asked to judge for how long stimuli (emotional faces: fearful, happy, neutral, taken from a standardized battery, NimStim OR simple shapes) remained on the screen. They then have to make a button press denoting the relative duration of these stimuli, compared to durations they learned.

Memory (Healthy N=80; Patient N=90 from each patient group, 20 of which will overlap with the patients in the Perception experiments because they will be run in the same session) These memory tasks will also include the below noted groups.

Morris Water Maze task (Healthy controls without war-zone exposure N=30, Healthy controls with war-zone exposure N=30, PTSD from war-zone exposure N=30) In one procedure, participants navigate a virtual reality water maze with a joystick to learn the location of an escape platform. Participants perform a series of trials. On some trials, the platform is visible to allow them to encode its location, and on other trials, they must retrieve its location by using spatial information in the virtual environment.

Behavioral Pattern Separation Task (Healthy controls without war-zone exposure N=30, Healthy controls with war-zone exposure N=30, PTSD from war-zone exposure N=30) The BPS Task consists of two phases; in the first encoding phase, participants assign an "indoor/outdoor" verdict to pictures of neutral objects. Following this encoding phase, participants complete a memory test (i.e., the retrieval phase), where they must identify pictures of neutral objects as ''Old'', ''Similar'', or ''New''. One-third of the objects in the retrieval phase are "Old" or exact repetitions of the objects presented in the encoding phase; one-third of the objects are "New", not presented during the encoding phase; and one-third of the objects are perceptually "Similar" to the objects presented during the encoding phase, but not identical (i.e., lures). We are particularly interested in the

lure object responses and the rates at which participants correctly identify these as ‘‘Similar’’, avoiding the inclination to identify these as ‘‘Old’’. Identifying the lure objects as ‘‘Old’’ (i.e., overgeneralization) is likely driven by pattern completion processes. In contrast, discriminating the lure objects from the “Old” objects requires a distinct representation—a hallmark of pattern separation. The encoding and retrieval phases will be presented under safe and threat contexts (i.e., threat of unpredictable shock) to investigate the role of experimental threat on pattern separation and pattern completion processes.

In a separate procedure, 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). Subjects will also be asked to simply view the stimuli (without responding) as well as remember words or International Affective Picture System (IAPS) pictures that they were presented with previously after a short delay. IAPS pictures include both positive and negative images. Examples of pleasant pictures include baby faces, ice cream, puppies, fireworks, and erotic pictures. Examples of unpleasant pictures include car accidents, guns, surgery, snakes, tumors, and bleeding faces. Subjects will be told that these pictures distressing and they will be reminded of the option to withdraw from the experiment at any time.

Resting State (Healthy N=40; Patient N=60 from each patient group, all of which will overlap with some of the patients in the Memory and Perception experiments because they will be run in the same session) This task will also include (Healthy controls without war-zone exposure N=30, Healthy controls with war-zone exposure N=30, PTSD from war-zone exposure N=30)
Subjects will be asked to lie still with their eyes open and stare at a fixation cross for 6-9 minutes. They will be asked to remain as still as possible and not fall asleep. During a resting state run, subjects will not engage in any task. However, on some rest runs, they will be under the threat of a shock they have already experienced. On other runs, they will be told they will not receive a shock.

Passive Presentation (Healthy N=60)
These procedures involve simple, passive experience (visual, auditory, tactile) to measure sensory cortical responses under threat and safe conditions. Participants are asked to remain attentive to whether they are at risk to receive shock or safe from shocks but do not need to respond to the stimuli.

Loss Aversion “Motivation” Task (Loss aversion: Healthy N=30, Patients N=30; development of versions of the task: Healthy N=60)
This paradigm provides a quantitative measure (lambda) of sensitivity to loss, and is designed after the work by Tom et al., 2007. This task requires subjects to decide between taking or dismissing a series of monetary gambles.
In the clinic, versions of this task will also be developed. These modifications of the task have several goals. (1) Enhanced salience of stimuli: The main task is

fairly dry, presenting just numbers. We would like to pilot a similar task with more salient stimuli, such as dollar bills, or thermometer-type indicators of amount. (2) Manipulate probability: this task is currently only probing magnitude of 50% chance of winning or losing hypothetical money. We expect that probability (level of uncertainty in choice) plays an important part in decision-making, next to magnitude of incentives. Therefore, we would like to modify the task to manipulate probability. For example, instead of a fair coin-toss scenario, we would present a biased coin-toss, which could favor either losses or gains. (3) Manipulate the self-agency: anxiety is known to enhance salience of events that are determined by one's own actions. We are interested in understanding to what extent anxiety affects self-agency. To examine this aspect, we would pilot a task in which some incentives will not depend of the participants' choice, but will be set by the experimenter. These various versions of the task will be piloted in the clinic. The most successful manipulations would then be brought to the fMRI setting.
The need to use real money is based on feedback from participants which indicated lack of motivation in the absence of real money for this task, which can be boring. The use of real money will make the gains and losses more salient. The weaker than expected effect of loss aversion with use of token money is the reason to request the use of real money. While the weak effect of hypothetical money is clearly seen in our pilot data, the literature supports this notion. Most neuroimaging studies clearly show increased activation of reward pathways with increased value of monetary rewards (see review Richards et al., 2013).

The determination of the amount of money disbursed will be as follows. First, to provide a more real-life situation and enhance motivation to gamble and engagement in the task, we will provide an initial endowment of $20.00 that can be lost during the task. This endowment will make the potential losses real. At the end of the task, we will randomly select three gambles from the total number of gambles (n=512) presented to the participants. To do so, we will ask participants to randomly pick 3 numbers between "001" and "512". We will then identify the gambles corresponding to these 3 gambles and add the gains and losses from these 3 gambles. Participants can earn up to $50.00 or lose up to $20.00 (entire endowment). We have already used this strategy in previous protocols (e.g., 01-M-0192).

Motivation/ "Monetary Incentive Delay" Task (Healthy N=150; Patients N=30):

Participants will perform one of two versions of the "Monetary Incentive Delay" task. During the first version of the task (N = 80 HVs on the 3T scanner and N= 30 HVs on 7T scanner), we will examine the effect of threat of shock on goal-driven/ motivational stimuli. We will use a paradigm during which subjects make speeded responses to a central target under conditions of potential monetary gain, loss or neutrality during threat of shock and during safety. During each trial of the task, participants will first see one of three shapes, either a circle, a square, or a triangle, then wait for a variable time interval, and finally respond to a white box

with a button press. Trials beginning with a circle indicate that the subject will win money if s/he presses the button fast enough once the white box appears; trials beginning with a square indicate that the subject will lose money if s/he does not press the button fast enough once the white box appears; trials beginning with the outline of a triangle indicate that the subject will neither lose nor win money, independent of task performance. Participants will perform this version of the Monetary Incentive Delay task within the MRI scanner.

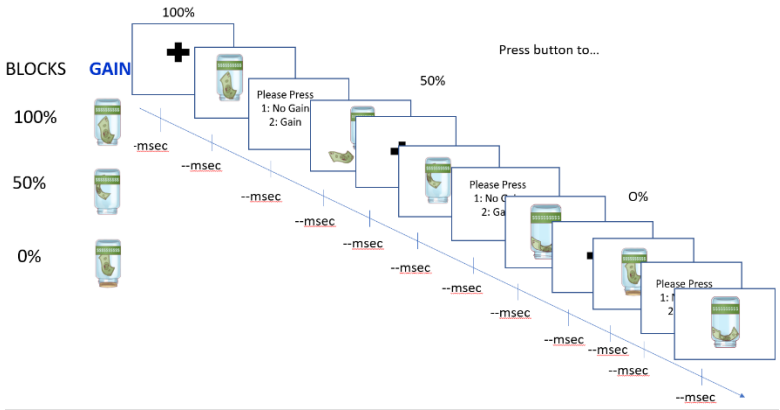
During the second version of the task (N = 40 HVs), we will examine the impact of monetary incentives and aversive stimuli on goal-driven / motivational processing. We will use a paradigm during which subjects make speeded responses to a central target during four types of trials. Accurate responses to the central target will determine whether participants: 1) win money (gain trials), 2) avoid losing money (loss trials), 3) avoid the presentation of an aversive electrical stimulus (aversive trials). During 4) even trials, participants will never receive monetary or aversive stimuli, regardless of performance. During each trial of the task, participants will first see a cue denoting the type of trial, then wait for a variable time interval, and finally respond to a white box with a button press. Participants will perform this version of the Monetary Incentive Delay task within the MEG scanner.

During both versions of the modified Monetary Incentive Delay task, the shape at the beginning of the trial serves as a signal characterizing the trial type. Additionally, participants will receive feedback after each trial indicating whether they succeeded in responding to the target fast enough. The duration of the white box is adjusted such that each participant succeeds on approximately 66% of his or her responses. Participants will be informed that “Your performance during each trial will determine whether you win money or lose money. However, the task may become more difficult or less difficult over time. Please try your best at all times.”

Participants will begin with an initial endowment of $20.00 and will perform a task which includes both gain trials and loss trials. Successfully responding to a target during a gain trial will result in more money being added to the participant’s total. Unsuccessfully responding to a target during a loss trial will result in money being subtracted from the participant’s total. As such, the participants total winnings can increase or decrease depending on performance during gain trials and loss trials, respectively. $0 serves as a floor (i.e. participants can lose their total endowment but cannot win negative amounts of money). All participants will receive $10 in compensation regardless of their performance during the task. Although participants can lose their entire $20 endowment, performance during the Motivation/ "Monetary Incentive Delay" Tasks has no impact on compensation from participation in other parts of the study. We have used this strategy in this and previous protocols ("Loss Aversion" task and 01-M-0192). The need to use real money is based on feedback from participants in the

"Loss Aversion" task, which indicated lack of motivation in the absence of real money. The use of real money makes the gains and losses more salient.

