Title page

Cognitive vs. emotional psychopharmacological manipulations of fear vs. anxiety

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Total requested accrual

0 Patients (separately describe patient groups)

300 Volunteers

Project Uses Ionizing Radiation: **x No** ☐ Yes (attach *RSC/RDSC documentation)*

- ☐ Medically-indicated only
- ☐ Research-related only
- ☐ Both

IND/IDE ☒ **No** ☐**Yes** *(attach FDA documentation)*

Drug/Device/#_____

Sponsor: _____

Durable Power of Attorney **x No** ☐ Yes

Multi-institutional Project **x No** ☐ Yes

Institution_____ FWA #_____

Date of IRB approval_____ *(attach IRB documentation)*

Data and Safety Monitoring Board **x No** ☐ Yes

Technology Transfer Agreement ☐No **x Yes**

Agreement type and number: Human DTA 2019-0418

Expiration Date: 09/2022

Confidential Disclosure Agreement **x No** ☐ Yes

Samples are being stored x No **Yes**

Flesch-Kincaid reading level of consent form:

Startle test consent 8.9

Study consent 8.9

fMRI study consent 8.6

- Précis

Objective: The overall aim of this protocol is to examine the effect of pharmacological manipulations of affective and cognitive processes on anxiety and task performance. Ultimately, the goal is 1) to provide insight into the relative influence of cognitive and affective states on anxiety, 2) generate theoretical models that can be applied to a better understanding of the interaction between cognition and emotion, 3) develop a better screening approach to candidate anxiolytics, and 4) help formulate novel therapeutic interventions for clinical anxiety.

Excessive or inappropriately sustained anxiety and fear lead to the most common group of psychiatric disorders. A number of theoretical models have been proposed to understand the mechanisms engaged in these maladaptive behaviors. Most recent emphasis has focused on the synergistic contribution of cognitive and emotional processes. Our laboratory has been instrumental in delineating aspects of behavioral and neural processes that are associated with fear and anxiety, using psychophysiological and neuroimaging measures of fear and anxiety. Evidence shows that levels of anxiety modulate cognitive performance, such as working memory or perceptual discrimination, and that, conversely, cognitive engagement influences severity of experimentally induced anxiety. The exact contribution of emotional processes vs. cognitive processes to the experience of anxiety is not clear, similarly to the neural mechanisms underlying these interactions.

In this protocol, we propose to manipulate pharmacologically separately cognitive and emotional processes to dissociate their contribution to fear/anxiety, while using state-of-the-art measures of anxiety derived from translational work. Indeed, we already developed integrative experimental models of fear and anxiety via the manipulation of predictable and unpredictable shock, respectively. We already employed successfully these models to measure anxiolytic and anxiogenic effects of various compounds such as alprazolam, citalopram, hydrocortisone, and oxytocin in healthy participants.

In the initial version of this Protocol, we proposed in a first step (step-1) to start with a simple proof-of-concept study, using two pharmacological compounds in a double-blind randomized parallel design, each preferentially acting respectively on the cognitive (20 mg, methylphenidate) or affective (propranolol) domain, and using a single cognitive process (working memory). In a second step (step-2), we proposed to extend this work to the fMRI to examine the cognitive correlates of the effects seen in the step-1 behavioral study, specifically with 20 mg methylphenidate. Whereas the comparison among three drugs was planned for the electrophysiology study, we planned to study only the drug that improves cognition in the fMRI. The reason we focused on methylphenidate in step 2 was that our overall goal was to study the effect of improving cognitive functions on anxiety using neuroimaging. To reach this goal, we planned to use different approaches to boost cognitive functions in the coming years, including psychopharmacology, direct current stimulation, mindfulness. Methylphenidate was our first psychopharmacological study towards this objective. Future work will also expand to other compounds and cognitive processes, as well as vary the strategy to induce anxiety. In the initial study, anxiety was induced using the threat of shock, while participants performed the task. We examined in step-1 whether 1) the

reduction of induced-anxiety with propranolol improved cognitive performance, and 2) the facilitation of cognitive performance with methylphenidate reduced induced-anxiety. In step-2, we proposed to identify the neural mechanisms underlying the effects of methylphenidate, the drug having beneficial effects on cognitive function.

The results of the step-1 and step-2 studies were in the expected direction but did not reach statistical significance. We therefore decided to conduct similar studies but with a higher dose of methylphenidate, 60 mg instead of 20 mg. We initially submitted an amendment (AMD 10) that was accepted by IRB to use 60 mg MPH only in the fMRI study (Step-2). However, we now have decided to also use 60 mg MPH in the behavioral study (Step-1). The initial version of the behavioral study used 3 treatments, placebo, MPH, and propranolol. We will drop the propranolol treatment because we did not find any significant effect of this treatment on anxiety, cognition, or the interaction of cognition and anxiety. The objective of the study remains the same.

Study population: Medically and psychiatrically healthy adult males and females, aged 18 to 50 years.

Design: The study is a double-blind design. For each of the two studies (i.e., step-1/ behavioral and step-2/fMRI), two groups of healthy participants will come for one experimental session, following a screening session. During this session, they will be asked to perform a working memory task under the threat of shock, i.e., while anticipating unpleasant electric shocks. Each group will receive one drug challenge, either placebo or methylphenidate (60 mg).

Outcome measures: In step-1, the primary outcome measures are the startle reflex and performance on the working memory task. In step-2, the primary outcome measures are the startle reflex and the cerebral fMRI blood-oxygen-level dependent (BOLD) responses. For both step-1 and step-2, secondary measures include skin conductance, heart rate, and subjective measures of anxiety.

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List of Abbreviations:

dB Decibel
DSM Diagnostic and Statistical Manual of Mental Disorders
ECG Electrocardiogram
MPH Methylphenidate
PLA Placebo
PRO Propranolol

SBP Systolic blood pressure
WM Working memory

1. Introduction

a. Interaction between Anxiety and Cognition

A large literature documents the influence of anxiety on cognition (Eysenck et al., 2007), and conversely the influence of cognition on anxiety (McRae et al., 2009; Van Dillen and Koole, 2007), the latter being exploited for the treatment of anxiety disorders (Clark and Beck, 2010). This interaction suggests that anxiety disorders include pathology in functional networks that mediate emotion and cognition (Sylvester et al., 2012). Indeed, some of the prominent cognitive problems of anxiety are mediated by impaired cognitive function, independent from disruption in affective processes. Anxious individuals complain of being easily distracted and of having difficulty concentrating, while population-based studies report impairments in executive functioning and episodic memory across anxiety disorders (Airaksinen et al., 2005). Consequently, pathological anxiety may not only reflect primary overactive emotional reactivity but also independent cognitive impairment. For example, attentional bias for threat and increased distractibility associated with anxiety could reflect weak top-down attentional control rather than increased bottom-up influence.

It is well-established that anxiety can impair cognition, and that cognitive strategies can be used to reduce anxiety. For example, one effective way to down-regulate anxiety is to immerse oneself in a cognitive task (Vytal et al., 2012). Tasks that engage working memory appear to be especially efficient at reducing anxiety, probably because of competition for attentional resources (Vytal et al., 2012). However, the underlying mechanisms that mediate the interaction between anxiety and cognition are unclear. This protocol aimed at clarifying this question using a pharmacological approach.

As a first step, we will use a threat induction method combined with a working memory (WM) task to test the interaction of anxiety state with WM in healthy adults. Specifically, we propose to employ our well-established, translational, within-subjects state anxiety induction method (induced-anxiety) to collect a physiological measure of anxiety intensity (Davis et al., 2010; Grillon, 2008). This method consists of the induction of anxiety through the application of unpredictable electrical shocks, and the quantification of anxiety level by assessing the startle blink reflex measured using EMG methodology. We also select a WM task, which we have shown to be reliably impaired by induced-anxiety (Vytal et al., 2012; Vytal et al., 2013).

