**Study Title:** Cannabinoid Control of Fear Extinction Neural Circuits in Post-Traumatic Stress Disorder

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## *A. Background and Specific Aims*

### Significance

An inability to suppress inappropriate fear responses is the hallmark of anxiety disorders, such as post-traumatic stress (PTSD)[1,2]. A common, empirically-validated approach to treat this disorder is Prolonged Exposure Therapy (PE)[3], one component of which involves repeated exposure to fear-linked cues to produce "extinction" of fear (clinically referred to as exposure leading to desensitization) and to prevent avoidance responses to these cues[4]. This exposure-based learning can be modeled in the laboratory, in both animals and humans, using Pavlovian fear conditioning models in which fear is first linked to a previously innocuous cue (conditioned stimulus; CS) and then decreased by presenting the CS alone (producing extinction). *Unfortunately, one major limitation of extinction is that it is a temporary phenomenon and extinguished fear can re-emerge*[5-8]. Fear extinction and its recall have become the prime translational neuroscience target for the treatment of PTSD and other anxiety disorders[9-11].

Although PE is an effective first-line treatment for PTSD, approximately 20-30% of treatment completers continue to have a PTSD diagnosis, slightly more (30-40%) fail to achieve a stringent criterion for good end-state functioning[12,13], and some fail to complete treatment (20.5%)[14]. Even fewer respond to first-line pharmacological treatments, such as selective serotonin reuptake inhibitors (SSRIs)[15,16]. The Institute of Medicine in 2007 concluded that little empirical evidence exists to support pharmacological treatment for PTSD; therefore new treatments are desperately needed[17]. *Enhancing the neural and neurochemical substrates of inhibitory fear learning could solve this challenge and improve PTSD treatment outcomes*[9-11].

**What are the neural circuits mediating fear extinction?**

Convergent evidence from rat and human work have elucidated that discrete, yet anatomically and functionally interconnected, brain structures are critical for extinction learning and the retention of extinction memory (amygdala [AMYG], ventromedial prefrontal cortex [vmPFC], and hippocampus [HPC])[6,18-35]. At acquisition, sensory information about the CS and the aversive unconditioned stimulus (US) converge at the AMYG and become associated (i.e. yielding the fear memory) and translated into conditioned responses of fear (CRs)[21,22]; of note the AMYG may also be involved in extinction learning[23-25]. Indeed, AMYG activation has been correlated with fear responses during conditioning in human subjects based on functional magnetic resonance imaging (fMRI) studies[26,36,37]. Prefrontal brain regions that interconnect with the AMYG, particularly the vmPFC, are important for retention and retrieval of extinction memories and consequent attenuation of fear CRs perhaps via inhibiting AMYG output neurons[26-32]. In humans, vmPFC activation during extinction recall and vmPFC thickness both correlate with magnitude of extinction retention[26,33,38,39]. In addition, the magnitude of task-dependent functional coupling between the AMYG and vmPFC has been shown to be negatively correlated with intensity of subjective reports of negative affect[40]. Similarly, HPC activation is associated with successful retrieval of extinction memory and is positively correlated with vmPFC activation during extinction recall in humans[33,34]. Interestingly, increased functional connectivity between the AMYG and the HPC has been attributed to the persistence of memories for emotionally arousing events in humans[41-45]. These lines of convergent evidence suggests that how these regions interact with one another may mediate the control, or lack thereof, of fear regulation in humans.

**What is the neurobiological basis of PTSD?**

PTSD is characterized by altered emotional responses following trauma exposure (e.g. combat, assault, and disasters). Patients with PTSD not only experience intense negative emotional reactions when reminded of their trauma but also report exaggerated arousal (poor sleep, restlessness, hypervigilance), anhedonia, social withdrawal, and decreased emotional expressivity ("emotional numbing"). Characterizing the neural basis of these diverse, distorted emotional responses poses a major challenge to contemporary psychiatric research. Functional neuroimaging techniques have focused primarily on the study of brain function related to fear