ValSal Task (Healthy controls N = 46, Anxiety participants, N=46)
The goal of this experiment is to extend the question of the mechanisms underlying symptoms of anxiety disorder, particularly those implicated in motivated behavior including aversion to uncertainty and avoidance behavior. The ValSal task was designed to probe two related, but distinct, processes that are integral to motivated behavior, i.e., valence and salience. Valence refers to the positive vs. negative values tagged to stimuli/situations. For example, a gain of $5.00 is a positive value, and a loss of $5.00 is a negative value. Salience refers to the subjective importance of stimuli/situations. For example, a gain of $5.00 may have the same subjective importance as a loss of $5.00, both being more important than no loss/no gain. The ValSal task uses monetary values to assay these processes at the neural (fMRI) level.
The task lasts 25 min long and comprises two runs. Each run consists of 4 blocks. Two blocks feature positive valence trials, and two blocks feature negative valence trials. The order of the blocks is counterbalanced across runs, i.e., positive-negative-positive-negative, or negative-positive-negative-positive.
Each block presents 16 trials. There are 6 types of trials. The positive blocks present 3 types of trials: 100% probability of $5.00 gain, 50% probability of $5.00 gain, and 0% probability of $5.00 gain. The negative blocks present 3 parallel types of trials: 100% probability of $5.00 loss, 50% probability of $5.00 loss, and 0% probability of $5.00 loss. Types of trials are randomized across each block. For each block, there are 4x100%trials, 4x0% trials and 8x50% trials, for a total of 128 trials for the whole task.
Each trial is 8 s long and consists of 3 screens: (1) a 2 s cue signaling the type of trial (e.g., 50% change of gain), (2) a key-press delay screen, and (3) a 2 s feedback screen. An intertrial interval (ITI) of 2 s separates the trials. An illustration of the composition of the trials is presented in **Figure** below.



This task can be modified in terms of the nature of the positive vs. negative stimuli. This could be important to distinguish among different types of anxiety (e.g., social anxiety vs. generalized anxiety).

Squeezer Task/ Follow-up to ValSal Task (Healthy controls = 40, Anxiety participants = 40)

The valence/salience study targets the circuits associated with the coding of valence and salience, including medial temporal structures (amygdala and extended amygdala and dopaminergic pathways (e.g., ventral tegmental/substantia nigra). However, preliminary analyses of the salience/valence study revealed the limits of the design, which, for the sake of simplicity, does not permit to assess instrumental responses to valence and salience stimuli. The salience/valence design provides essential knowledge on the circuits evaluating valence and salience, but not the responses to such stimuli that motivate behavior. This complementary study fills this gap by querying motivation operationalized as the amount of motoric effort extended to approach or avoid incentive stimuli (see example in Pessiglione et al. 2008). We will use the grip force measure (described on page 37 of this protocol) to compare motivation coding between adults with anxiety and healthy adults to complete our initial valence/salience study.

It has been shown that effort is modulated by incentive magnitude (e. g. effort towards $10 > effort towards $1) and that this modulation most likely originates from structures of the basal ganglia. Reports on how anxiety modulates effort, particularly in function of incentives, are few, and inconsistent. To address this gap in knowledge, we propose a new task that uses grip force as a proxy to motivation, based on (Pessiglione et al. 2008). The task timeline is depicted in Fig.1: while in the MRI scanner, the participant will hold a hand dynamometer ("squeezer"). The task lasts 20-30 minutes. After device calibration, baseline recordings and instructions, the participant will work on 5 runs of two blocks of 6 trials with a 5s break in between, making up a total of 72 trials. Next, they will complete 19 questions detailing their experience. This debriefing questionnaire is uploaded into iRiS. The task ends with a message telling the participant that they won a total amount (will be made fixed for all participants, e.g., $25).

There will be two types of trials in which the participant will squeeze to win (positive valence trials) or to avoid losing (negative valence) a monetary amount. See Fig. 2 for an example. The valences and amounts are pseudo-randomized a priori, so all participants go over the same experiment timeline. The trial timeline is: first, participants see a fixation cue for 1s. Next, they are cued with the valence ("Avoid lose" or "Try to win") together with the amount at play ($1, $5 or $10) for a random duration of 1.75, 2, or 2.25s. Next, participants respond by squeezing and holding for as much as they want, up until the dash reaches the maximum level or the screen times out, whichever happens first. The farther up they can bring the dash, the more money they are able to win/avoid lose. The trial ends with a feedback screen that displays how much money they won/lost that lasts 1.5s.

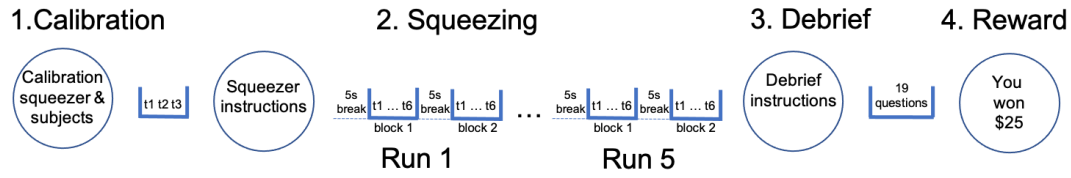


Figure 1. Task design.

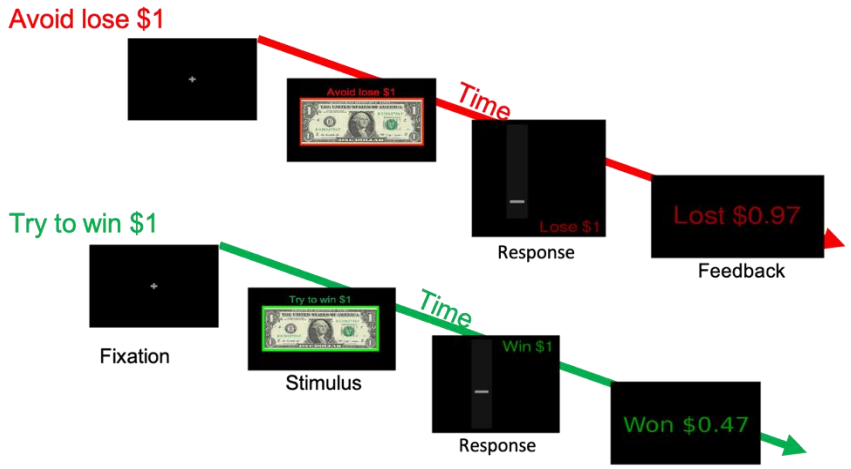


Figure 2. $1 trial timelines. Top: negative valence. Bottom: positive valence.

Active Avoidance of Signaled Threat Paradigm (Healthy controls without war-zone exposure N=90, Healthy controls with war-zone exposure N=30, PTSD from war-zone exposure N=30).

Participants will perform a modified version of the active avoidance of signaled threat paradigm (Gorka, LaBar, & Hariri, 2016), during which, they will hold down a button and view sequentially presented pairs of colored squares. The squares will differ based on the color's hue (low, medium, and high). Participants will be instructed to lift their finger when they see 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 in safe and threat conditions, and each block will consist of a series of "go" and "stop" trials.

We will use independent stair-case procedures to titrate task difficulty so that all participants achieve a ~75% accuracy during go trials for each condition. During the threat condition, participants will receive an electrical shock whenever they make a mistake. Participants will be informed that "Your performance during each trial will determine whether or not you receive an electrical shock. However, the task may become more difficult or less difficult over time. Please try your best at all times." During the safe condition, participants will never receive an electrical shock. The duration of each electrical shock will not last longer than

100 milliseconds. The frequency of delivery for electrical shocks will be based on performance, but a minimum of 10 seconds will separate each electrical shock. No participant will receive more than 10 electrical shocks in this substudy regardless of their performance. The amplitude of the electrical shock will be selected by the participant, through the shock workup protocol (see section 8, Electric shocks: work-up), to correspond to a level which the participant perceives to be tolerable, yet aversive. The shock intensity will not go above the 100 mA maximum shock intensity outlined in the protocol (see section 4iv., Electric shocks as unconditioned stimulus). These parameters have been well tolerated by participants in our previous studies.

Healthy controls without war-zone exposure will perform the task in the MEG or MRI scanners. Healthy controls with war-zone exposure and participants with PTSD from war-zone exposure will perform the task in the MRI scanner. Differences in accuracy and reaction times between the safe and threat condition will serves as measures of active avoidance, and will be used to determine whether persons with PTSD exhibit impaired or facilitated behavioral responses to threat. Indices of neural circuit function will be used to determine whether persons with PTSD exhibit altered neural responses during active avoidance, and whether changes in neural circuit function are associated with individual differences in active avoidance behavior (i.e. accuracy and reaction time).

Additional Procedural Description

fMRI and MEG investigations of threat

These experiments will have a similar design for both the event-related fMRI and event-related MEG. Separate groups of subjects will be run in the fMRI experiment (consisting of just one session in the MRI scanner) and in the MEG experiment (including the acquisition of a structural MRI scan). The fMRI experiment may be performed in a 3-Tesla or 7-Tesla fMRI machine depending on the availability of the 7T scanner.

The primary focus of the study will be the selective facilitation of processing and learning of threat-related information (Baas et al., 2002; cf. Weinberger et al., 1995; Thiel et al. 2002). A visual or auditory stimulus that indicates whether or not an electric shock and/or loud sound stimulus may be anticipated will be presented in a repetitive manner to allow averaging over enough trials to achieve acceptable signal-to-noise ratio.

The first run will consist of a baseline measurement to determine processing of the visual or auditory cues before these are associated with a threat manipulation. Subsequently, the subjects will receive an instruction that links one of the stimuli to the possibility of shock and/or loud sound stimulus reinforcement. The second run will be again a series of presentation of these stimuli, but with occasional reinforcement of the instruction by presenting a shock and/or loud sound stimulus during the presentation of the threat cue. During both runs participants may be

asked to use an MRI compatible fiber optics response box to rate their level of anxiety at multiple time points. Additional runs that include the procedures listed above (Perception, Memory, Resting State, and Passive Presentation) will be applied such that participants will be tested during 2-6 runs, depending on the number of tasks. Experimental procedures will not exceed 6 functional imaging runs in a single session.

Previous experiments (Baas et al., 2002) proved that reinforcement of 1 in 16 threat cues was sufficient to maintain expectation of shock throughout the experiment. Previous experiments have also shown that sufficient signal to noise ratio was achieved with event-related potential measures with about 200 presentations of each stimulus condition. This amounts to administration of about 12 to 15 electrical shocks during the experiment. This is the same duration of the experiment and total number of shocks as proposed as the experimental procedure proposed in the amendment to protocol 01-M-0185. The discomfort to the patient will not be increased by adding MEG measurements. MEG measurements are non invasive, painless, and are used routinely in clinical practice, including in children and infants.

MEG assessment of the cold pressor test

This MEG experiment will involve exposure to an acute stressor, i.e., immersion of hand into ice-cold water for 1min, to increase baseline anxiety. The procedure consists of a 5 min interval, during which the subject has his hand immersed up to the wrist level in water at room temperature (70°F). After this interval the subject is asked to immerse the same hand up to the wrist level in ice water for 60 seconds. Ice water with temperature of 33-36°F is prepared by mixing 2 liters of crushed ice and 2 liters of tap water in a plastic foam bucket shortly before the procedure takes place. After 60 seconds the hand is taken out of the ice water and dried with paper towels. Subsequently, we will measure autonomic responses and neural responses with MEG during resting periods to assess the effects of stress exposure.