To identify factors that influence the relation between anxiety and cognition, we will attempt to separately manipulate anxiety and cognition using pharmacological agents that modulate preferentially cognitive or affective processes. We will use methylphenidate (MPH) for modulating cognition, and propranolol (PRO) for modulating physiological arousal. *This is a proof-of-concept study that tests two hypotheses:*

1) A treatment, which improves cognitive functioning, can also reduce anxiety.

2) *A treatment, which reduces anxiety symptoms, can also improve cognition*.

b. Effects of Anxiety on cognitive performance

Several studies have shown that anxiety impairs performance on tasks of executive function and working memory (Vytal et al., 2012; Vytal et al., 2013). A general account of the detrimental effect of anxiety on cognitive performance is competition for resources (Eysenck et al., 2007). Specifically, the performance on a cognitive task, together with the experience of being anxious, is equivalent to being engaged in a dual task, i.e., anxiety-related processes act as a secondary task that take up resources away from the primary cognitive task. *This protocol seeks to better understand the nature of the anxiety-related processes responsible for interfering with cognitive performance*.

It is well-established that anxiety is not a homogeneous process. At least two components of anxiety have been traditionally recognized one is cognitive and the other is physiological. The cognitive component, also termed anxious apprehension, involves worry and anxiety-related thoughts. The physiological component, also termed anxious arousal, includes interoceptive feeling state, tension, and nervousness. So far, the cognitive component has been assumed to play a key role because worries and anxious thoughts are believed to take up working memory resources (Eysenck et al., 2007). However, it is also likely that cognitive performance is also affected by the distracting influence of anxious arousal. Indeed, drugs that reduce anxious arousal, such as the beta-blocker PRO, are used to treat performance or test anxiety (i.e., public speaking, stage fright) (see below).

According to this bimodal model, the manipulation of either the cognitive component or the physiological component of anxiety could reduce interference of anxiety on ongoing activity (i.e., by reducing anxiety or improving attention). In this protocol we will examine whether psychopharmacologically reducing the physiological component of anxiety improves cognitive performance. We hypothesize that pharmacological blockade of physiological signs of anxiety with the beta-blocker PRO will reduce the detrimental effect of anxiety on WM performance. Since it is not possible to rule out potential direct cognitive effects of PRO, we adopt a design that will permit to measure such potential effects and control for them statistically using ANOVAs. Because of the specific effect of PRO on test-anxiety, and the unclear mode of action of this drug, the Step-2 will not test this drug in the fMRI.

c. Effects of Cognition on Anxiety (Step-1 and Step-2)

The effects of cognition on anxiety generate substantial interest because of their potential therapeutic benefits (Clark and Beck, 2010; Robinson et al., 2013). Several studies have shown that anxiety and threat-related physiological effects can be down-regulated by the use of various cognitive strategies such as self-distraction, reappraisal, or WM load (Dvorak-Bertsch et al., 2007; Kalisch et al., 2006; King and Schaefer, 2011). Specifically, this effect appears to have the greatest impact when the task involves high cognitive (King and Schaefer, 2011) or perceptual load (Doallo et al., 2006). Moreover, therapeutic techniques based on this assumption (e.g., cognitive behavioral therapy, CBT)

are highly effective in reducing anxiety in patient populations (Chambless and Gillis, 1993). These findings suggest that when attentional demands are high, task performance takes precedence over anxiety-related processes (e.g., worry, interference from physiological arousal). Therefore, engaging in a WM task can successfully relieve anxiety. However, there is large individual variability in the ability to engage task-related WM processes when anxious. Specifically, attention control mechanisms play a key role in the ability to stay on task and screen out anxiety-related processes. In fact, poor attention control has been proposed to be a vulnerability factor for anxiety disorders (Bishop, 2009). This raises the possibility that improving attention-control pharmacologically could not only minimize the detrimental effect of anxiety on performance, but could also minimize attention to anxiety-related processes. This leads to the hypothesis that MPH may help alleviate anxiety during task performance under threat. Since it is not possible to rule out potential direct anxiolytic effects of MPH (see section Page 10, MPH and anxiety), we adopt a design that will permit to measure such potential effects and control for them statistically using ANOVAs. Because of the clear effects of MPH on cognition in general, and the well-known mechanism of action of this drug, we plan to test it in the fMRI in our Step-2 study.

d. Previous work: Effect of induced-anxiety on n-back WM tasks

Working memory refers to a temporary storage system that can be used to encode, rehearse and manipulate information in mind (Postle, 2006). One of the most commonly used WM tasks is the n-back paradigm (where subjects respond to successive stimuli based on whether they match the stimulus 1, 2, or 3 trials back etc.), because cognitive load or task difficulty can be parametrically modulated. Findings regarding the effects of anxiety on WM consistently report performance impairment (Eysenck et al., 2007). However, little is known about the effect of performing a WM task on anxiety.

We have examined the effect of induced-anxiety evoked by threat of shock on n-back WM in several studies (Vytal et al., 2012; Vytal et al., 2013). Anxiety was probed by examining startle reactivity to brief and loud sounds. Startle is a well-established translational measure of anxiety and is reliably increased (fear-potentiated startle) by anticipation of aversive stimuli such as shock (Davis et al., 2010).

We found that induced-anxiety impaired performance in the low load WM tasks (1- and 2-back) but not in the high load WM task (3-back) (Fig. 1). In addition, as expected, induced-anxiety increased the magnitude of startle, a phenomenon termed *fear-potentiated startle.* Most notably, the fear-potentiated startle was reduced linearly as WM load increased (Fig. 2). In other words, as task difficulty increased, anxiety decreased. These findings are consistent with the hypothesis that under low load, task performance does not take up all processing resources. Remaining resources are then available to process threat-related stimuli, including worry and physiological arousal. However, when these resources are depleted by high-load working memory tasks, anxious apprehension and anxious arousal has no workspace with which to operate, and their effects on performance are eliminated.

These results illustrate the interaction between anxiety and cognition and provide support for the two following predictions regarding the effect of PRO and MPH.

- First, one would expect that reducing anxious arousal with PRO should reduce fear-potentiated startle during induced-anxiety regardless of whether subjects are involved in a task or not. Importantly, reducing fear-potentiated startle (i.e., reducing anxiety) during induced-anxiety should also improve WM performance.
- Second, improving attention control with MPH during induced-anxiety should help subjects focus on the task and ignore threat-related processes. This should reduce anxiety (fear-potentiated startle) during task performance and minimize the detrimental effect of anxiety on performance.

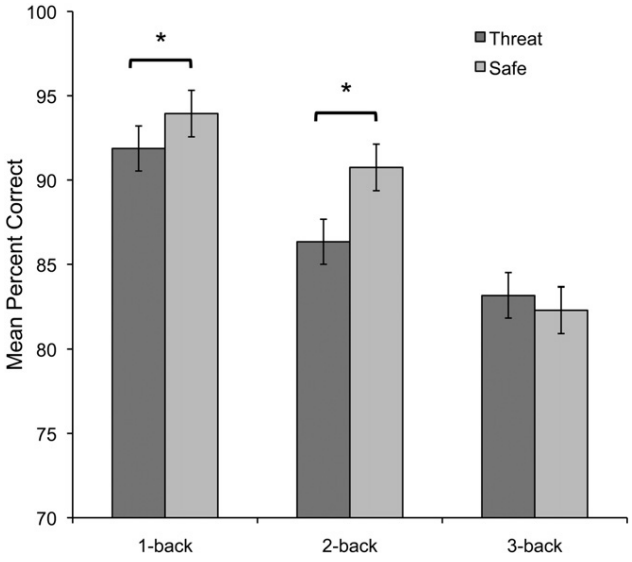

Fig. 1: N-back performance during threat and safe: performance was impaired during threat vs. safe in low-load task, but not in the high load task (Vytal et al., 2012).