perception and response, and have consistently implicated dysfunctions in the above mentioned limbic-prefrontal network. In particular, many studies have shown AMYG hyperactivity in PTSD in response to trauma-related and unrelated negative stimuli[46-58]. Exaggerated AMYG reactivity observed in PTSD has been posited to be at least in part, a result of insufficient top-down regulation from the vmPFC, consequently leading to hyperarousal and deficits in extinction retention as well as the inability to suppress attention and responses to trauma-related stimuli[59-62]. For example, exaggerated AMYG reactivity is negatively correlated with responses in the dorsal and vmPFC across individuals with PTSD[47,56]. Although less commonly implicated, abnormal HPC function and diminished HPC volumes in PTSD patients have been associated with deficits in contextual processing, as well as memory impairments and neuroendocrine dysregulation[47,63-68]. *Poor extinction recall and vmPFC-HPC dysfunction displayed by patients with PTSD could undermine the efficacy of the therapeutic effects of exposure[59,69-75].*

**Can we enhance fear extinction?**

Exciting new evidence from studies in rodent models of fear suggest that activation of the cannabinoid (CB) system within brain structures important for extinction may regulate extinction learning and retention. For instance, drugs that block type 1 CB receptors or genetic deletion of CB1 receptors, within these structures, prevents fear extinction, whereas activation of these same receptors, via agonists, such as $\Delta^9$-tetrahydrocannibinol (THC), can lead to facilitation of extinction[76-86]. In addition, drugs that increase the level of endogenous cannabinoids during extinction, not only enhance extinction retention, but also impair the return of extinguished fear in rats[77]. Recently PI Rabinak showed that pre-extinction administration of THC facilitates extinction of conditioned fear in humans using a similar behavioral design as that proposed in the current K01 project[87]. In particular, participants that had received placebo during extinction learning exhibited spontaneous recovery of fear to a CS that was previously extinguished, whereas THC attenuated spontaneous recovery of fear (Fig 1; Rabinak et al.[87]). Of note, THC did not affect within-session extinction learning, but only influenced the ability to successfully recall extinction memory when compared to PBO, suggesting that THC affects the ability to maintain and/or successfully retrieve extinction memory. These findings are consistent with pre-clinical studies in rats[76,77,79,81,82,84,85] and *provides the first evidence that pharmacological enhancement of extinction recall is feasible in humans using cannabinoid system modulators.*

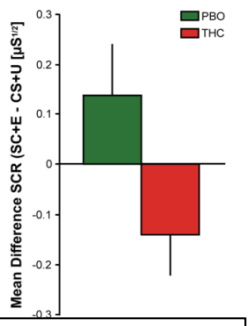


**Fig. 1: THC reduces fear recovery during extinction recall; Rabinak et al.[87]**

**How might the cannabinoid system affect fear extinction?**

In the brain, endocannabinoids (eCBs) from the postsynaptic cell diffuse in a retrograde fashion to activate presynaptic CB1 receptors, densely localized within AMYG, vmPFC, and HPC[88-91], which in turn inhibit presynaptic release of neurotransmitters[79,92]. It has been hypothesized that during extinction learning eCB activation of CB1 receptors within the AMYG decreases activity in local GABAergic networks, which leads to a disinhibition of principal neurons and finally to the extinction of conditioned fear responses[79,83]. Interestingly, intra-basolateral AMYG infusion of CB1 agonists enhances retention of inhibitory training (memory consolidation)[93]. On the other hand, activation of CB1 receptors within the vmPFC during extinction induces neuronal plasticity within the vmPFC, and subsequently increases inhibition on brain areas involved in the expression of conditioned fear responses (e.g. AMYG)[81]. In addition, HPC CB1 receptor activation enhances glutamatergic neurotransmission, which may support long-term extinction memory formation (consolidation)[78].

Studies conducted in our laboratory using fMRI found that oral THC (vs. placebo/PBO) attenuated AMYG[94] and enhanced mPFC (unpublished) activity to threat stimuli. Moreover, work by PI Rabinak has shown that THC modulates mPFC-AMYG activation and connectivity during emotional processing[91]. Although not directly related to fear extinction learning per se, these data demonstrate that THC effects on brain response to threat can be localized to fear-related AMYG-mPFC circuitry. Consistent with these findings we have preliminary fMRI data to suggest that THC facilitates retention of extinction memory in healthy humans via increased activation of vmPFC and increased vmPFC-HPC functional connectivity[95] (**see 'Preliminary Data'**). Collectively, these findings are exciting because they suggest that *the efficacy of extinction learning and retention can be enhanced via increasing activity of CB1 receptors within the neural circuits involved in extinction processing, and prompt translational investigation in PTSD patients with impaired extinction memory recall and associated aberrant vmPFC-HPC function.*