Alprazolam/threat study

This will be a double-blind cross-over design; each subject will receive each drug treatment (placebo, 1 mg alprazolam). Subjects (healthy only) will be tested on 2 occasions on separate days as follows:

1) fMRI/MRS/MEG/placebo
2) fMRI/MRS/MEG/Alprazolam

For the initial study involving only MEG scanning after alprazolam and placebo administration, subjects will be tested during periods of threat, when they anticipate unpredictable shocks, and during safe periods. Tasks during MEG scanning include passive presentation of visual (3 cycles per degree, square-wave

gratings) and auditory stimuli (pure tones) and memory tasks (n-back, virtual reality) to evoke gamma responses. Following this initial study, a new sample of subjects will be tested in similar way during fMRI, MEG, and MRS scanning.

Recruitment, exclusion/inclusion, and safety precaution for this study are the same as in protocol 10-M-0049, which examines the effects of various compounds, including Alprazolam, on psychophysiological responses during anticipation of shock. Administration of the drug/placebo will take place in an outpatient clinic or a Day Hospital of the Clinical Center by a certified healthcare provider (nurse, nurse practitioner, or physician) and under the supervision of the medical staff of the Clinical Center. Participants will remain in the clinic or Day Hospital of the Clincal Center for 2 hours to allow the drug to take effect, and will return to that site following the testing procedures, which take place in the NMR center. A certified healthcare provider will be present for testing in the NMR center, and participants will be monitored (but not recorded) via a video camera. There will be two-way communication between the testing/patient room and the experimenter room. This study has been completed.

v. Study procedures

This protocol includes an fMRI study of cognition and emotion without threat of shock (2.5 hours; 90 minutes in the scanner), an fMRI threat of shock study (2.5 hours; 90 minutes in the scanner), an MEG cold pressor study (1.5 hours), and an MEG threat study (2.5 hours), an alprazolam/threat MEG study (5-6 hours, 2 sessions), and an alprazolam/threat fMRI/MEG/MRS study. An additional telehealth consenting visit may be added to each during the pandemic. The alprazolam studies have been completed. Participants will not necessarily complete all these components of the study but will initially be recruited for one component and then invited to participate in additional components as needed. MEG and alprazolam studies will only recruit healthy subjects. Healthy volunteers and patients may undergo the wrist shock task in 7T or 3T fMRI machine.

After initial telephone screening, individuals who appear to qualify for inclusion will be invited for an in-person screening at the NIH.. On the screening day, subjects will be asked to sign an informed consent for screening protocol 01-M-0254. A medical and psychiatric history will be taken and a physical exam will be conducted. Subjects will also be asked to fill out various questionnaires of mood and anxiety. Subjects will complete informed consent for protocol 02-M-0321 prior to study procedures. Questionnaires may be administered on the day of the study procedures through the Clinical Trial Survey System (CTSS)/ Clinical Trials Database (CTDB).

We estimate that 1186 healthy subjects and 686 patients will be needed: about 30 subjects per experiment (in at least 6 studies), so that high-quality data is acquired from at least 15-20 subjects per experiment, with enough subjects to account for

data lost to artifact or subject withdrawal. Subjects will be told that the study examines their responses to anticipation of shock. In addition, to pilot an adaptation of this procedure to make it suitable for children, adolescents and patients, we will test 12 more subjects (healthy adults) using a mildly aversive air blast to the neck instead of aversive shock. Air blasts have been used as unconditioned stimuli successfully to examine explicit cue fear but not contextual anxiety (Grillon et al., 1999; Pine et al., 2001). The original procedure will employ threat of shock to maximize the chance of finding significant effects. Shocks are inherently more anxiogenic than air blasts. The air blast manipulation will be piloted in the psychophysiological laboratory.

Anxiety, mood & subjective measures

We will use Mood and Anxiety Symptom Questionnaire-Short Form (MASQ-SF), Spielberger State/Trait Anxiety Inventory (STAI, Spielberger, 1983), Penn State Worry Questionnaire (PSWQ, Meyer et al., 1990), the Beck Anxiety Inventory (BAI, Beck et al., 1988), and the Profile Of Mood States questionnaire (POMS, McNair et al., 1971). It will be noted whether subjects have had previous experience with fMRI, because previous experience may lead to a lower baseline anxiety. To evaluate the effect of several experimental parameters, analogue scales will be employed. Visual analogue scales will be used for measurements outside of the scanner (a 10 cm long, indicating at the left-hand side 'none', right 'extremely high'; the middle (at 5 cm) will be scored as neutral). First, the subjects' level of discomfort induced by the shock will be evaluated after shock sample administration. On the test day, subjects' anticipation of the aversiveness of the shocks will be measured. Non-analogue measures 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). Participants may enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. CTSS will not require real-time monitoring.

Subjective measures in the scanner

After each run subjects will be asked to retrospectively indicate their level of fear on a numeric scale from 1-100 during the absence of the visual cue in all three conditions (neutral, predictable, unpredictable), and in the presence of the cues in the predictable condition. The instruction texts that indicate these conditions will be projected on the screen one by one and the subject will be asked to give their rating orally through the intercom or by pressing response buttons.

Function Magnetic Resonance Imaging

The parameters for acquisition of fMRI data that we will start out with in the current study will be as follows. First, a sagittal localizer scan will be acquired to orient subsequent scans. Second, the functional scans will be acquired. Although BOLD signal drop-out during EPI sequences is more severe at higher field, preliminary analysis of 7T data confirms that the signal obtained is adequate to image our primary regions of interest, the BNST and dorsal nuclei of the

amygdala. These scans will include series of contiguous 4 mm axial slices extending covering the entire brain, parallel to the AC-PC. These scans will use a 64 x 64 matrix with echoplanar single shot gradient echo T2* weighting (TR=2000 ms; TE=40 ms; FOV=200 mm; 64 x 64 matrix, 3.75 mm voxels). This slice thickness and voxel size has been optimized on the NIH scanner for obtaining changes in BOLD measures in the human amygdala (Dr. Pessoa, personal communication). These parameters have also yielded good results for the amygdala, the lower orbital cortex, and the anterior cingulate cortex, in terms of minimizing signal susceptibility artifacts. However, if we fail to see reliable activations using these parameters in the pilot subjects, we may need to acquire a more limited number of slices focused on the amygdala and prefrontal regions (axial oblique angle). We are aware that on restricting the field of view in this manner, the analyses will have to be restricted to ROI analyses. Finally, a high-resolution T1-weighted anatomical scan of the whole brain will be acquired using a magnetization prepared gradient echo sequence (MPRAGE). This yields 0.9 x 0.9 x 1.2 mm^3 voxels optimized for gray-white-CSF contrasts. Finally, a set of standard perfusion scans will be acquired to evaluate cerebral vasculature structure and function. Participants may be scanned in the 3-Tesla or 7-Tesla fMRI scanner.

GABA MR Spectroscopy

The procedure will be similar to that implemented in 05-M-0006. Subjects will be scanned on a 3 Tesla GE whole body scanner using a GE resonator head coil that provides a homogenous radiofrequency field and capability of obtaining spectroscopic measurement from cortical regions. The voxel will be located on the basis of a MPRAGE structural MRI acquired in the same scan session. GABA will be measured using an interleaved PRESS-based J editing method. Individual peak areas will be fitted using MRUI (http://carbon.uab.es/mruiwww), which performs time domain spectral. Concentrations of GABA, choline, N-acetyl aspartate (NAA), and co-edited Glx (i.e., glutamate and glutamine) will be expressed in mmol/liter (mM) referenced to concentration of creatine. This creatine referencing method has been used in the field for over a decade and has been validated by a number of research groups. The spectroscopy data will be processed in two steps. First the unedited spectra will be fitted for the amplitudes of choline, creatine, and NAA. Secondly the GABA at 3.0 ppm and co-edited Glx-2 at around 3.8 ppm will be extracted from the edited spectra and fitted accordingly. The GABA signal will be corrected for macromolecule contaminations (Shen, Rothman, & Brown, 2002). At experimental conditions optimized for GABA editing, a small fraction of Glx-2 at 3.8 ppm and Glx-4 at 2.4 ppm will be co-edited because of their J couplings to the Glx-3 signal at 2.1 ppm. The clean co-edited Glx-2 signal will be used for measurement of Glx as its intensity is proportional to the total concentration of Glx. The GABA signal closest to the Glx-2 peaks resonates at 3.0 ppm.

Visual and auditory threat

For the MEG recordings, the participant will either sit or lie supine in the shielded recording room with her head in the helmet. Brain magnetic fields will be recorded with the 275-channel OMEGA system. The 275 SQUID sensors are uniformly distributed, in a grid, over the inner surface of the helmet that covers the entire head with provisions for the eyes and ears. Visual and two-way audio communication with the participant will be maintained throughout the session. Head position within the magnetometer will be determined continuously during the scan by digitizing the position of three indicator coils that are attached to the preauricular and the nasion fiducial points. The positions define the coordinate system for the signals and allow for detection of head movement artifacts. Digital photographs of the fiducial points will also be taken to localize the same points on the participant's anatomical MRI scan. Participants in the MEG experiment will be invited back for a short MRI session, in which a structural MRI scan will be acquired, to be used in the source localization of the MEG data.

Eyetracking in MEG
Subjects are situated in the seat of the MEG, and the camera of the eye-tracking unit (SR Research Ltd. Ottawa, Canada) is moved into place. Afterward, the subject undergoes a calibration procedure where they are instructed to fixate on repeated presentations of a randomly positioned fixation cross. Afterward, the eye-tracking camera passively monitors the position of the subject’s gaze, and the diameter of the subject’s pupil. The unit also creates events at the onset and offset of each blink or saccade.

Scanning: On the day of the scanning session, subjects will again fill out state questionnaires, and visual analogue scales to assess anticipation of the experiment. Electrodes for shock administration and for skin conductance monitoring will be attached. Subjects will then enter the scanner. They will be informed of all conditions via a display monitor in the scanner. The conditions are as follows: neutral (no shocks), predictable (shocks only during display of stimulus cue) and unpredictable (shocks can be administered at any time). During these three conditions, scans will be made in the presence and the absence of the stimulus cues. Shock reinforcements will occur during as few runs as possible.

Psychophysiological testing: Following scanning, the experiment may be repeated in a psychophysiological laboratory, where we will assess their startle, skin conductance, and heart rate responses during the different threat conditions. The psychophysiology will likely take place on a different day.