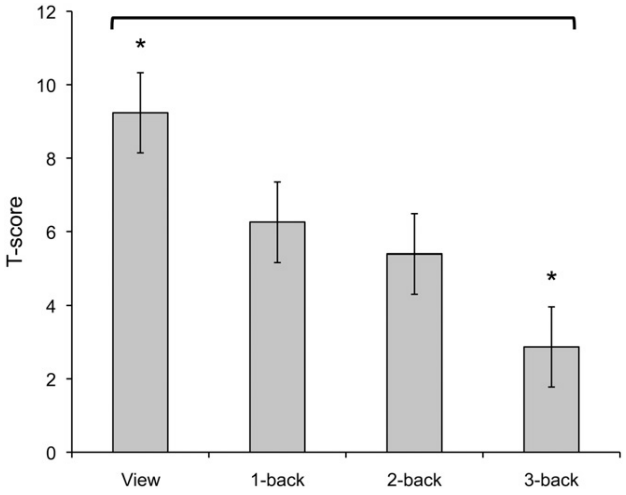

Fig. 2: Fear-potentiated startle (i.e., difference in startle reactivity between the threat and safe conditions) during the view condition (no task) and during the n-back task (Vytal et al., 2012)

Effects of MPH on Cognition and Anxiety

Methylphenidate primarily acts by blocking the dopamine transporter (DAT), which removes excessive dopamine from the synaptic cleft, resulting in increases of extracellular dopamine levels (Volkow et al., 2002). It also blocks norepinephrine transporter (NET) but is viewed more generally as an indirect dopamine agonist.
It is the first-line medication treatment of attention deficit hyperactivity disorder (ADHD). In this clinical context, MPH decreases symptoms of inattention and hyperactivity (Faraone and Buitelaar, 2010).

MPH on Working Memory:
As reviewed below, most of the experimental and clinical evidence supports a beneficial effect of MPH on working memory.
Generally, MPH has been shown to enhance the efficiency of cognitive processing (Swanson et al., 2011; Tomasi et al., 2011; Volkow et al., 2008). A large body of work evidences MPH positive effects on attention tasks (see review (Swanson et al., 2011). The literature on the effects of single dose MPH on working memory is scarcer and mostly involved treatment of children and adolescents with ADHD. However, findings tend to evidence improved performance (see reviews (Swanson et al., 2011). For example, MPH single dose (for children >25kg, 3 single MPH doses 10 mg, 15 mg, and 20 mg) improved auditory–verbal WM. Of note, this study was conducted in ADHD children with and without anxiety. MPH-related WM performance improvement was not seen in the comorbid ADHD-anxiety group (Bedard and Tannock, 2008). A similar finding was reported in ADHD children with and without anxiety receiving single MPH doses of 0.3, 0.6 and 0.9 mg/kg (Tannock et al., 1995). Because of the dearth of studies examining the MPH effects on WM in clinical anxiety, or even high anxiety state, it is difficult to interpret the role of comorbid anxiety in ADHD children as decreasing the efficacy of stimulants on WM improvement. One study in healthy adults reported that a single 40mg MPH dose improved performance on a visual–spatial WM task, particularly in those adults with lower baseline WM performance (Mehta et al., 2000a). Taken together, these data suggest a beneficial effect of MPH on working memory, but this effect may be dependent on individual factors, including baseline WM capacity and clinical anxiety.

MPH on Anxiety:
As reviewed below, there is a dearth of studies of the effect of MPH on anxiety. The few studies reviewed below suggest that MPH might have some anti-anxiety effects. However, these studies in humans are weak and are confounded by the ADHD status of the participants. From a clinical perspective, MPH is not used to treat anxiety, and there is no consensus that MPH would influence anxiety in patients with ADHD who are treated with MPH. Here, we will be able to test the direct effect of MPH on anxiety using the fear potentiated startle measure in the absence of WM.

Animal models suggest that MPH at 3mg/kg may have anti-anxiety effects (Zhu et al., 2010). Similarly, MPH seems to reduce state anxiety in ADHD patients clinically treated with MPH (e.g., (Barrickman et al., 1995; Bouffard et al., 2003). However, this effect may be secondary to improved cognition. To our knowledge, no human studies have examined MPH single doses on anxiety, not only clinical anxiety, but also anxiety state. Accordingly, no studies have assessed MPH effects on startle reflex or fear-potentiated startle reflex. The closest works concern pre-pulse inhibition or exposure to positive and negative emotionally evocative distractors. The findings are confounded by the clinical population (ADHD) and the use of emotionally salient distractors (Ashare et al., 2010). The latest study reported normalization of startle response to these evocative stimuli after MPH treatment in ADHD adults.

Based on this literature review, we predict that single MPH dose compared to placebo will improve WM memory performance in a safe condition, but even more so in the induced-anxiety condition by reducing attention to the threat context in favor of performance on the task (Fig. 3). In addition, this will lead to reduced anxiety state during the threat context in the MPH vs. placebo condition (Fig. 4).

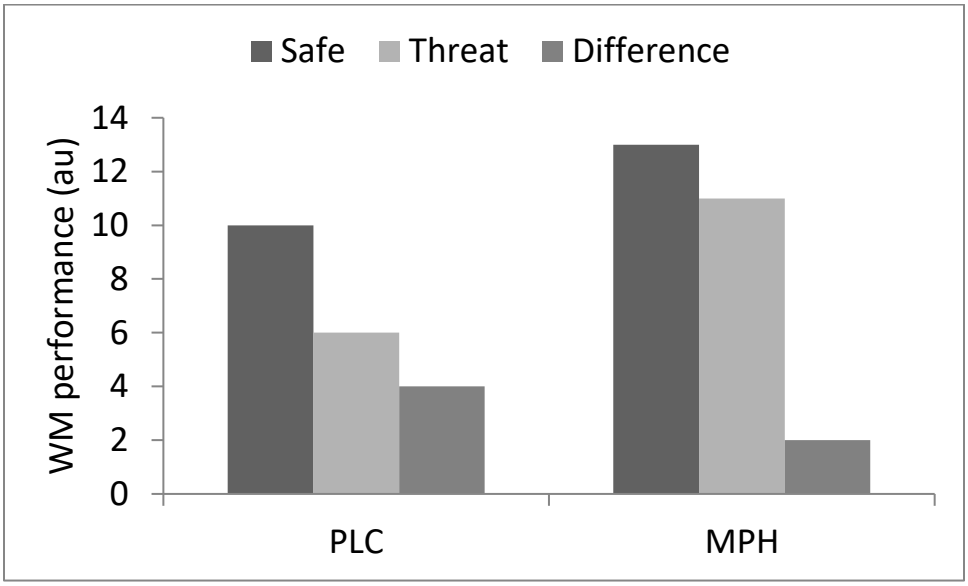

Fig. 3: Predicted effects of MPH on WM (arbitrary units, au) working memory performance compared to placebo in a safe and threat context (y axis represents WM accuracy, higher number representing better performance). MPH is expected to reduce the detrimental effect of induced-anxiety on performance. (Difference is the difference score threat minus safe).