**Can cannabinoids facilitate fear extinction in PTSD?**

An early placebo-controlled study showed that nabilone, a synthetic THC, dramatically reduced anxiety in anxious patients[96]. These anxiolytic effects of eCB enhancers have sparked interest in CB1 receptors as a pharmacological target for treating anxiety disorders[97-100]. Given that extinction retention deficits and vmPFC-HPC dysfunction have been observed in patients with PTSD, and that enhancing cannabinoid transmission helps extinction recall, the cannabinoid system is a promising target for improving the learning that goes on in therapy and perhaps increasing the efficacy and durability of PE in treating PTSD (e.g., shortening treatment while strengthening and prolonging gains). However, direct tests of cannabinoid effects on extinction recall and associated neural circuits have not yet been conducted in PTSD patients. *Therefore, the primary goal of the proposed project is to test the hypotheses that administration of an exogenous CB1 agonist will 'rescue' deficits in fear extinction recall in PTSD patients and that these effects will be mediated by increased activation and functional connectivity of vmPFC and HPC.*

To address the issues mentioned above we propose the following specific aims:

**Specific Aim 1: To assess the effects of THC on extinction memory recall and vmPFC and HPC activation and connectivity in controls (healthy controls, HC; trauma-exposed non-PTSD controls, TEC).** *Hypothesis 1A: Relative to PBO, THC will decrease SCRs and US expectancy ratings to a CS that was previously extinguished (CS+E) during an extinction recall test in controls. Hypothesis 1B: Relative to PBO, THC will enhance regional activation in the vmPFC and HPC to the CS+E during an extinction recall test in controls. Hypothesis 1C: Relative to PBO, THC will increase functional coupling between the vmPFC and HPC to the CS+E during an extinction recall test in controls.*

**Specific Aim 2: To compare extinction memory recall success and vmPFC and HPC activation and connectivity between PTSD patients and controls (HC/TEC).** *Hypothesis 2A: Relative to controls, PTSD patients will show increased SCRs and US expectancy ratings to the CS+E during an extinction recall test. Hypothesis 2B: Relative to controls, PTSD patients will show attenuated regional activation in the vmPFC and HPC to the CS+E during an extinction recall test. Hypothesis 2C: Relative to controls, PTSD patients will show less functional coupling between the vmPFC and HPC to the CS+E during an extinction recall test.*

**Specific Aim 3: To investigate whether THC can correct extinction memory recall impairments and aberrant vmPFC and HPC activation and connectivity in PTSD patients.** *Hypothesis 3A: Relative to PBO, THC will decrease SCRs and US expectancy ratings to the CS+E during an extinction recall test in PTSD patients, similar to levels observed in controls (HC/TEC). Hypothesis 3B: Relative to PBO, THC will enhance regional activation in the vmPFC and HPC to the CS+E during an extinction recall test in PTSD patients, similar to controls (HC/TEC). Hypothesis 3C: Relative to PBO, THC will increase functional coupling between the vmPFC and HPC to the CS+E during an extinction recall test in PTSD patients, similar to extent of connectivity observed in controls (HC/TEC).*

***B. Preliminary Data***

The preliminary data here: 1) establish the capacity of the PI to carry out fMRI studies of fear extinction learning and THC challenge in humans; 2) provide evidence that THC facilitates extinction recall in healthy humans; and 2) provides evidence collected by the PI that replicate findings of extinction recall deficits in PTSD patients and support the hypotheses and feasibility of the proposed study.