Electric shocks as unconditioned stimulus (US): Electric shocks will be used as the US. 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, feet or ankles. Given high heterogeneity of skin resistance and pain threshold between participants, each shock will have intensity up to 100

mA and will be delivered as trains of 1-2 ms pulses. The total shock duration will not exceed 500ms. For each subject a level of shock that is tolerable, but uncomfortable will be selected (see shock workup below, section 8, Electric shocks: work-up). The shock is generally described by participants as rather anxiogenic and unpleasant, but tolerable. We have been using shocks in various protocols at the NIMH since 2000 without major complains. Participants will have the opportunity to withdraw from the study if they wish at any moment, and are informed explicitly about this option in the informed consent. Our experience is that over 95% of subjects who received the shock chose to participate in the experiment.

Startle Reflex: The acoustic startle stimuli will be a 40-ms burst of white noise (103 dB) with instantaneous rise time. Auditory stimuli will be delivered binaurally via headphones. The eyeblink component of the startle response will be measured by recording electromyographic (EMG) activity of the left orbicularis oculi muscle as we have done in all of our previous psychopharmacology studies.

Motion induced by the shock reinforcements: To reduce the impact of the motion artifacts on the data caused by the reaction to a shock (typically a startle response, i.e., a jerk-like motion), as few shocks as possible will be given during the experiment. . The normal procedures for movement artifact detection and correction will be employed (AFNI/ SPM). However, due to movement induced by the shocks there may be a substantial change in position of the head during the shocks, which would change the head position in the scanner. The alignment procedure of functional images with respect to each other work well with motion up to 1 or 2 voxel sizes (see http://afni.nimh.nih.gov/afni/edu/README.registration, and Cox and Jesmanowicz, 1999).

The runs in which the shocks are delivered will be closely evaluated with respect to the occurrence of motion artifacts, by examining the data visually, and by relying on the parameters provided in the dedicated software (AFNI/ SPM) that yield measures of amount of movement. TRs in which excessive movement occurs will be discarded. If either based on the visual inspection or based on the movement parameters movement appears excessive, the data from that run will be discarded. The AFNI software can successfully correct movement up to the order of about a voxel size (Cox and Jesmanowicz, 1999).

An alternative strategy is to prevent head movement in the scanner by the use of a bite-bar. Our experience suggests that movement associated with shock reinforcement is marginally higher than the levels of movement observed across functional imaging studies, in general. This leads to a slightly higher than average amount of data rejection in our studies involving shock reinforcement. Nevertheless, the use of a bite-bar does not appear warranted because of the additional baseline anxiety and muscle stress caused by restraining the subjects in this way.

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.

v.i. **End of participation:** Participation in this study will end after the completion of each visit, following an assessment for adverse reactions to experimental procedures and a psychiatric interview when clinically indicated. Participants will remain under the care of their medical provider. Additionally, depressed patients will receive care as outlined under the direction of Dr. Zarate and the mood disorders program staff. Medical care will not be offered at the completion of study procedures. Participants will be informed of any findings that require further evaluation. Clinical MRI brain scans are completed one time per year, and sent to radiology for assessment. In rare cases when an abnormality is discovered, participants are offered further assessment at the NIH via a neurology consult. Minor abnormalities with no clinical significance to participants may not be shared.

b. Clearly identify which procedures are research and which are clinical care

N/A

c. Identify any medications/ devices requiring IND/IDE

NSR devices:

- Acoustic Startle
- Shock Device
- 7T fMRI

d. Identify if radiation is medically-indicated or for research only

N/A

e. Identify relationship of this study to other protocols (include if subjects are required to participate in other protocol)

N/A

5. Management of Data and Samples:

Storage

Any saliva samples collected will be stored in Building 10, Room 3D55. These samples will be assayed for salivary cortisol levels as well as for alpha-amylase activity.

Information is stored using a confidential case number (subject research code number), and no identifiers (name, address, phone number, etc.) are placed on data that could allow direct linking of database information to individual subjects. Additionally, data will be kept in password-protected computers. Samples are kept in locked storage. Only study investigators have access to the samples and data. Any loss or destruction of samples will be reported to the IRB. Samples will be destroyed at the end of the study.

Data and sample sharing plan

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

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

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

6. Additional Considerations:

a. Research with investigational drugs or devices

Acoustic startle, shock device, and 7T fMRI 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.

The 7T fMRI 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 7T fMRI 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 7T fMRI 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 7T fMRI, 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 7T fMRI 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 7T fMRI studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for 7T fMRI within these limitations.

b. Gene therapy

N/A

7. Risks and Discomforts:

<u>fMRI</u>
MRI is widely regarded as a safe, noninvasive procedure for visualization of brain tissue. The risks involved with fMRI are the same as those involved in standard anatomic MRI, since these three procedures rely on the same physical properties of brain tissue. This study will be performed on an FDA approved 3T scanner at the NIMH.

MRI at 3 Tesla is a routine clinical procedure, and issues regarding radio frequency deposition, time varying magnetic fields, and the static field at 3 Tesla do not require detailed discussion.

MRI at 7 Tesla (ultra high field strength) is less commonplace but becoming more so, as there currently are approximately 60 high field scanners in global operation. fMRI at 7T poses the minimal risk of vertigo-like sensations during movement in and out of the bore, but subjects in one study rated their discomfort of such sensations well below the length of scan session, which is the same for 7T as for 3T (Theysohn, et al. 2008). Furthermore, tissue heating from radiofrequency deposition is a minimal concern at small flip angles for fMRI at high fields (Gonzales-Castillo, et al 2011). Essentially, the risks of shocks in the scanner environment, with 7T as well as 3T, include heating of the electrodes with discomfort or burns. These risks, however, are actually less likely in the 7T scanner than

in 3T MRI scanner because in the 7T scanner the RF field does not extend to the whole body, as it does with the 3T.

MRS

There is no known hazard to the exposure of magnetic waves during MR imaging. MRS does not introduce additional risks compared to fMRI (the gradient noise is much milder with MRS than MRI).

Electric shocks: work-up

Electric shock poses minimal risk to participants. The shocks will be delivered through two disk electrodes located on one of the subjects' ankles or feet or wrist. Prior to testing, a shock work up procedure will be used to allow us to control for heterogeneity of skin resistance and pain threshold between participants and to administer a range of mildly painful stimuli in an ethical manner during the task itself. Shocks with step increases in amplitude will be administered up to a level tolerable to each participant, while participants will provide subjective ratings of shock intensity. The initial shocks are of mild intensity while participants familiarize with the setup. Up to three series of shocks will be administered (up to 20 shocks in total), as repeated escalation allows participants to adapt to initial anxiety about the shocks, since thereafter ratings are more consistent (Seymour et al., 2004, 2005). Participants are explicitly informed of their right to discontinue the research without consequence at any point during the consent process and then again following the sample shock procedure. Our experience is that over 95% of participants choose to participate in the experiment following the sample shock workup. Anticipation of the shock increases subjective anxiety and enhances physiological arousal.

Electric shock within the MRI scanner

Introduction of electrical wires that are connected to the subject in the strong magnetic field of the scanner may constitute an additional risk. The main risk associated with administering shocks is the introduction of electrode wires directly in the RF- (radio frequency) field of the magnet. Wires will not be exposed to the radio frequencies induced in the RF head coil. The shock stimulator equipment will be outside the magnetic area, and a plastic and copper cable is taken through the wall for connection to the subject electrodes. To eliminate the possibility that electrode wires will enter the RF-field from subject movements, shock electrodes will be attached to either the foot or ankle region. Similarly, electrodes for skin conductance recording in the scanner will be attached to the sole of the other foot. Area between the wires will be minimized by twisting the wires together, and coiling of the wires will be prevented by inspection prior to each session (i.e., wires run without loops). Studies employing wires for electrical stimulation (e.g., Disbrow et al., 1998) or for psychophysiological measurement (skin conductance; Büchel et al, 1998; LaBar et al., 1998 etc) have been reported in the literature.

MEG

MEG is a noninvasive procedure to measure brain responses by recording magnetic fields and electrical potentials outside the head with sensors,. No known risks is associated with this procedure.

Eyetracking in MEG
The unit is a passive monitoring system, and nothing is attached to the subject. There are minimal risks associated with this procedure.

Startle stimulus
The auditory stimuli that will be used in the startle studies are 40-ms duration 103 dB white noise. Auditory startling sounds of much higher intensities are frequently used in startle studies. Sounds of higher intensities and longer duration are also widely used in aversive conditioning in human subjects, where they serve as unconditioned stimuli. The short duration (40 ms) of these sounds minimizes risk of hearing loss (Blumenthal et al., 2005). In addition, a white noise is safer than a pure tone. The PI has been involved in similar studies and collaborations involving over 1000 of subjects with no adverse reactions. The auditory stimulus may trigger a migraine.

Cold Pressor test
The cold pressor test is a simple, safe and reliable procedure, widely used to determine cardiovascular reactivity, pain perception and stress response in healthy subjects, as well as in clinical conditions such as hypertension or depression (Velasco et al. 1997, Kelly et al. 1998). There are no known risks associated with the cold pressor procedure.

Psychophysiological recording
The psychophysiological measures that will be obtained are non-invasive, requiring the administration of no 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 since the salt concentration of the paste is similar to that of human sweat. The risk is equivalent to that of an EEG recording.

Alprazolam
Alprazolam (Xanax™) is a benzodiazepine widely used in the treatment of anxiety disorders or for the short-term relief of anxiety symptoms. For chronic treatment of anxiety disorders, the PDR (2000) recommends a daily dose up to 4 mg, given in divided doses. Plasma levels are proportionate to the dose given. Peak concentration in the plasma occurs in one to two hours following administration. For dose 0.5-3.0 mg, peak levels of 8.0 to 37 ng/mL are observed. Mean plasma elimination half-life is 11.2 hours. Contraindications include sensitivity to benzodiazepines. Side effects in patients using chronic alprazolam include insomnia, drowsiness, fatigue, headache, involuntary movement, nausea, and memory difficulties. Besides its effect on anxiety in clinical populations, alprazolam has also been frequently used to test the effects of benzodiazepines on experimental anxiety at doses of 0.5 and 1 mg. This study has been completed.

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, & Vaitl, 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.

Delayed Treatment (patients only)
Although participants will not be taken off any medication for the purposes of this study, we will only include those patients who are not currently taking any psychotropic medications, with the exception of PTSD participants only. Thus patients included in this study may be at risk of increased symptoms because of the absence of psychopharmacologic treatment. During the initial psychiatric assessment, clinical staff will inform patients that delaying psychopharmacological treatment may increase their risk of increased symptoms and that such treatment is readily available at mental health care providers outside of the NIH. PTSD participants may continue on antidepressants and benzodiazepines.