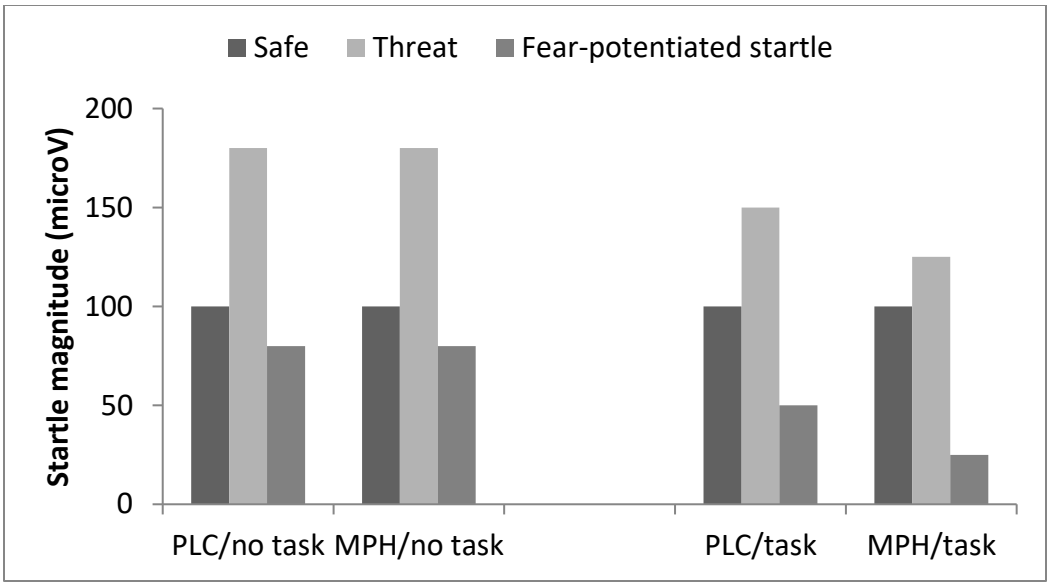

Fig. 4: Predicted effects of MPH on fear-potentiated startle (difference score threat minus safe) compared to placebo in the absence of task and during the low load WM task. Performing the task is expected to reduce the magnitude of fear-potentiated startle. MPH is expected to 1) have no or minimal effect on startle reactivity or fear-potentiated startle in the no task condition but 2) reduce fear-potentiated startle in the WM task condition.

Effects of PRO on Cognition and Anxiety

Propranolol hydrochloride (PRO) is a synthetic beta-adrenergic receptor-blocking that crosses the blood-brain barrier. It is primarily used for its peripheral effects in the treatment of hypertension, cardiac arrhythmia, essential tremor and migraine (Gleiter and Deckert 1996 pharmacopsychiat). It is also a first line of treatment of stage fright (James et al., 1978; Neftel et al., 1982), test anxiety (Faigel, 1991) and post-traumatic stress disorder (Pitman et al., 2002; Reist et al., 2001; Taylor and Cahill, 2002). Whether PRO also improves cognitive performance in tandem with reducing stage fright is unknown (e.g., (Beversdorf et al., 2002)).

PRO on WM:

Overall, the review below indicates the absence of or the potential for weak negative effects of PRO on WM. We will be able to test this cognitive effect by assessing the effect of PRO on WM in the safe condition.

A few studies of acute administration of single doses of PRO suggest impaired memory performance in healthy adults. A 25mg PRO single dose administered to young healthy volunteers (16 subjects per drug condition) has been shown to slow reaction time in a numerical working memory task (Müller et al., 2005). This was particularly true for individuals with low state anxiety rating at baseline. The trend for a preferential slowing of manipulation times in less anxious subjects by PRO is in accordance with an inverted U-model suggested for performance on working memory (Kimberg et al., 1997; Mattay et al., 2000) and task-related brain activity (Mattay et al., 2003). It can be assumed that

less anxious subjects have lower endogenous noradrenaline levels and only this group's performance will fall under the left threshold after PRO, whereas the more anxious subject have higher noradrenaline levels and their performance stays within the normal range, even after PRO treatment. Consistent with the results of Muller et al's study, PRO affected cognitive functions positively in high-anxiety subjects and negatively in low-anxiety subjects, when subjects were required to give a brief speech (Hartley et al., 1983).

For completeness, a body of literature describes the influence of PRO on the memory of emotional stimuli, the most consistent effect being the blockage of the memory advantage conferred by the emotional load associated with stimuli (Cahill et al., 1994; Reist et al., 2001). Conversely, PRO fails to show any effect on memory of neutral material. This selective negative effect on memory for emotionally-laden stimuli has been ascribed to the blockade of the peripheral somatovegetative feedback to the CNS, as described by the 'somatic-marker hypothesis' (Bechara et al., 2005). However, PRO's central effects on beta adrenergic receptors within the prefrontal cortex cannot be excluded.

In summary, findings with acute PRO administration in humans at doses of 25 mg to 180 mg report most consistently either no effects or negative effects on memory performance. No studies have yet examined how the blockade of somatovegetative responses to fear/anxiety affects working memory. However, PRO improves WM performance in the presence of negative distractors (unpleasant pictures), an effect that is partially mediated by cortisol and that has been attributed to a lessening of the arousal component of the emotional distractors (Oei et al., 2010). Based on the hypothesis of the "distracting" role of vegetative inputs and physiological arousal, we expect improved working memory during stress under PRO.

PRO on anxiety:
The studies reviewed below evidence the efficacy of PRO in reducing the physiological markers of anxiety (e.g,, during stage fright), and, in turn, mitigate the negative emotional response to threat situations or stimuli. There are no controlled studies showing increased anxiety under PRO.
As already mentioned, PRO is currently used to treat test-anxiety (Faigel, 1991) and acute stage fright (Brantigan et al., 1979). Propanolol reduces the physiological correlates of anxiety (i.e., anxious arousal), including startle EMG (Morgan et al., 1993), by blocking changes in anxiety-related autonomic reactivity. Accordingly, the beneficial effects on acute anxiety have been related mainly to PRO's peripheral action that blocks somatovegetative changes associated with stress, i.e., palpitations, sweating, shortness of breath. However, it is not clear whether this blockade of anxiety-related physiological arousal is solely based on peripheral effects. Of note, PRO is much less efficient in affecting the cognitive component of anxiety (worry) (Gottschalk et al., 1974; Papadopoulos et al., 2010), perhaps suggesting that its main effects on anxiety are not centrally mediated. Accordingly, we expect reduced fear-potentiated startle and subjective response to threat under propanol.

Based on this literature review, we predict that a PRO single dose administration, compared to placebo, will not (or minimally) affect WM memory performance in a safe

condition, but will block or reduce the negative effect of threat in the induced-anxiety condition (Fig. 5). In addition, PRO, compared with PLA, will reduce startle in the threat condition (Fig. 6).

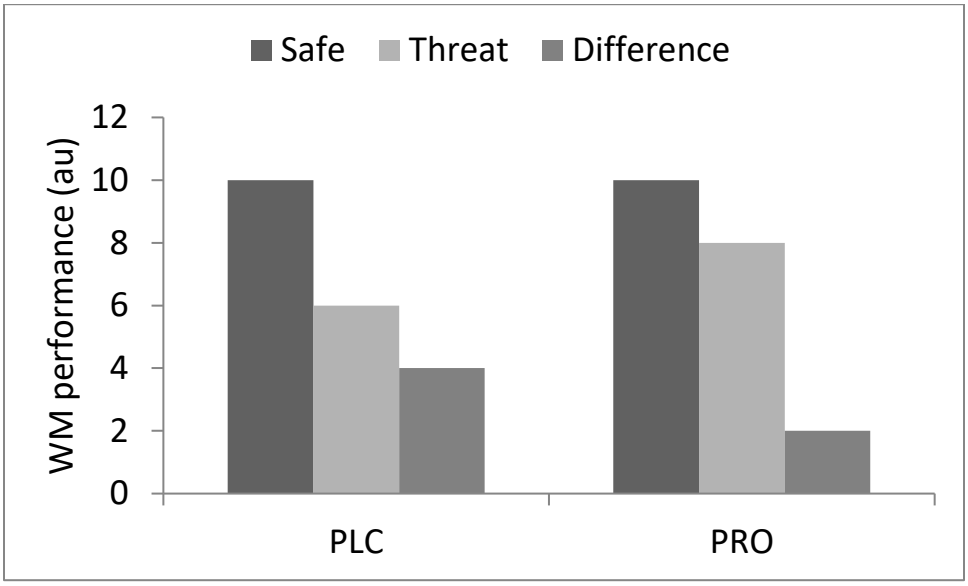

Fig. 5: Predicted effects of PRO on working memory performance compared to placebo in a safe and threat context (y axis represents WM accuracy, higher number representing better performance). PRO is expected 1) to have no or minimal effect on performance in the safe condition, but 2) to reduce the detrimental effect of induced-anxiety in the threat condition. (Difference is the difference score threat minus safe).