Impaired Extinction Recall and Dysfunction Activation of vmPFC and HPC during Extinction Recall in PTSD

To assess fear extinction recall in PTSD, Milad and colleagues have developed a fear conditioning-extinction paradigm and has shown less activation in vmPFC and HPC and increased SCRs in PTSD patients (vs. trauma-exposed non-PTSD controls) during extinction recall in response to the a previously extinguished CS+ (CS+E) compared to a non-extinguished CS+ (CS+U)[70]. The PI has collected SCR data as a measure of fear in healthy controls (HCs; n = 15) and PTSD patients (n = 14) in a behavioral pilot study of fear extinction learning and recall using the study design proposed here. Similar to Milad et al.[70], when recall of extinction was tested 24 hours after extinction learning PTSD patients exhibited recovery of fear, as evidenced by increased SCRs

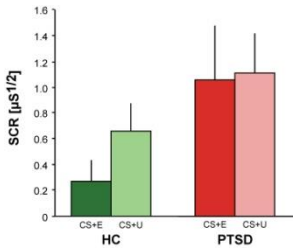


**Fig. 2: Deficits in extinction recall in PTSD**

to the CS+E that did not differ from SCR to the CS+U, as compared to HCs, which displayed lower SCRs to the CS+E (Fig. 2).

THC Facilitates Retention of Extinction Memory via Increased Recruitment of the vmPFC and HPC

The PI has previously demonstrated that 7.5 mg oral THC facilitated retention of extinguished fear and prevented spontaneous recovery of fear in healthy humans[87] (see Fig. 1). Recently, in a separate study, the PI has collected fMRI data investigating the effect of cannabinoids on the underlying neural circuitry involved in the recall of extinction memory in 28 healthy adult volunteers using the study design proposed here. Preliminary fMRI results suggest that THC administration during extinction learning subsequently increases vmPFC activation and functional coupling (psychophysiological interaction; PPI) with the HPC to the CS+E (vs. the CS+U) during recall of extinction memory as compared to PBO[95] (Fig. 3).

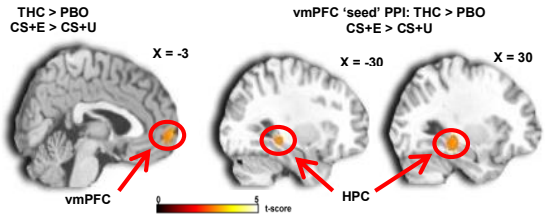


**Fig. 3: THC increases vmPFC activation and functional HPC-connectivity at extinction recall: Rabinak et al.[95]**

***C. Research Design and Methods***

*C.1. Overall Study Design:* In a randomized, double-blind, placebo-controlled, between-subjects design, we will couple a standard Pavlovian fear extinction paradigm in fMRI with an acute pharmacological challenge with oral dronabinol (synthetic THC) or placebo (PBO) 2 hours *prior to extinction learning* in patients with PTSD, trauma-exposed controls without PTSD (TEC), and non-exposed healthy controls (HC) and test extinction memory recall 24 hours after extinction learning. This provides the most translational and critical test to determine if consolidation effects of THC will facilitate subsequent extinction recall in PTSD patients.

This fMRI protocol adopts a similar event-related design developed and validated by Milad et al.[101,102], which manipulates context using an ABBA design and will be conducted over 4 study visits. Stimulus parameters will be similar to those conducted by Milad et al.[102]. Each "context" will consist of different images of a neutral scene in which the conditioned stimuli will appear. Conditioned stimuli (CSs) will consist of emotionally neutral objects or facial expressions and unconditioned stimuli (US) will be an adverse stimulus (e.g. loud noise [103]; a mild electric shock to the wrist[36,101,102,104-110]). During all sessions, our primary measures of conditioned fear will be measures of psychophysiological responding via electrodes, as well as US expectancy ratings for each CS trial[103]. All experimental phases (Extinction, Extinction Recall/Fear Renewal), except Fear Acquisition, will be conducted in the fMRI scanner with simultaneous psychophysiological recordings and US expectancy ratings. Fear Acquisition will be conducted in one our testing rooms at the Eugene Applebaum College of Pharmacy & Health Sciences (EACPHS) building.

*C.2. Nature of Participant Population:* The number of participants expected to participate in the study is 150 [50 patients with PTSD and two controls groups individually matched (age, gender, education): 50 trauma-exposed subjects without PTSD (also matched on trauma-exposure) and 50 non-exposed subjects]. The sample size is required to reach at least 15-20 participants in each experimental group (6 groups). Based on prior experience we expect that data from about 30 participants (5 per group) will be not be usable due to either failure to complete all sessions or poor skin conductance recordings. Participants of both genders will be included in the study and the age range of participants will be from 21-45. Persons below 21 or above 45 are excluded because beyond this range age may influence response to the drugs. Furthermore, the relatively small age range is intended to reduce variation in performance on the behavioral task. There are no enrollment restrictions based upon race or ethnic origin. Candidates will undergo a structured clinical psychiatric interview[111-113] and will fill out questionnaires related to their general physical health, current medications, with a detailed section on current and lifetime drug and alcohol use.