Storage/sharing of data and samples: There is minimal risk that data or samples could be identified. To minimize this risk, data and samples that are shared will be de-identified. Data and samples will be sent with a code. This linking code will be kept at the NIH.

Grip force measures: This measure is not expected to increase risks in human subjects. Gripping hard may cause muscular fatigue or soreness but is not expected to increase risk of injury or harm to the subject. The fatigue or soreness is expected to be mild and self resolve without treatment.

8. Subject Safety Monitoring:

a. Parameters to be monitored

General Subject Monitoring

Over the course of the outpatient visits to the NIH, Clinical Center, the Principal Investigator, Associate Investigators, Independent Study Monitor, Research Nurse Coordinator, and attending clinical staff will monitor the participants for distress, discomfort, and desire to discontinue the study. Participants will be provided with the contact information of the Principal Investigator should concerns arise in between visits.

Alprazolam Subject Monitoring

For the alprazolam experiment, a certified healthcare provider (nurse, nurse practitioner, or physician) will be on hand throughout the procedure and will be responsible for participants' discharge. A sedation assessment, the Observer's Assessment of Alertness/Sedation Scale (Chernik 1990), will be administered by the certified healthcare provider prior to and following the procedure before discharge. The certified healthcare provider will also contact the participant the following day after each testing session to ensure that there are no adverse reactions to the drug or experimental procedures. This study has been completed.

Increased symptom severity
If, during the course of assessing a participant, a study staff member identifies a significant worsening of symptoms, a mental health provider currently treating the participant will be contacted, and the necessary treatment steps (e.g., hospitalization, referral to a care provider, etc.) will be offered.

Risk for self-harm: Participants will be monitored for risk for self-harm via a history and physical exam and clinical interview on Visit 1, and subsequently during all study visits. If a participant is considered a positive screen for self-harm, the participant will not be able to leave the premises until evaluated for safety and study procedures will be terminated for that day. If following the safety/mental health evaluation it is determined that a participant is at risk for self-harm, the participant will be excluded from the study. Participant transfers to alternative medical facilities will be conducted according to NIH, Clinical Center Guidelines. For off-hour psychiatric emergencies, the study investigator's voice message instructs participants to call 911 or visit the nearest Emergency Department. If a participant leaves a voicemail in distress, the clinical team will attempt to make contact with the participant within one business day. If contact by phone is successful, the participant will be triaged by phone and offered a list of mental health resources. If the participant does not respond to calls, the clinical team will try calling an alternative number if available.

b. Toxicity tables/ criteria to be used

N/A

c. Criteria for individual subject withdrawal
Participants will be withdrawn from the study if the Principal Investigator determines a serious adverse event is related to the study procedures or if the severity of an adverse event warrants withdrawal. Participants will also be withdrawn if they develop high levels of anxiety, fear, or panic during/from any shock experiments. Participants will also be excluded from the study, if they become at risk for self-harm. Additional criteria include withdrawal of consent and/or participant decision, non-compliance with protocol procedures, and lab results that preclude participation (i.e., positive drug and/or pregnancy result). This study nor the NIH will pay for outside treatment.

9. Outcome Measures:

a. Primary

Functional magnetic resonance imaging (fMRI), measuring the event-related haemodynamic response related to neural activity in the brain.

Magnetoencephalography (MEG) a neuroimaging technique employed in the current protocol to measure the event-related magnetic fields produced by

electrical activity in the brain via extremely sensitive devices such as superconducting quantum interference devices (SQUIDs).

b. Secondary

Psychophysiological measures of anxious arousal including the skin conductance response (SCR), heart rate, respiration, and EMG measures of the fear-potentiation of the startle reflex.

Salivary cortisol assays to measure the stress response to the cold pressor, saliva will be collected at several intervals throughout the session with plain cotton swab Salivettes. All samples will be frozen and stored at -70ºC until assayed in one of the freezers located in bldg 10, 2D corridor. On the day of assay, the Salivette tubes will be thawed at room temperature and centrifuged at 3000g for 10 minutes. Cortisol concentration in saliva will be measured by enzyme immunoassay (Salimetrics LLC, State College, PA). Management and analysis of saliva samples will be done by Roman Duncko, MD, in the Section on Genetic Developmental Epidemiology. Samples will be number coded, stored for approximately a year, and then properly disposed in medical waste.

Self-reported measures of anxiety, level of risk, and CS-US contingency awareness.

10. Statistical Analysis:

a. Analysis of data/ study outcomes

Analysis of functional imaging data involves a series of initial steps including realignment (i.e., correction for subtle head movement). Hypotheses regarding task-associated changes in the BOLD signal will initially be tested by analyzing task-related hemodynamic responses in four regions-of-interest (ROI) selected based on hypothesized areas of activation: the amygdala, the dorsal anterior hippocampus (Williams et al., 2001), the anterior insula, and the anterior cingulate. These ROI will be defined using AFNI (Analysis of Functional NeuroImages; http://afni.nimh.nih.gov/afni/). The ROIs will be defined on each subjects' anatomical MRI image, and the corresponding changes in BOLD data will be extracted from fMRI images that have been coregistered to the anatomical MRI. Correction to control for inflation of α (Type I error) will be applied to the tests performed on these 8 regions (4 regions in each hemisphere).

In addition, a voxel-wise analysis will be performed to investigate activations outside of these regions using SPM 8 (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm/). Spatial transformation and smoothing will be performed prior to the voxel-wise analysis. This analysis will assess activations in areas outside the ROIs defined *a priori*, as well as to localize the voxel-coordinates for the peak difference between conditions within the primary ROIs. We will model the effects of presenting the cues depending on the background, and of presenting the background itself on BOLD activity.

The factor context (neutral, predictable, unpredictable) will be entered as a blocked factor to model the background state during these 2-min conditions. In addition, the transient response to the cues will be modeled with respect to the onset of the cue. The hypothesis is that in the areas critical for cue fear there will be a transient response to the cue in the predictable relative to the neutral and unpredictable condition. This model of analysis constitutes a mixed block/event-related design.

All experiments except for those that include patient groups will use within-subject designs, such that each participant will experience each comparison condition of interest (note: this does not imply that ALL subjects will participate in ALL procedures, just that the procedures in which they elect to participate will include the conditions necessary to perform the intended analyses). Experiments that include patients will operate both within-subject and between-subject designs; the within-subject factors being the experimental conditions, and the between-subject factor being subject group (patient or healthy control).

b. Criteria for significance

For voxel-wise analyses on normalized whole brain data, we will control false positive rates per map at α=.05, using random-effects models (see e.g., Zarahn, 2000; Zarahn et al., 2000; Pine et al., 2001; 2002).

c. Power analysis

Sample size of 15 subjects with good quality data (no major artifacts or other problems) is usual for previous published studies on activation of limbic structures, such as Phelps et al. (1999; N=12), and Whalen et al. (1998; N=10). Although power computations are possible for ROI analyses using conventional statistical tests in which average BOLD signal intensities are treated as dependent variables, because the current study constitutes a pilot experiment that has not been previously performed, the mean and standard deviation of the changes in BOLD responses that will be obtained using the proposed methods are not known. In general, with sample sizes as small as n=10 and for alpha = .05 (two-tailed), power exceeds .80 to detect effect sizes of 1.2 in one pre-specified brain region. Fortunately, most fMRI studies of neurobiologically meaningful task-associated changes in BOLD signal document larger effects than this. For patient subtypes, (i.e., GAD, SAD, panic, phobia, PTSD, and MDD) an N of 30 will be targeted for each study. This larger sample size will provide us with a greater range of symptom severity in order to conduct correlation analyses with regional brain activity and it will buffer against potentially higher rates of attrition (excessive motion, symptom-related dropout) in our psychiatric samples.

For the Time Perception, cognitive task, a power analysis was conducted to determine the number of participants that will be used for that sub-study. In a previous fMRI experiment, our largest effect size (η^2=0.24) was for brain activation in the insula under our anxiety manipulation. Assuming a somewhat smaller effect size (η^2=0.20) due to regression to the mean, if we set power at .90 and experiment-wise, two-tailed alpha at 0.05. Based on these parameters, we will need 46 subjects per experiment. Assuming 4

subjects to initially adjust the experimental procedure, a N of 50 subjects will be the target for the experiment.

For the first modified version of the *Motivation/ "Monetary Incentive Delay" Task,* we propose to collect 80 participants on the 3T fMRI scanner in order to assess the role of between subject factors such as gender and trait anxiety on neural responses to incentives. Assuming a medium effect size (R^2=0.15), a power of 0.8, and an alpha of 0.05, power analyses suggest that we would require a sample of 75 participants to detect an association employing two-tailed multiple regression analyses. Assuming that five participants are lost due to head motion, we will need to collect 80 participants to detect a medium sized effect of gender or trait anxiety on these neural responses.

For the first modified version of the *Motivation/ "Monetary Incentive Delay" Task,* we propose to collect 30 participants on the 7T fMRI scanner in order to assess the role of small midbrain structures in reward/threat processing. Assuming a slightly larger effect size due to the improved signal to noise ratio of 7T imaging (Cohen's d_z=0.6), a power of 0.8, and an alpha of 0.05, power analyses suggest that we would require a sample of 24 participants to detect a difference between conditions employing two-tailed paired T tests. Assuming five participants are lost due to head motion. we will need to collect 30 participants to detect a medium sized effect of trial type on neural responses to incentives.

For the second modified version of the *Motivation/ "Monetary Incentive Delay" Task,* we propose to collect 40 participants on the MEG scanner to assess anticipatory responses to aversive and monetary motivational stimuli. Assuming a medium effect size (Cohen's d_z=0.5), a power of 0.8, and an alpha of 0.05, power analyses suggest that we would require a sample of 34 participants to detect a difference between conditions employing two-tailed paired T tests. Assuming five participants are lost due to head motion, we will need to collect 40 participants to detect a medium sized effect of trial type on anticipatory neural responses.

Collectively, the three research projects listed above (80+30+40=150) will increase our accrual from 30 to 150 participants for the *Motivation/ "Monetary Incentive Delay" Task*.

For the first modified version of the *Active Avoidance of Signaled Threat Paradigm*, we propose to collect 45 healthy participants without war-zone exposure on the fMRI scanner. Assuming a medium effect size (Cohen's d_z=0.5), a power of 0.8, and an alpha of 0.05, power analyses suggest that we would require a sample of 34 participants to detect a difference between conditions employing two-tailed paired T tests. Assuming ten participants are lost due to head motion and poor performance, we will need to collect 45 participants to detect a medium sized effect of trial type on neural responses during avoidance.