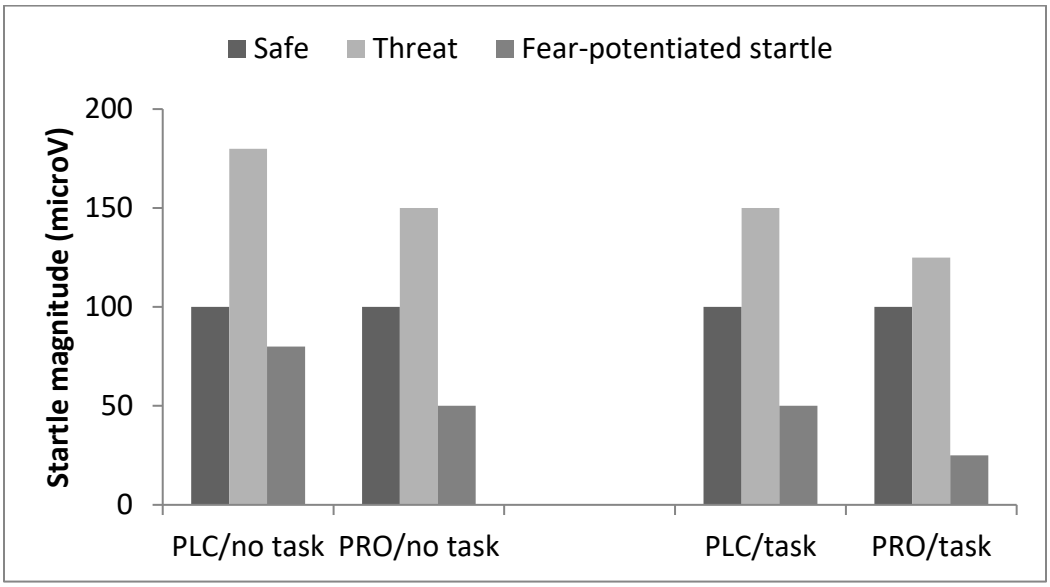

Fig. 6: Predicted effects of PRO on fear-potentiated startle (difference score threat minus safe) compared to placebo in the absence of task and during the low load WM task. Performing the task is expected to reduce the magnitude of fear-potentiated startle. PRO is expected to reduce fear-potentiated startle in the no task and the task conditions.

The 40 mg single dose propranolol did not have any significant effects on either measures of anxiety or cognitive performance variables when compared to placebo in step-1 of this study. Given the lack of significant findings, the propranolol portion of the study is now complete and will not be included in the step-2 fMRI study.

In step-2 fMRI, the single dose of methylphenidate 20 mg did not produce anticipated physiological effects (i.e. increased heart rate), nor changes in neural activity that survived correction for multiple-comparisons. A recent study by Schmidt et al. noted that methyplphenidate (60 mg) enhanced cognitive performance more than other tested stimulants (Schmidt et al 2017). Thus, a follow-up substudy to the step-2 fMRI will test neural activity on a higher dose (60mg). The follow-up study will continue to test the initial aims and hypotheses of the step-2 fMRI study.

Results of the 1st version of the Protocol with 20 mg MPH

Results of the behavioral study have been published (Ernst, M., et al. 2016). "The effects of methylphenidate and propranolol on the interplay between induced-anxiety and working memory." Psychopharmacol **233**: 3565-3574.). Findings were twofold: (1) Methylphenidate blocked anxiety interference only on the 3-backWM performance, while propranolol and placebo had no effects on anxiety-WM interference, and (2) contrary to hypothesis methylphenidate did not reduce anxiety-potentiated startle. The fMRI data are still being analyzed but there is no clear effect.

2. Study objectives

The objectives of the study are to examine the effects of single dose administration of MPH on (1) induced-anxiety (measured with the startle reflex) evoked by the threat of shock, (2) performance on working memory, and (3) the cerebral fMRI blood-oxygen-level dependent (BOLD) responses.

We will test the hypotheses (see also Figures above) that:

- Methylphenidate
    - Prevents or minimizes the impairment in WM caused by the threat of shock
    - Does not affect anxiety evoked by the threat of shock per se (threat of shock at rest), but enhances the anxiety-reducing effect of performing a task (threat of shock while performing a WM task)

3. Subjects

a) Description of study population:

The subjects will be healthy adult male and female volunteers ages 18-50. There will be 2 groups, MPH, and placebo (PLA) for step 1 behavioral study and step-2 fMRI study. The target number of completers for the step-1 behavioral study will be N=28 per group for a total of 56 completers. Subjects who drop out will be replaced. We anticipate that about 15%-20% of subjects (N=10) will either drop out from the study or will have poor data due to low startle reactivity or problem with the recording of the data. The total accrual number for step-1 will be 66 (see power analysis).

For step-2, there will be two groups: MPH and PLA. The target number of completers for the fMRI study will be N=30 per group for a total of 60 completers. Subjects who drop out will be replaced. We anticipate that about 25%-30% of subjects (N=15) will either drop out from the study or will have poor data due to low startle reactivity or problem with the recording of the data. As a result the total accrual number for step-2 will be 75 subjects (see power analysis). The total accrual for the follow-up step-2 fMRI study will also be 75 subjects with a target of 60 completers (n = 30 per group).

The accrual ceiling will be 300 healthy volunteers.

NIH, but not NIMH, employees may participate. NIH employee participation is guided by intramural institute policy.

b) Inclusion criteria

- Ages 18-50
- Males and females
- Subjects give their own consent

c) Exclusion criteria

- Clinically significant prior exposure to medications, that based on the investigator's judgment, may impact the study, such as Ritalin (MPH).
- Any significant medical or neurological problems (e.g. cardiovascular illness, respiratory illness, neurologic illness, seizure, etc.)
- Raynaud syndrome
- IQ < 80
- Sinus bradycardia (P<45), or tachycardia (P>90)
- Significant ECG abnormality (i.e., greater than first-degree block etc.) as determined by investigators judgment
- High or low blood pressure (SBP>140 or SBP<90; SDP<50 or SDP>90)
- A first-degree family history of mania, schizophrenia, or other psychoses based on verbal reports
- Significant past psychopathology (e.g., hospitalization for psychiatric disorders, recurrent depression, suicide attempt, psychoses)

- Current psychiatric disorders according to DSM-V
- Current alcohol or substance use disorder
- History of moderate or severe alcohol or substance use disorder
- Current use of psychotropic medication
- Impaired hearing
- Pregnancy or positive pregnancy test
- Neurological syndrome of the wrist (e.g., carpal tunnel syndrome) for shocks to be delivered on affected arm.
- Breastfeeding
- Significant lab abnormalities (i.e., CBC with differential, acute care and mineral panel, hepatic panel, thyroid panel)
- Positive urine toxicology screen
- You have been in another study with an experimental medication within the previous month
- For physiological/clinic participants: Small startle reactivity (a change in EMG activity that is less than 3 times the baseline EMG activity)
- Current use of anti-acid medications
- Employee of NIMH or an immediate family member who is a NIMH employee.
- For fMRI participants: Any medical condition that increases risk for fMRI:
  - Any metal implants (clips, screws, plates, pins, etc) or metal fragments cause by injuries or metal working
  - Any sort of medical implants (aneurysm clips, pacemaker, insulin pump, Hickman line, etc.)
  - Permanent eye liner and tattoos above the neck
  - Patients who have difficulty lying flat on their back for up to 90 min in the scanner
  - Participants who are uncomfortable in small closed spaces (have claustrophobia) and would feel uncomfortable in the MRI machine

4. Study Design and Methods

a) Study overview

This protocol will examine the effect of PLA and MPH in step-1 psychophysiological study and step-2 fMRI study on WM during experimentally-induced anxiety in a double-blind between-subject design. The study involves 1 visit lasting up to 6 hours. The increased dose of MPH follow-up psychophysiological and fMRI studies will be conducted similarly to the initial studies.