*C.2.1. Inclusion of Women and Minorities:* Women and minorities will be included in the study. Sex/gender, racial/ethnic, or socioeconomic group are not expected to affect the results of the experiments, therefore there are no limitations set on any of these groups for inclusion in the study. Volunteers will not be excluded on the basis of gender or minority/ethnic/racial status. Approximately half of the sample (see below) will be women, and thus, females will be well-represented. Based on Wayne State University's participant recruitment area, which includes the city of Detroit, Wayne County, and surrounding suburban areas; we plan to recruit and obtain data from a diverse ethnic/racial sample comprised of the following minority composition. According to the U.S. Census Bureau 2012 statistics, the following groups are represented in percent in Wayne County:

Female, 51.9%; White 54.4% (White not Hispanic, 49.8%); Black, 40.1%; Asian, 2.8%, Hispanic/Latino, 4.2%; American Indian and Alaska Native persons, 0.5%; Native Hawaiian and Other Pacific Islander, 0.1%; ≥ 2 races, 2.2% (For reference: http://quickfacts.census.gov/qfd/states/26/26163.html).

However, pregnant participants will be excluded from participation because there is insufficient data to assure safety of the fetus during dronabinol exposure, as well as during MRI scanning. In addition, nursing mothers will be excluded from the study because dronabinol is concentrated in and secreted from human breast milk and is absorbed by the nursing baby.

*C.2.2. Inclusion of Children:* Those under the age of 21 are excluded from this protocol because by definition provided by the National Institutes of Health, these participants are classified as "children" and exclusion of this group eliminates the potential ethical complications of administering dronabinol, a controlled substance, to children. Knowledge as to the effects of this drug in "adults" are limited and no studies have been conducted in children to determine what effects this drug would have, thus making administering this drug to children risky. In addition persons below 21 are excluded because ages below this cutoff may influence response to the drugs and increase variation in performance on the behavioral task.

*C.2.3. Vulnerable Populations:* This study will include women of child-bearing potential and college students. However, we will conduct urine pregnancy tests at the screening visit and before each scanning session to make sure that women are not pregnant. In addition, participation of college students in this study is completely voluntary, as it is for all participants, and will not be required by any course.

*C.2.4. Recruitment and Attrition Considerations:* Participants will be recruited from the WSU Psychiatric Clinic, Wayne State University's campus, and surrounding community via several sources including: newspaper advertisements, flyers, and webpage advertisements. Current staff in the department and Department of Psychiatry will be informed about the study to pass along the information (in flyer format) to their participants. Once a participant is in contact with the project coordinator, the coordinator will explain the study to the participant and schedule the participant to come in for a screening and orientation session (Visit 1) at the EACPHS building.

*C.3. Study Visits*

*C.3.1. Screening & Study Entry (Visit 1):* After the experimenter reviews the consent form with the participant and answers any question he/she may have, the participant will sign the informed consent document. Candidates will undergo a structured clinical psychiatric interview to determine eligibility for the control groups (HC/TEC) or the PTSD group. Milad and colleagues[114] have recently reported that a high level of estrodial in women significantly facilitates fear extinction recall as compared to women with low levels of estrodial. Therefore, to control for possible hormonal facilitation during extinction we will schedule women to complete the experimental session approximately 1 week before the onset of menses; when estrogen levels are low. The Health Questionnaire participants complete during the screening visit includes a section that asks women to report when their last menstrual cycle began and the approximate duration of their cycle. We will use this information to schedule women for the experimental session approximately 1 week before the onset of menses. All participants will undergo a urine drug (and pregnancy test for women) at the screening visit and must be negative to enroll in the study.