For the second modified version of the *Active Avoidance of Signaled Threat Paradigm*, we propose to collect 45 healthy participants without war-zone exposure on the MEG scanner. Assuming a medium effect size (Cohen's d_z=0.5), a power of 0.8, and an alpha

of 0.05, power analyses suggest that we would require a sample of 34 participants to detect a difference between conditions employing two-tailed paired T tests. Assuming ten participants are lost due to head motion and poor performance, we will need to collect 45 participants to detect a medium sized effect of trial type on neural responses during avoidance.

Collectively, the two research projects listed above (45+45=90) will increase our accrual from 30 to 90 healthy participants without war-zone exposure for the *Active Avoidance of Signaled Threat Paradigm*.

d. Accrual number
Our ceiling is set at 1952 participants. This will account for the addition of patients (726) as well as a separate group of 1226 healthy matched (age, sex, IQ) controls.

11. Human Subjects Protection:

a. Subject selection

i. *Statement of equitability*
This study provides equal opportunity to participate for all eligible persons and prohibits discrimination on the basis of race, ethnicity, color, religion, creed, sex, sexual orientation, gender identity, national origin, ancestry, disability (unrelated to exclusion criteria), genetic information, or handedness. Participants above the age of 50 will be excluded to achieve some homogeneity in our sample and thereby reduce the variability of the neurobiological signal across subjects. An age limit of 50 years provides for the inclusion of the majority of participants we recruit, without compromising the results by including older adults who have been shown to exhibit functional reorganization in the brain (Reuter-Lorenz, & Lustig, 2005). The initial age limit was set at 40 years old because it resulted in a relatively homogenous sample, without unnecessarily excluding subjects. This limit has since become too stringent, and based on similar studies (e.g., Blair et al. 2008; Blair et al., 2006; Hsu et al., 2010), we have increased our age limit to 50 years old.

ii. Rationale for selection if not equitable
We plan to enroll men and women in 1:1 ratio.

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 except American Indians, who are less prevalent. Since 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 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.

Participants who do not speak English fluently will be excluded because our primary instruments are not validated in languages other than English.

b. Justification for inclusion/ exclusion of children

We will not enter subjects under age 18 because of ethical concerns about exposing them to threat of shock. Though shock stressor is 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 younger children. 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/exclusion of other vulnerable subjects, e.g. cognitively impaired, pregnant, mentally ill

Pregnant females will be excluded due to the risk of electric shock and MRI during pregnancy.

We have specific hypotheses regarding the effects of stress on different psychiatric populations, and the data that we gather will have implications for clinical intervention as well as basic understanding of behavioral and neural differences associated with the different subtypes of mood and anxiety disorders. Patients will undergo clinical assessment and care as outlined above, and will be consented, enrolled, and retained as participants only if they a) fully understand the procedures and risks involved, b) are willing to participate, c) do not display any contraindication to participating (see exclusion criteria above), and d) do not display significant psychiatric symptom increase during periodic clinical assessment throughout the study.

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

Participants will be asked to abstain from smoking and from consuming caffeinated beverages including coffee, tea, and soft drinks for at least 1 hour prior to testing. They will also be instructed not to drink alcohol on the night prior to testing and on the day of testing. To reduce any associated withdrawal symptoms, participants will not be asked to completely stop smoking or consuming caffeine for the entire duration of this study.

Participants who are currently on certain types of psychotropic medications will be excluded from study participation (exceptions made for PTSD participants only). To reduce any associated withdrawal symptoms, patients will not be taken off medications for the purpose of the study

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

shocks have been very well tolerated by 100's of past participants in our psychophysiology experiments, we decided to again use electric shocks in our neuroimaging investigations described herein.

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 MRI session and exclude all participants who are pregnant.

f. Informed consent procedure

Informed consent for the study will be obtained in-person at the initial study visit or using NIH-approved Telehealth platforms prior to any diagnostic or medical procedure. AIs listed on the KSP form are qualified to obtain consent for the studies..

All consent forms will be approved by the Institutional Review Boards (IRB) at the NIMH.

Subjects can be invited back to participate in future experiments. If a subject is not contraindicated in any way (e.g., contraindication cases include: a patient who enters into treatment and thus is not eligible, or a subject who does not wish to participate in other aspects of the study), he or she will be invited back for additional testing. Subjects will undergo informed consent each time they participate in a different experiment in this study, and will be explicitly reminded that they are not required to participate in additional procedures. Further, all participants will be re-consented, even for repeat procedures, when more than two months have passed since they were last consented. Full SCIDs will be performed for all subjects annually; if a SCID was done within the year and the subject returns to participate in another study, then a credentialed staff clinician will spend time with them to do a brief assessment of current symptoms and functioning.

The consent forms include a checklist which 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

14. Consent Documents and Process:

a. Who will obtain consent?

Study investigators designated as able to obtain consent are listed on the study personnel sheet.. All study investigators obtaining informed consent have completed the National Institute of Mental Health, Human Subjects Protection Unit "Elements of Successful Informed Consent" training.

b. *How will consent will be obtained if special procedure is needed?*

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 before signing.

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. The process for documenting signatures on an electronic document is described below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the following electronic platform to obtain the required signatures:

- iMedConsent platform (which is 21 CFR Part 11 compliant)

Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse. Electronic signatures (i.e., the "signature" and a timestamp are digitally generated) will not be used.

c. If special documents are needed (minor assent, Braille, another language, etc).

N/A

15. Data and Safety Monitoring:

a. Monitoring plan for the study as a whole

Data and Safety Monitoring Plan – Independent Monitor
This protocol will have an independent safety monitor, Dr. Joyce Chung. She has no involvement in protocol implementation or investment in the outcome of the study. The PI will provide, every four months, study data including enrollment, study progress, and outcome data to the independent safety monitor. The PI will report serious, unexpected adverse events and deaths related to the protocol's experimental procedures to the independent study monitor at the same time as they are reported to the IRB, the IC Clinical Director and other NIH officials.

The independent monitor will provide the PI with a summary report of findings, including recommendations for continuation or topping of the study. The PI submits this report to the Office of the Clinical Director and the IRB after each review occurs.

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. Data and safety issues are reviewed at biweekly laboratory meetings with all research and clinical staff.

c. Criteria for stopping the study or suspending enrollment or procedures:
Study enrollment will be stopped or suspended for any potentially related serious adverse event, until the Principal Investigator, Clinical Director, the Independent Study Monitor, and the IRB determine there is no risk to continue.

16. Quality Assurance:

Quality assurance monitor

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

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

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

a. Reporting/ non reporting of expected AEs

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

18. Alternatives to Participation:

a. Treatment/ therapeutic alternatives should be listed. If none, so state.

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:

Information will be stored using a confidential case number (subject research code number), and no identifiers (name, address, phone number, etc.) will be placed on data that could allow direct linking of database information to individual subjects. Additionally, data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

Special precautions: Confidentiality will be protected to the extent possible under existing regulations and laws, but cannot be guaranteed. De-identified results from this study will be posted on cctrials.gov.

21. Conflict of Interest:

a. Distribution of NIH guidelines: The NIH conflict of interest guidelines were distributed to all investigators.

b. Conflict of interest: There are no conflicts of interest to report for NIH investigators. Non-NIH investigators will abide by the conflict of interest policies of their own institutions.

c. Role of a commercial company: There is no commercial company or sponsor for the study.

22. Technology Transfer:

A tech transfer agreement (2018-0101) with SmartMedTek LLC is in place between Dr. Grillon and Dr. Zeffiro.

23. Research and Travel Compensation:

In compliance with federal regulations, active duty service members or other federal employees who participate in this study while on duty may only be compensated for blood sample collection. They will be compensated at a rate of $50 for the single sample collection. Active duty participants will travel on invitational orders. Participants who are non-active duty/non-federal employees (or active duty/federal employees on leave with command approval) will be compensated for research-related discomforts and inconveniences in accordance with NIH guidelines.

b. *Amount of compensation (hourly rate, inconvenience units and maximum for study)*

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.

VOLUNTEER PAYMENT SCHEDULE PER TESTING SESSION

Procedure	Duration	Inconveni-ence units	Total units	Total pay
Testing (fMRI)				
OUTPATIENT Visit				
Time	1.5-2 h	1	4	$40
FMRI scanning incl. shocks	90 min	6	6	$130
TOTAL				$**170**
Testing (MEG)				
OUTPATIENT Visit				
Time	1-1.5 h	1	4	$40
MEG scanning incl. shocks	1-1.5 h	6	6	$60
MRI scan	30-45 min			$70
TOTAL				$**170**

Testing (Alprazolam) – Completed				
OUTPATIENT Visit				
Time	*5-6 h*	*1*	*20*	*$200*
MEG scanning incl. shocks	*1-1.5 h*	*6*	*6*	*$60*
MRI scan	*30-45 min*			*$70*
TOTAL (x2 MEG sessions)				***$590***

Additional compensation may be provided during the "Loss Aversion" testing session. This can amount up to an additional $50.00. Subjects may also be compensated an additional $20 if they complete an extra visit for Telehealth consent instead of in-person consenting.

c. *Travel compensation (append travel form as attachment)*

No travel compensation will be given to participants in this protocol.

24. References:

Ameli R, Ipp C, Grillon C (2001). Contextual fear-potentiated startle conditioning in humans: replication and extension. Psychophysiol, 38, 383-390.

Armony JE, Dolan RJ (2001). Modulation of auditory neural responses by a visual context in human fear conditioning, NeuroReport, 12(15), 3407-3411.

Baas JMP, Böcker KBE, Kenemans JL, Verbaten MN (2002). Threat-induced cortical processing and startle potentiation. *Neuroreport*, 13(1): 133-7

Baas JMP, Grillon C, Böcker KBE, Brack AA, Morgan CA, III, Kenemans JL, Verbaten MN (2002). Benzodiazepines have no anxiolytic effect on cue-specific fear-potentiated startle in humans. Psychopharmacology, 161: 233-247.

Barlow DH (2000). Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. Am Psychol 2000, 55(11): 1247-63.

Beck AT, Steer RA (1987), BDI: Beck Depression Inventory. New York: The Psychological Corporation, Harcourt Brace Jovanovich, Inc.

Beck AT, Epstein N, Brown G, Steer RA (1988). An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol, 56, 893-7.

Benkelfat C, Bradwejn J, Meyer E, Ellenbogen M, Milot S, Gjedde A, Evans A (1995). Functional neuroanatomy of CCK4 –induced anxiety in normal healthy volunteers. Am J Psychiatry, 152(8), 1180-84.