First, we will measure individual startle responses for the psychophysiological study only. We do not use auditory startle as a threat condition for the fMRI study due to the noise of the fMRI scanner. Because there is a large inter-individual variability in startle response, and some individuals do not show reliable startle responses we will complete an auditory startle test prior to the study tasks. We need to make sure that we study only

individuals who show reliable startle response. Thus, the criterion to be eligible for the psychophysiological drug study is a measurable startle response for all 9 startles used during the startle test visit. A measurable startle response should be 3 times the baseline EMG activity.
If participants passed the startle test (all 9 measurable startle responses) they will move on to the drug portion of the visit.

Participants will be assigned to one of two treatments, 1) PLA, or 2) 60 mg MPH. For participants in the behavioral study, they will complete study tasks in the outpatient clinic. For participants in the fMRI substudies, they may be instructed to lay in the fMRI machine for up to 90 minutes while completing the study tasks. For both the behavioral substudies and fMRI substudies, the tasks will consist of performing a working memory task under safe conditions and under threat conditions (anticipation of shock). Startle will be used to examine subjects' anxiety level.

b) Recruitment methods

All recruitment materials (paper or web ads, flyers and listserv announcements) will be IRB approved prior to use.

- Advertisements will be placed in publications such as university newspapers, city newspapers or magazines and local gazettes. We will also post flyers on the NIH campus as well in community sites such as local eateries, small businesses, and public libraries.
- Many universities are now paperless, and we may post web ads on their electronic newsletters. We may advertise on local buses. We will use the Public Relations and Public Liaison (PRPL) list of volunteers. Lastly, recruitment efforts will include listservs provided by PRPL as appropriate for our target audience. An example is the Club PCR site which is part of the Recreation and Welfare (R&W) Association targeting young scientists on the NIH campus.
- 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 also 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.
- We may also identify potential healthy volunteers through the NIMH protocol titled "Recruitment and Characterization of Research Volunteers for NIMH Intramural Studies."

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

c) Screening methods

There are three steps before participants are able to consent to the drug study.
1- Phone Screening: There will be an *initial phone screening* to explain the study and exclude subjects meeting exclusion criteria. Individuals who appear to qualify for inclusion will be invited for the second step, the Medical and Psychiatric Screening.
2- Protocol 01-M-0254 screening (Medical and Psychiatric Screening): This screening comprises a psychiatric interview (SCID), a physical exam, questionnaires and computer tasks, all covered under *protocol 01-M-0254.* Participants who are cleared to continue are invited to the third step, the Startle test.

d) Drug Study design & procedure

Subjects will participate in a single testing session in our laboratory in the Clinical Center. The scans will be performed in the NMR center. In step-1, they will be randomly assigned to either PLA or MPH (60 mg) to achieve 2 groups of 28 completers each (see section ii. *Description of the various components*). In step-2, they will be assigned to either PLA or MPH (60 mg), and each group will include 30 completers. In follow-up studies to the step-1 behavioral and step-2 fMRI, the subjects will take either PLA or 60mg of MPH. Each group will include 30 completers for both follow-up studies. The design is double-blind, i.e., neither the participant nor the investigators will know what drug the subject is taking.

At the start of the study visit for the follow-up behavioral study, each participant will have a startle test. The participants are tested 9 times with the startle procedure. They need to have a measurable startle for each of the 9 measurements to be continue onto the study itself. A measureable eyeblink response is defined as a change in EMG activity that is 3 times baseline EMG activity calculated as the average of the 20 ms that precedes the delivery of the startle stimulus. EMG values below this level will be considered as no startle response. The startle test will not be performed for the follow-up fMRI study.

Participants will also receive electric shocks to try out the shocks. They will be given 3-5 sample shocks to determine the level of shock for the experiments.

The timeline is presented first, and the various procedures are detailed afterwards.

i. Study visit

- Subject signs the study consent
- Urine pregnancy for females
- Completes questionnaires to qualify cognitive styles (i.e., attention control, BRIEF)
- Self-administered questionnaires
    - Spielberger state anxiety #1
    - Mood Rating Scale #1
- Blood pressure, heart rate, VAS #1
- Placement of the recording electrodes
- Assessment of baseline startle #1
- **Drug administration at time 0 min**
- Self-administered questionnaires (~80 min after ingestion)
    - Spielberger state anxiety #2
    - Mood Rating Scale #2
- Blood pressure, heart rate, VAS #2
- Assessment of baseline startle # 2
- **Threat of shock experiment (~90 min post drug administration)**
- Placement of shock electrodes
- Shock work up procedure
- Instructions
- WM under shock threat / safety block 1
- WM under shock threat / safety block 2
- Blood pressure, heart rate and VAS #3
- Questionnaires including a debriefing of the experiment
- End of experiment; recording of side effects and release of subjects by a nurse or MD.

ii. fMRI study visit

- Subject signs the study consent
- Urine pregnancy test for women
- Completes questionnaires to qualify cognitive styles (i.e., attention control, BRIEF)
- Self-administered questionnaires
    - Spielberger state anxiety #1
    - Mood Rating Scale #1

- Blood pressure, heart rate, VAS #1
- Placement of the recording electrodes
- Assessment of baseline startle #1
- Drug administration at time 0 min
- Self-administered questionnaires (~80 min after ingestion)
    - Spielberger state anxiety #2
    - Mood Rating Scale #2
- Blood pressure, heart rate, VAS #2
- Assessment of baseline startle # 2
- Subject enters the fMRI machine
- T12 Threat of shock experiment (~90 min post drug administration)
    - Placement of shock electrodes
    - Shock work up procedure
    - Instructions
    - Resting State task
    - WM under shock threat / safety block 1
    - WM under shock threat / safety block 2
- Subject exits the fMRI machine
- Blood pressure, heart rate and VAS #3
- Questionnaires including a debriefing of the experiment
- End of experiment; recording of side effects and release of subjects by a nurse or MD.

ii. Description of the various components

Psychopharmacological challenges:

This is a double-blind study. Neither the participant nor the investigators will know what drug the subject is taking. The blinding is done by the pharmacy. Both compounds will be placed in capsules of similar appearance. The NIH pharmacy will randomize the administration of drugs across subjects.

Dose, Timing and Side Effects
MPH
A 60 mg oral dose is selected because of its documented effectiveness in improving cognitive efficiency in adults. This dose complies with the maximum FDA approved daily dose, and single dose MPH studies have used 60 mg without any serious adverse events (e.g., Schmidt et al 2017, Hysek et al 2014).

A delay of 90 minutes between dose administration and cognitive testing will be used to optimize MPH effects, based on MPH pharmacokinetics. Peak plasma levels of methylphenidate are reached after 1 to 2 hours, and plasma half-life is 2 to 4 hours (Gualtieri et al., 1982).

The most common side effects associated with Ritalin are rapid heart rate, palpitations, nervousness, restlessness, insomnia, dry mouth, constipation, nausea, diarrhea, loss of appetite, headache, irritability, and elevation of blood pressure. MPH cardiovascular and subjective effects will be monitored regularly. Cardiovascular measures will include heart rate and blood pressure, which will be recorded prior to drug administration, and then every ½ hour. Subjective ratings will be collected prior to drug administration, prior to cognitive testing, and at the end of the study, and they will consist of ratings on visual analogue scales (Bond and Lader, 1974).