C.3.1.1. DNA secondary exploratory analysis (Visit 1): Experimenters will collect a one-time buccal sample using the Epicentre Buccal DNA extraction kit in order to analyze cannabinoid genetic variation and its influence on the efficacy of cannabinoid use in this RCT. The samples will be stored for up to 50 years in Dr. Kyle Burghardt's locked laboratory located at the Eugene Applebaum School of Pharmacy at Wayne State University for future studies investigating biomarkers related to PTSD treatment and response. Experimenters will swab the inside of the subject's mouth (on their cheek) approximately 20 times on each side to obtain the buccal samples. Consent to obtain, prepare and store DNA samples is contained within the main consent document and participants have the ability to opt out of the DNA collection component of the trial.

*C.3.1.2. Informed Consent:* A written summary, in lay terms, of the research project will be provided to the participants in the written informed consent document that the participants will review. The consent document will inform the participants of the voluntary nature of the study procedures, the purpose of the study, the procedures to be followed, the duration of the study, the risks associated with MR scanning, exposure dronabinol, as well as the potential benefits to the community at large. Participants will agree in the consent not to take other drugs for 24 hours before and 12 hours following each session and not to operate any machinery requiring concentration for 12 hours following the fear extinction session in which drug may be

administered. Women will agree that they are not pregnant and not planning to become pregnant. Participants will be informed that blood alcohol levels and urine samples will be obtained prior to the extinction session. Written informed consent will be obtained by the PI or designated research staff and the participant will receive a copy of the signed consent form. The current protocols and informed consent form will be approved by the Institutional Review Board (IRB) at Wayne State University. Participants will be informed that they can discontinue participation at any time without penalty.

*C.3.1.3. Diagnostic Assessment:* Semi-structured interview for general diagnostic assessment include the Structure Clinical Interview for DSM-IV[111-113]. Civilian post-traumatic stress disorder will be confirmed by additional reliable probes, such as the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5)[115], Life Events Checklist for DSM-5 (LEC-5)[116], and the PTSD Checklist for DSM-5 (PCL-5)[117]. Self-report measures including the State-Trait Anxiety Inventory (STAI)[118], Emotion Regulation Questionnaire (ERQ)[119], Attentional Control Questionnaire (ACQ)[120], Hamilton Depression Scale (HAM-D)[121], Hamilton Anxiety Scale (HAM-A)[122], and Beck Depression Inventory (BDI-II)[123-126] will be used to corroborate and validate clinician-administered scales. Diagnostic interviews will be done by MINI/CAPS-trained clinical rating staff (MD/PhD/MSW) employed at the Department of Pharmacy Practice. While most interviews will be conducted by one rater, inter-rater reliability studies will be conducted.

*C.3.1.4. Study Criteria:* Inclusion Criteria: All participants must be: a) able to give informed consent; b) physically and neurologically healthy as confirmed by a comprehensive medical history; c) age between 21-45 years old; d) right-handed. In addition, to be included as a PTSD candidate, subjects must have a current PTSD diagnosis (related to civilian trauma). To be included as a trauma-exposed control without PTSD (TEC), subjects will have experienced a civilian trauma without a PTSD diagnosis. To be included as a non-exposed healthy control (HC) or TEC, subjects must be free of a lifetime diagnosis of Axis I or Axis II disorder. Exclusion Criteria: Subjects in any group (PTSD, TEC, or HC) with the following are excluded from the study: a) clinically significant medical or neurologic condition; b) less than a high school education; c) lack of fluency in English; d) night shift work; e) currently pregnant (positive pregnancy test) or planning pregnancy or lactating (women); f) unwilling/unable to sign informed consent document; g) inability to tolerate small, enclosed spaces without anxiety (e.g. claustrophobia), as determined by self-report and a preliminary session in a mock scanner; h) left-handed; i) presence of ferrous-containing metals within the body (e.g., aneurysm clips, shrapnel/retained particles); j) under 21 or over 45 years of age; k) anticipation of a required drug test in the 4 weeks following the study; l) positive urine drug test/alcohol breathalyzer; m) current or past allergic or adverse reaction or known sensitivity to cannabinoid-like substances (Dronabinol /Marijuana/Cannabis/THC, cannabinoid oil, sesame oil, gelatin, glycerin, and titanium dioxide); n) if they have participated in an experiment involving shocks in the last 6 months. In addition, PTSD subjects with the following will be excluded: a) primary comorbid anxiety disorder (defined by which disorder was the more debilitating and clinically salient); b) life history of bipolar disorder, schizophrenia, or presence of an organic mental syndrome, mental retardation, or pervasive developmental disorder; c) current alcohol/drug abuse or dependence; d) current major depressive disorder; e) current suicidal ideation; f) diagnosis of an Axis II personality disorder; g) concomitant treatments with psychotropic/psychoactive medications (including beta-adrenergic blockers, SSRI, benzodiazepines, tricyclic/mono-amine oxidase inhibitor [MAOI] antidepressants, lithium, antiepileptic/anticonvulsants, neuroleptic/antipsychotics, etc.) or in the past two weeks (8 weeks for fluoxetine and 4 weeks for MAOIs) before screening; h) currently receiving exposure-based therapy for PTSD. Note: Although a current history of drug/alcohol abuse/dependence and major depression is an exclusion criterion, a past history of these conditions will not be an exclusion criterion for this study. This is because past history of these conditions is frequently present in PTSD subjects. Thus, exclusion of such subjects would yield a biased and unrepresentative sample of PTSD subjects. If a PTSD subject is currently taking a psychoactive medication at the time of the screening visit and/or the experimental visits, but are still symptomatic despite taking the medication they will be included; unless the medication would interfere negatively with the study drug. Control subjects (TEC and HC) with any current or past Axis I psychiatric disorder; including alcohol/substance abuse or dependence disorder will be excluded.