Blair, K., Shaywitz, J., Smith, B., Rhodes, R., Geraci, M., Jones, M., McCaffrey, D., Vythilingam, M., Finger, E., Mondillo, K., Jacobs, M., Charney, D. S., Blair, J. R., Drevets, W.C., & Pine, D. S. (2008). Response to Emotional Expressions in Generalized Social Phobia and Generalized Anxiety Disorder: Evidence for Separate Disorders. American Journal of Psychiatry, 165(9), 1193-1202. doi: 10.1176/appi.ajp.2008.07071060.

Blair, K., Marsh, A. A., Morton, J., Vythilingam, M., Jones, M., Mondillo, K., Pine, D. S., Drevets, W. C., & Blair, J. R. (2006). Choosing the Lesser of Two Evils, the Better of Two Goods: Specifying the Roles of Ventromedial Prefrontal Cortex and Dorsal Anterior Cingulate in Object Choice. The Journal of Neuroscience, 26(44), 11379-11386.

Blanchard, Caroline D., Hynd, A. L., Minke, K. A., Minemoto, T., & Blanchard, R. J. (2001). Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defense patterns of non-human mammals. *Neuroscience & Biobehavioral Reviews*, *25*(7–8), 761–770. https://doi.org/10.1016/S0149-7634(01)00056-2

Blanchard RJ, Yudko EB, Rodgers RJ, Blanchard DC (1993). Defense system psychopharmacology: an etiological approach to the pharmacology of fear and anxiety. Behav Brain Res, 58, 155-165.

Blumenthal, T. D. et al. (2005) Committee report: Guidelines for human startle eyeblink electromyographic studies. Psychophysiology 42, 1–15.

Büchel C, Morris J, Dolan RJ, Friston KJ (1998). Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron, 20, 947-957.

Bush G, Luu P, Posner MI (2000). Cognitive and emotional influences in anterior cingulate cortex. Trends in Cognitive Sciences, 4(6), 215-222.

Charney DS, Drevets WC (2002). Neurobiological basis of anxiety disorders. In Davis, Charney, Coyle, Nemeroff (Eds), Neuropsychopharmacology: The fifth generation of progress. American college of neuropsychopharmacology.

Chernik, D.A., Gillings, D., Laine, H., et al. (1990). Validity and reliability of the Observer's Assessment of Alertness/Sedation scale: study with intravenous midazolam. *Journal of Clinical Psycopharmacology, 10*, 244–251.

Chua P, Krams M, Toni I, Passingham R, Dolan R (1999). A functional anatomy of anticipatory anxiety. NeuroImage 9, 563-571.

Cornwell BR, Garrido MI, Overstreet C, Pine DS, and Grillon C (2017).The Unpredictive Brain Under Threat: A Neurocomputational Account of Anxious Hypervigilance.*Biol Psychiatry.* *82*(6):447-454.

Cox RW, Jesmanowicz A (1999). Real-time 3D image registration for functional MRI. Magnetic Resonance in Medicine, 42:1014-1018.

Davidson RJ, Irwin W (1999). The functional neuroanatomy of emotion and affective style. Trecns Cogn Sci, 3(1):11-21.

Davis M (1998). Are different parts of the amygdala involved in fear versus anxiety?

Davis M (1992). The role of the amygdala in fear and anxiety. Ann Rev Neurosci, 15, 353-375.

De Lissnyder, Derakshan, N., De Raedt, R., & E., Koster, E. H.W. (2010). Depressive symptoms and attentional control in a mixed antisaccade task: specific effects of depressive rumination. Cognition and Emotion, 24, 264–280.

Derakshan, N. & Eysenck, M.W. (2010). Introduction to the special issue: Emotional states, attention, and working memory. Cognition & Emotion 24(2), 189-199.

Der-Avakian A, Markou A (2012). The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci, 35, 68-77.

Disbrow E, Buonocore M, Antognini J, Carstens E, Rowley HA (1998). Somatosensory cortex: a comparison of the response to noxious thermal, mechanical, and electrical stimuli using functional magnetic resonance imaging. Human brain mapping, 6, 150-159.

Drevets WC (2000). Neuroimaging studies of mood disorders. Biol Psychiatry, 48, 813-829.

Drevets WC (1999). Prefrontal cortical-amygdalar metabolism in major depression. Ann NY Acad Sci, 29(877): 614-37.

Drevets WC, Videen TO, Snyder AZ, MacLeod AK, Raichle ME (1994). Regional cerebral bloodflow changes during anticipatory anxiety. Soc Neurosci Abstr, 20(1):368.

Drevets WC, Videen TQ, MacLeod AK, Haller JW, Raichle ME (1992). PET images of blood flow changes during anxiety: correction. Science, 256(5064), 1696.

Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion. 7*, 336-353.

Fales, C. A., Becerril, K. E., Luking, K. R., & Barch, D. M. (2010). Emotional-stimulus processing in trait anxiety is modulated by stimulus valence during neuroimaging of a working-memory task. *Cognition and Emotion*, *24*(2), 1-23.

Filipek P, Richelme C, Kennedy D, Caviness V (1994). The young adult human brain: an MRI-based morphometric analysis. Cereb Bortex, 4, 344-60.

Frijda, N. H. (2009). Emotion experience and its varieties. *Emotion Review*, *1*(3), 264–271.

Garcia R, Vouimba RM, Baudry M, Thompson RF (1999). The amygdala modulates prefrontal cortex activity relative to conditioned fear. Nature, 402, 294-296.

Gonzales-Castillo J, Roopchansingh V, Bandettini PA, Bodurka J (2011). Physiological noise effects on the flip angle selection in BOLD fMRI. NeuroImage 54, 2764-2778.

Gorka, A. X., LaBar, K. S., & Hariri, A. R. (2016). Variability in emotional responsiveness and coping style during active avoidance as a window onto psychological vulnerability to stress. *Physiology & Behavior*, *158*, 90–99. https://doi.org/10.1016/j.physbeh.2016.02.036

Grillon C (2002). Associative learning deficits increase symptoms of anxiety in humans. Biological Psychiatry, 51, 851-858.

Grillon C (1996). Context and startle: effect of explicit and contextual cue conditioning following paired versus unpaired training. Psychophysiology (abstract) 33:S41.

Grillon C, Ameli R (1998): Effects of threat and safety signals on startle during anticipation of aversive shocks, sounds, or airblasts. Journal of Psychophysiology 12, 329-337.

Grillon C, Ameli R, Goddard A, Woods S, Davis M (1994): Baseline and fear-potentiated startle in panic disorder patients. Biological Psychiatry 35:431-439.

Grillon C, Ameli R, Woods SW, Merikangas K, Davis M (1993). Measuring the time-course of anxiety using the fear-potentiated startle reflex. Psychophysiol, 30:340-346

Grillon C, Ameli R, Woods SW, Merikangas K, Davis M (1991): Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. Psychophysiol 28:588-595.

Grillon C, Davis M (1997): Fear-potentiated startle conditioning in humans: Explicit and contextual cue conditioning following paired vs. unpaired training. Psychophysiol, 34, 451-458.

Grillon C, Dierker L, Merikangas KR (1998a): Fear-potentiated startle in adolescents offspring at risk for anxiety disorder. Biol Psychiatry, 44, 990-997.

Grillon C, Merikangas KR, Dierker L, Snidman N, Arriaga RI, Kagan J, Donzella B, Dikel T, Nelson C (1999). Startle potentiation by threat of aversive stimuli and darkness in adolescents: a multi-site study. Int J Psychophysiol, 32(1), 63-73.

Grillon C, Morgan CA, Davis M, Southwick SM (1998b): Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. Biol Psychiatry, 44, 1027-1036.

Grillon C, Dierker L, Snidman N, et al. (1999). Startle potentiation by threat of aversive stimuli and darkness in adolescents: a multi-site study. Int J Psychophysiol 32, 63-76.

Grillon C, Morgan CA (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf war veterans with posttraumatic stress disorder. J Abn Psychol 108, 134-142.

Hariri AR, Bookheimer SY, Mazziotta JC (2000). Modulating emotional responses: effects of a neocortical network on the limbic system. NeuroReport, 11(17), 43-48.

Heimer L, Harlan RE, Alheid GF, Garcia MM, De Olmos J (1997). Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. Neurosci, 76(4), 957-1006.

Helfinstein SM, Fox NA, Pine DS. (2012) Approach-withdrawal and the role of the striatum in the temperament of behavioral inhibition. Dev Psychol. 48, 815-26.

Hembree, R. (1988). Correlates, causes, and treatment of test anxiety. *Review of Educational Research*, 58, 47-77.

Holland PC and Bouton ME (1999). Hippocampus and context in classical conditioning. Curr Opin Neurobiol, 9, 195-202.

Hsu, D. T., Langenecker, S. A., Kennedy, S. E., Zubieta, J.-K., & Heitzeg, M. M. (2010). fMRI BOLD responses to negative stimuli in the prefrontal cortex are dependent on levels of recent negative life stress in major depressive disorder. Psychiatry Research: Neuroimaging, 183(3), 202-208. doi: 10.1016/j.pscychresns.2009.12.002.

Isenberg N, Silbersweig D, Engelien, A, Emmerich S, Malavade K, Beattie B, Leon AC, Stern E (1999). Linguistic threat activates the amygdala. PNAS, 96, 10456-59.

Kalisch, R., Wiech, K., Critchley, H. D., Seymour, B., O'Doherty, J. P., Oakley, D. A., Allen, P., & Dolan, R. J. (2005). Anxiety reduction through detachment: subjective, physiological, and neural effects. *Journal of Cognitive Neuroscience*, 17, 874–883.

Kandel ER (1983). From metapsychology to molecular biology: explorations into the nature of anxiety. Am J Psychiatry, 140(10): 1277-93.

Kelly CB, Cooper SJ (1998). Plasma norepinephrine response to a cold pressort test in subtypes of depressive illness. *Psychiatry Res, 81*, 39-50.

Kim JJ, Fanselow MS (1992): Modality-specific retrograde amnesia of fear. Science 256:675-677.

Krain AL, Gotimer K, Hefton S, Ernst M, Castellanos FX, Pine DS, Milham MP. (2008). A functional magnetic resonance imaging investigation of **uncertainty** in adolescents with anxiety disorders. Biol Psychiatry 63(6):563-8.

LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed trial fMRI study. Neuron, 20, 937-945.

LaBar KS, LeDoux JE, Spencer DD, Phelps EA (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. J Neurosci, 15, 6846-55.

Lang PJ, Davis M, Öhman A (2000). Fear and anxiety: animal models and human cognitive psychophysiology. J Abn Psychol 61, 137-159.

Lang PJ, Ohman A, Vaitl D (1988) The international affective picture system (photographic slides). Center for research in psychophysiology, University of Florida

LeDoux JE (2000). Emotion circuits in the brain. Ann Rev Neurosci, 23, 155-184

Leotti, L. A., & Wager, T. D. (2010). Motivational influences on response inhibition measures. *Journal of Experimental Psychology: Human Perception and Performance*, *36*(2), 430.