Design
A between-group repeated measures design will be used. Subjects will be assigned randomly, in double blind to one of three drugs (PLA or MPH) in step-1 and in step-2. The NIH pharmacy will establish the random order within females and males.

fMRI: The study may be done using Magnetic Resonance Imaging (MRI). MRI is painless and safe. Participants with any magnetic metal implants (such as pacemakers) will be excluded. The participant will lie on a table inside the scanner for up to 90 minutes while doing the task. During the scan, the participant may be asked to lie still for up to 90 minutes at a time. While in the scanner the participant will hear loud knocking noises and will be fitted with earplugs or earmuffs to muffle the sound. The participant will be able to communicate with the MRI staff at all times during the scan and may ask to be moved out of the machine at any time.

Resting State Procedure: In the fMRI scanner, subjects may be asked to lie still with their eyes open and stare at a fixation cross for 10 minutes. They will be asked to remain as still as possible and not fall asleep.

Psychophysiological procedures and threat of shock

- Startle stimulus & response

The startle reflex will be elicited with a 102 dB white noise (40-ms duration) delivered via headphone. The eyeblink component of the startle reflex will be recorded binaurally with two AgCl electrodes placed under one eye. Eyeblink responses will be scored in the 20-120 ms window following the onset of the startle stimulus. This is included in the behavioral study only. The auditory startle will not be done in the fMRI scanner.

- Autonomic measures

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

- Electric shock

The shocks will be delivered through two disk electrodes located on fingers, arm, or wrist. The shock will have an intensity between 1.0-5 mA and duration of 100-300 ms. Shock will be delivered at a level that is judged by the subject as unpleasant but not painful. Study shock levels will be determined during participant screening prior to the first experimental session.In very rare occasions, subjects have experienced symptoms that may be related to the shock. A participant with a condition called “cubital tunnel syndrome,” a repetitive motion injury similar to carpal tunnel syndrome, indicated

worsening of his syndrome over the months subsequent to his participation. Another participant reported pain in her arms for several hours after testing. The pain was no longer present the next day. It is unclear whether these symptoms were due to the shocks. Nevertheless, subjects with neurological symptoms of the wrist and arms will be excluded from the study.

The experiment will consist of alternating periods (e.g., 1-3 min) of safety and shock threat conditions during which subjects will perform the N-back WM task.

N-Back WM task
Stimuli are presented one at a time on a screen. Participants are instructed to remember one, two, or three stimuli back from the current stimulus on the screen as we have done in the past (Vytal et al., 2012).

Questionnaires:
We will use various questionnaires to assess the subjective effect of treatment on mood, anxiety, and sedation, and the perceived aversiveness of the shocks during testing using visual analog scales. These include State-Trait Anxiety Inventory (Spielberger, 1983) and the Mood Rating Scale (Bond and Lader, 1974).

The non-analogue measures from these reports may be completed by the participant on the Clinical Trial Survey System (CTSS) online system. AIs may then collect data from the Clinical Trials Database (CTDB) for the purposes of this study. Participants may enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. No new questionnaires will be used with CTSS and there are no actionable items with use of CTSS requiring real-time monitoring.

e. End of participation
Participants are discharged after an assessment and clinically cleared by a physician or nurse. As we work only with healthy volunteers, there are no other follow up procedures barring adverse effects. Adverse effects are followed by the medically responsible physician or nurse practitioner and medical care will be provided if needed. Otherwise, the participant is discharged at that time.

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

5. Management of Data and Samples

a. Storage
Phone screenings with personal identifying information will be kept in a secure private office locked at all times. When participants are admitted the phone screenings are placed into a binders and moved to a locked cabinet.

All electronic records will be kept confidential to the extent permitted by law. Participants' names and other personal identifying information will be stored in

electronically secured databases at the study sites. These databases are password protected and only study personnel will be given a password. Results will be published as group data without the use of characteristics that would identify individual subjects.

b. 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. Consent for sharing was added with Amendment H. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

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

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

6. Additional Considerations

a. Research with investigational drugs or devices.

Acoustic startle and shock device used in this protocol are considered non-significant risk (NSR) devices and will only be used within published guidelines.

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

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

The shock device (electrical stimulator) does not meet criteria for a Significant Risk device as outlined Under 21 CFR 812.3(m), as an investigational device that:
1. Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device is not an implantable device.
2. Is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.
3. Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject
Response: The shock device, as used under this protocol is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.
4. Otherwise presents a potential for serious risk to the health, safety or welfare of a subject
Response: The shock device has been in use numerous for decades and have been cleared by the FDA. Safety guidelines have been developed and updated allowing its dissemination to a wide range of clinical and non-clinical settings. The FDA has generally waived pre-IDE inquiries for shock studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for shock device within these limitations.

Methylphenidate: On 10/2/13, we received IND exemption for the drug's use in this protocol.

b. Gene Therapy. N/A

7. Risks/ discomforts

a. Study medications

Methylphenidate: Adverse effects include non-specific side effects (e.g., stomach ache, headache, nausea, dizziness), as well as nervousness and insomnia. Decreased appetite is also common. Occasional palpitations, blood pressure and pulse changes (both up and down) have been reported. The 60mg dose in the step-2 follow up study is on the higher end of dosing. Subjects are more likely to experience cardiovascular side effects such as palpitations, tachycardia, and a rise in blood pressure than those getting lower doses. Sudden death has also been reported in chronic treatment with MPH, but a causal link with MPH has not been established. (http://www.fda.gov/drugs/drugsafety/ucm277770.htm). No sudden death has ever been reported after a single dose of MPH.

b. Testing:

fMRI: 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 procedures rely on the same physical properties of brain tissue. Potential risk of heart rhythm disturbances exists for patients with a history of heart rhythm abnormalities or those who have certain types of pacemakers. A substantial risk to persons who have metallic objects inside their bodies exists, as the magnet in the scanner can cause these to move. Pregnant women should not undergo MRI because of the possible harmful effects to the fetus. fMRI scanner is very noisy for this reason participants wear ear plugs, or headphones that are designed to reduce the noise impact of the scanner. The enclose space of the scanner can be uncomfortable. Participants have access to a panic button at all times and can press this to stop the scan and be removed from the scanner.

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

Auditory startle stimulus: The auditory stimuli that will be used in the startle studies are 40-ms duration 102 dB white noise. Auditory startling sounds of much higher intensities are frequently used in startle studies. Sounds of higher intensities and longer duration are also widely used in aversive conditioning in human subjects, where they serve as US. The short duration (40 ms) of these sounds makes them safe (i.e., there is no danger of hearing impairment). In addition, a white noise is safer than a pure tone. The PI has been involved in similar studies and collaborations involving over 1000 of subjects with no adverse reactions.

Electric shock: The shocks will be delivered through two disk electrodes located on the median nerve, on the forearm, or on fingers. The shock will have an intensity of up to 5 mA and duration of 50-100 ms, as in our published studies. The PI has extensive experience with shocks. The shock is generally described by subjects as anxiogenic and moderately painful. The mean rating of aversiveness on a scale of 1 (not painful at all) to 10 (extremely painful) is about 5. Over 95% of subjects who experienced the shock chose to participate in the experiment.

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. 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.

c. Procedures to minimize risks

MRI: The potential risks related to MRI will be minimized as follows: 1) Claustrophobia associated with MRI will be reduced by explaining the nature of the procedure in detail prior to subject enrollment; and 2) a possible history of any intraocular, intra aural, intracranial, or intrathoracic metal will exclude the subject from the study. Earplugs will be given to each subject to wear during the scan to minimize discomfort and prevent any adverse effects on hearing resulting from the scanning procedure. Females of childbearing potential will undergo either serum or urine pregnancy testing to rule out pregnancy no more than 24 hours prior to each MRI session before the scanning procedures are initiated. During MRI scanning, the subject can communicate with the control room personnel via an intercom at the operating console. Thus, the subject can be removed immediately from the MRI scanner, if necessary.

8. Subject safety monitoring

The study timeline includes regular monitoring of blood pressure and heart rate as well as mental state. Subjects will be informed that they can withdraw from the study whenever they wish. Sample shocks will be administered prior to the study. At this point, subjects will be explicitly asked if they wish to continue. The experiment will also be stopped for any subject who exhibits signs of distress during any phase of the study. Subjects will be constantly monitored via closed circuit video by the AI. A credentialed staff member (RN, NP or MD) will assess the subject at the conclusion of the study or at any time if significant side effects develop. The subject will be withdrawn at anytime if unable to follow the rules for participation in this study or fails to meet exclusion criteria.

9. Outcome measures

The primary outcome measures include (1) anxiety measures: amplitude of the startle reflex; (2) cognitive measures: accuracy and reaction time on the WM task. In the functional magnetic resonance imaging (fMRI) substudy, the primary outcome measure includes the event-related haemodynamic response related to neural activity in the brain.

Secondary outcome measures include heart rate and skin conductance and responses to questionnaires.

10. Statistical Analysis

Analysis of the data: Startle and performance data will be analyzed with ANOVAs with repeated measures using Treatment (2 for step-1), condition (stress, no stress) and Load (low, high) as factors.

This design will help us detect and control potential effects of PRO on cognitive function, and, conversely, of MPH on anxiety. Specifically, to measure Drug effects on cognitive function, we will use a repeated measures ANOVA with Treatment (MPH, PLA) as the between-subjects factor and condition (stress, no stress) and Load (low, high) as the within-subjects factors on WM accuracy. To examine Drug effects on anxiety, we will conduct a repeated measures ANOVA with Treatment (MPH, PLA) and condition (stress, no stress) on startle EMG in the absence of task. This analysis will permit us to detect and control the effects of MPH on anxiety. For the fMRI analysis, only 2 drugs (MPH, PLA) will be analyzed following a similar approach as above. Specifically, to measure drug effects on anxiety, we will conduct a repeated measures ANOVA with treatment (MPH, PLA) and condition (threat, no threat) on startle EMG in the absence of task. No analyses will be conducted using both data from step-1 and data from step-2 data. Step-1 and Step-2 studies yield independent results.

Analysis of functional imaging data involves a series of initial steps including realignment (i.e., correction for head movement). Hypotheses regarding task-associated changes in the BOLD signal will initially be tested by analyzing task-related hemodynamic responses in five regions-of-interest (ROI) selected based on hypothesized areas of activation: the amygdala, the striatum, 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 10 regions (5 regions in each hemisphere).

In addition, a voxel-wise analysis will be performed to investigate activations outside of these regions. 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.

Hypotheses regarding the resting state study will be tested by analyzing the intrinsic connectivity of the networks involving the nodes (ROIs) examined in the task-based fMRI study. These nodes include the amygdala, striatum, dorsal anterior hippocampus, anterior insula, and anterior cingulate. The state of these networks at rest may inform their sensitivity to the pharmacological challenges and help predict their efficiency in mediating task performance.

Power analysis:
The main expected outcome measure is a decreased in the anxiety-potentiated startle following methylphenidate. Thus, we base the sample size calculation on the power analysis for obtaining an effect size sufficiently large to conclude to a significant difference between groups of the anxiety-potentiated startle among drug conditions.
To run the power analysis, we are using data from our initial study, Ernst et al (2016) . The mean and SD of anxiety-potentiated startle was 7 and 4.8 T-scores, respectively. We expect MPH to reduce anxiety-potentiated startle by about 50%, corresponding to an effect size (Cohen's d) of .72. Given an effect size of 0.72 and a power of 0.8, a total of 56 subjects or 28 per group will be needed to detect a significant difference in anxiety-potentiated startle between the two treatment groups. Given that we expect approximately 15-20% of subjects to have bad EMG recording, unanalyzable or small startle responses, or poor WM performance, we will recruit 68 subjects (34 per group).

Regarding the fMRI study of Step-2, we request the same number of subjects as in our initial Protocol, i.e., 30 per group, for each of the drug group. Given that we expect approximately 15-20% of subjects to have bad recording or poor WM performance, we will recruit 70 subjects (35 per group).

11. Human Subjects Protection

This study will be conducted in healthy adult males and females.

a) Subject Selection: Subject selection will be equitable and will include both men and women between the ages of 18-50 of diverse racial and ethnic backgrounds. As people get older the probability that they startle decreases. Because startle is our main independent measure it is important to test subjects who are likely to startle in order to collect analyzable data. Based on our experience, we made the decision that 50 would be the upper age limit to ensure usable data and thus justify the more than minimal risk.

b) Justification for exclusion of children: Children are excluded because of the aversive nature of the tests and the use of drugs. This is a more than minimal risk study with no prospect of direct benefit.

c) Justification for exclusion of vulnerable subjects. This study will include healthy volunteers only. All subjects must be able to provide their own consent. This study is above minimal risk and we do not want to enroll participants who do not understand the risk/benefit ratio of the study, particularly when there is no benefit to the participants. Vulnerable subjects will not be included because this is a more than minimal risk study, with no direct benefit.
For this reason, we exclude people with IQ lower than 80. We exclude and rule out pregnant women via a pregnancy test because of the unknown effects of the shocks and of the drugs on the developing fetus.
We will exclude NIMH employees to prevent bias from previous or current professional relations.
Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. This study collects sensitive information (e.g. drug and alcohol use, specific medical diagnoses). The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. Information about sensitive information (e.g. drug and alcohol use, specific medical diagnoses) will be in the participant's NIH medical record.

d) Justification of sensitive procedures: N/

e) Safeguards for vulnerable populations:
Neither participation nor refusal to participate as a subject will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. The NIH Information Sheet on Employee Research Participation will be available to employees considering enrollment.

12. Consent documents and process

a) Designation of those obtaining consent
Qualified staff for obtaining study consent only includes Dr. Monique Ernst and Emily Page, FNP.
Consent procedures

b) Informed screening consents for the study will be obtained in-person at the initial study visit, prior to any screening. Informed study consent for the study will be obtained in-person after screening. This will be conducted in a private room

c) Consent documents

The consent form contains all required elements. There is one consent form for the Step-1 clinic study and one for the fMRI study.

13. Data and Safety monitoring

a) Data and Safety monitor

Data and safety will be monitored by the Principal Investigator.

b) Data and safety monitoring plan

The principal investigator is responsible for participants in this protocol and will report all serious adverse events to the NIMH Clinical Director and the NIMH IRB within the guidelines set by the standards for clinical research within the NIH intramural research program. The PI will review data and safety parameters at least annually. The PI will document the data and safety review in the research records and at the time of continuing review.

a) Criteria for stopping the study or suspending enrollment

The study will be stopped if there is any Serious Adverse Event related to the research.

The PI and IRB will determine if changes are needed for the research to continue or if it will be closed.

14. Quality Assurance

a. Quality Assurance Monitor

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

b. Quality Assurance Plan

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

15. Adverse event and unanticipated problem reporting

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

16. Alternatives to participation

The alternative to participating in this study is not to participate.

17. Privacy

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

20. Confidentiality

a. For research data

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

b. For stored samples

N/A

c. Special Precautions: N/A

21. Conflict of Interest

a) Distribution of NIH Guidelines

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

b) Conflict of Interest

There are no conflicts-of-interest to report.

22. Technology Transfer

A tech transfer agreement (2019-0418) is in place between Dr. Ernst and Dr. Saggar. The agreement will be included in the data sharing spreadsheet.

23. Research and Travel Compensation

Volunteers will be compensated for time and research-related inconveniences based on NIH standards for time devoted to research projects. Travel time or expenses are not paid. NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

VOLUNTEER PAYMENT SCHEDULE

Procedure - Psychophysiology	**Duration**	**Amount**
Outpatient Visit for study session Drug, shock	5-8 hrs	$200
TOTAL	Up to 8 hrs	$200

Procedure - fMRI	**Duration**	**Amount**
Outpatient Visit for study session drug, fMRI ,shock	5-8 hrs	$210
TOTAL	Up to 8 hrs	$210

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