Levenson, R. W. (1994). Human emotion: A functional view. *The Nature of Emotion: Fundamental Questions*, 123–126.

Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., et al. (1999). Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. American Journal of Psychiatry, 156, 675–82.

McNair D, Lorr M, Droppleman L (1971). Profile of Mood States. Educational and Industrial Testing Service, San Diego, CA.

Meyer TJ, Miller ML, Metzger RL, Borkovec TD (1990). Development and validation of the Penn State Worry Questionnaire. Behav Res Ther, 28, 487-95

Morgan MA, LeDoux JE (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to acquisition and extinction of conditioned fear in rats. Behav Neurosci 109, 681-688.

Morris JS, Friston KJ, Büchel C, Frith CD, Young AW, Calder AJ et al. (1998). A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain, 121: 47-57.

Odling-Smee FJ (1975). The role of background stimuli during conditioning. Quat J Exp Psychol, 27, 201-209.

Öngür D, Price JL (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys, and humans. Cerebral Cortex, 10, 206-219.

Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M (2001). Activation of the left amygdala to a cognitive representation of fear. Nat Neurosci, 4, 437-41.

Phillips RG, LeDoux JE (1992): Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience, 106, 274-285.

Pine DS, Fyer A, Grun J, Phelps AE, Szeszko PR, Koda V, Li W, Ardekani B, Maguire EA, Burgess N, Bilder RM (2001). Methods for developmental studies of fear conditioning circuitry, Biol Psych, 50, 225-228.

Pine DS, Grun J, Maguire EA, Burgess N, Zarahn E, Koda V, Fyer A, Szeszko PR, Bilder RM (2002). Neurodevelopmental aspects of virtual reality navigation: an fMRI study. NeuroImage, 15, 396-406.

Pine DS, Grun J, Fyer A, Zarahn E, Koda V, Szeszko PR, Ardekani B, Li W, Bilder RJ (2001). Cortical brain regions engaged by masked emotional faces in adolescents and adults: an fMRI study. Emotion, 1, 137-47.

Pujol J, Lopez A, Deus J, Cardoner N, Vallejo J, Capdevilla A, Paus T (2002). Anatomical variability of the anterior cingulated gyrus and basic dimensions of human personality. NeuroImage, 15, 847-855.

Rauch SL, Savage CR, Alpert NM, Fischman AJ, Jenike MA (1997). The functional neuroanatomy of anxiety: A study of three disorders using PET and symptom provocation. Biological Psychiatry, 42, 446-52.

Reilly JP. (1989) Peripheral nerve stimulation by induced electric currents: exposure to time-varying magnetic fields. Med Biol Eng Comput.. 27, 101-110.

Reuter-Lorenz, P. A., & Lustig, C. (2005). Brain aging: reorganizing discoveries about the aging mind. Current Opinion in Neurobiology, 15(2), 245-251.

Richards JM, Plate RC, Ernst M. A systematic review of fMRI reward paradigms used in studies of adolescents vs. adults: the impact of task design and implications for understanding neurodevelopment. Neurosci Biobehav Rev. 2013, Jun;37(5):976-91.

Robinson, O. J., Krimsky, M., & Grillon, C. (2013). The impact of induced anxiety on response inhibition. *Frontiers in Human Neuroscience*, *7*, 69. https://doi.org/10.3389/fnhum.2013.00069

Robinson OJ, Letkiewicz AM, Overstreet C, Ernst M and Grillon C (2011) The effect of induced anxiety on cognition: threat of shock enhances aversive processing in healthy individuals. CABN, in press

Robinson OJ, Charney D, Overstreet C, Vytal K, Grillon C (2011) Salient and relevant: threat of electric shock alters the role of the extended amygdala in emotional face processing in humans, in submission

Robinson OJ, Cools R, Carlisi C, Sahakian BJ, Drevets W (2011) Ventral Striatum Response during Reward and Punishment Based Reversal Learning in Unmedicated Major Depressive Disorder, in submission

Scott-Parker B, Watson B, King MJ, Hyde MK (2012) The influence of sensitivity to **reward** and punishment, propensity for sensation seeking, depression, and **anxiety** on the risky behaviour of novice drivers: a path model. Br J Psychol,103, 248-67.

Shechner T, Britton JC, Pérez-Edgar K, Bar-Haim Y, Ernst M, Fox NA, Leibenluft E, Pine DS (2012). Attention biases, anxiety, and development: toward or away from threats or rewards? Depress Anxiety. 4, 282-94

Seligman MEP, Binik YM (1977). The safety signal hypothesis, in Operant-Pavlovian interactions. Edited by Davis H, Hurwitz HMB. New York, Hillsdale, pp 165-187

Simpson JR, Drevets WC, Snyder AZ, Gusnard DA, Raichle ME (2001). Emotion-induced changes in human medial-prefrontal cortex: II during anticipatory anxiety. PNAS, 98, 688-693.

Spielberger CD: Manual for the State-Trait Anxiety Inventory. Palo Alto, CA, Consulting Psychologist Press, 1983.

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion pro- cessing in anxiety-prone subjects. American Journal of Psychiatry, 164, 318–327.

Theysohn JM, Maderwald S, Kraff O, Moenninghoff C, Ladd ME, Ladd SC (2008) Subjective acceptance of 7 Tesla MRI for human imaging. Magn Reson Mater Phy 21:63–72

Thiel CM, Friston KJ, Dolan RJ (2002). Cholinergic modulation of experience-dependent plasticity in human ausitory cortex. Neuron, Vol. 35, 567-574.

Tom SM, Fox CR, Trepel C, Poldrack RA. (2007). The neural basis of loss aversion in decision-making under risk. Science. 315(5811):515-8.

Velasco M, Gomez J, Blanco M, Rodriguez I (1997). The cold pressor test: pharmacological and therapeutic aspects. *Am J Ther, 4(1),* 34-38.

Verbruggen, F., & Logan, G. D. (2008). Response inhibition in the stop-signal paradigm. Trends in Cognitive Sciences, 12(11), 418–424. https://doi.org/10.1016/j.tics.2008.07.005

Vytal, K, Cornwell, B., Arkin, N., & Grillon, C. (2011). Reciprocal Effects of Anxiety and Cognitive Load on the Acoustic Startle Reflex. Manuscript in preparation.

Waghorn, G, Chant, D., White, P., & Whiteford, H. (2005). Disability, employment and work performance among persons with ICD-10 anxiety disorders. *Australian and New Zealand Journal of Psychiatry, 39,* 55-66.

Walker D, Davis M (1997): Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci, 17, 9375-9383.

Whalen PJ, Rauch CL, Etcoff NL, McInerney SC, Lee MB, Jenike MA (1998). Masked presentations of emotional facial expressions modulateamygdala activity without explicit knowledge. J Neurosci, 18(1):411-18.

Weinberger NM (1995). Retuning the brain by fear conditioning. In Gazzaniga (Ed.), *The cognitive neurosciences*. Cambridge: MIT Press

Williams LM, Phillips ML, Brammer MJ, Skerrett D, Lagopoulos J, Rennie C, Bahramali H, Olivieri G, David AS, Peduto A, Gordon E (2001). Arousal dissociates amygdala and hippocampal fear responses: evidence from simultaneous fMRI and skin conductance recording.Neuroimage,14(5), 1070-9.

Yerkes, R. M., & Dodson, J. D. (1908) The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology, 18*, 459-482.

Zarahn E (2000). Testing for neural responses during temporal components of trials with BOLD fMRI. Neuroimage, 11, 783-796.

Zarahn E, Aguirre G, D'Esposito M (2000). Replication and further studies of neural mechanisms of spatial mnemonic processing in humans. Brain Res Cogn Brain Res, 9, 1-17.

Besnard, A., & Sahay, A. (2016). Adult Hippocampal Neurogenesis, Fear Generalization, and Stress. *Neuropsychopharmacology, 41*(1), 24-44. doi:10.1038/npp.2015.167

Bromley-Brits, K., Deng, Y., & Song, W. (2011). Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. *J Vis Exp*(53). doi:10.3791/2920

Deuker, L., Doeller, C. F., Fell, J., & Axmacher, N. (2014). Human neuroimaging studies on the hippocampal CA3 region - integrating evidence for pattern separation and completion. *Front Cell Neurosci, 8*, 64. doi:10.3389/fncel.2014.00064

Hasler, G., van der Veen, J. W., Grillon, C., Drevets, W. C., & Shen, J. (2010). Effect of acute psychological stress on prefrontal GABA concentration determined by

proton magnetic resonance spectroscopy. *Am J Psychiatry, 167*(10), 1226-1231. doi:10.1176/appi.ajp.2010.09070994

Lang, P. J., Ohman, A., & Vaitl, D. (1988). The international affective picture system (photographic slides). *Center for research in psychophysiology, University of Florida*.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*(5814), 961-966. doi:10.1126/science.1135801

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *Am J Psychiatry, 167*(1), 47-55. doi:10.1176/appi.ajp.2009.09030410

Muthukumaraswamy, S. D., Edden, R. A., Jones, D. K., Swettenham, J. B., & Singh, K. D. (2009). Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to visual stimulation in humans. *Proc Natl Acad Sci U S A, 106*(20), 8356-8361. doi:10.1073/pnas.0900728106

Muthukumaraswamy, S. D., Singh, K. D., Swettenham, J. B., & Jones, D. K. (2010). Visual gamma oscillations and evoked responses: variability, repeatability and structural MRI correlates. *Neuroimage, 49*(4), 3349-3357. doi:10.1016/j.neuroimage.2009.11.045

Niessing, J., Ebisch, B., Schmidt, K. E., Niessing, M., Singer, W., & Galuske, R. A. (2005). Hemodynamic signals correlate tightly with synchronized gamma oscillations. *Science, 309*(5736), 948-951. doi:10.1126/science.1110948

Shen, J., Rothman, D. L., & Brown, P. (2002). In vivo GABA editing using a novel doubly selective multiple quantum filter. *Magn Reson Med, 47*(3), 447-454.

Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia, 51*(12), 2442-2449. doi:10.1016/j.neuropsychologia.2012.12.014

25. Attachments/ Appendices:

An eligibility checklist, recruiting advertisement and screening questionnaire for Patient Recruitment Office has been attached to this protocol via PTMS.

26. Consent Forms:

This protocol includes three Healthy adult volunteer consents (one for fMRI studies and one for MEG studies,) and one Adult Patient consent form. Changes to these consents have been requested and subsequently addressed.