After screening, if eligible, the participant will continue with an orientation session to explain the procedures and schedule the sessions. Rules and conditions of participation will be carefully explained. We will query participants about any prior history of claustrophobia or anxiety in small spaces. Each subject will complete a detailed questionnaire regarding the presence of any metal implants or metal objects in their body, and other such contraindications for MRI scanning.

*C.3.2. Fear Acquisition (Visit 2):* During the Fear Conditioning (Context A) phase, there will be two CS+s that will be paired with the US using partial reinforcement. One of these CS+s will be extinguished during the

subsequent extinction phase (CS+E) whereas the other will not (CS+U). The CS+U serves as a within subject, no-extinction control. A third CS will be presented during conditioning *but* never paired with the US (CS-). Assignment of the slide as CS+E or CS+U (paired with US) and CS- (not paired with US) will be counterbalanced across subjects. The US will be delivered immediately following CS+ offset with no delay between the CS+ offset and the US onset. At the beginning of the session subjects will be told to look carefully at the slides because an unpleasant stimulus (US) may follow the slides. They will also be told that they should learn to predict whether the US will occur or not based on which slide was shown. To assess their predictions, subjects will be required to repeatedly rate their US expectancy using a button box on a scale from 1 to 3 (1 = certain that the US will be presented, 2 = certain that the US will not be presented, and 3 = uncertain whether the US will be presented) and subjects will be asked to repeatedly update their rating to reflect their current US expectancy. At the end of the session all participants will be explicitly told to remember what they had learned; in order to strengthen the retention of the CS-US association for the following days. This visit will be conducted at our research laboratory at the EACPHS building.

*C.3.3. Fear Extinction Session with fMRI (Visit 3):* At the beginning of this session all participants will undergo an alcohol breathalyzer test, urine drug (and pregnancy for women) test and all must come back negative before entering the fMRI scanner. During Extinction Learning (Context B), subjects will receive unreinforced presentations of the CS+E (no US) and CS-. As during Fear Acquisition, subjects will rate US expectancy and we will record psychophysiological responding. We will also ask participants to give a rating on the Subjective Units of Discomfort Scale (SUDS) at three time points throughout this session: before the session start, in the middle of the extinction session and at the end of the extinction session. This is scale is used to measure fear ratings/"subjective" anxiety[127,128]. This scale will help provide a measure for extinction of subjective fear. Participants will be explained the SUDS scale prior to getting into the scanner. The SUDS will be presented to the participants on the computer screen and the participant will be asked to report where they fall on the scale during that time point. In addition, all participants will complete three other tasks in the MRI scanner following the Extinction Phase:

*Emotional Regulation Task (ERT):* The ERT is a variant of the reappraisal-based emotion regulation task used by Davidson and colleagues[129], and Ochsnser and colleagues[130], which has been previously shown to effectively probe amygdala-vmPFC function and their interactions. In brief, the ERT employs active, voluntary regulation of negative emotion by cognitive reappraisal[32]. The stimulus set used to evoke negative affect includes highly aversive and arousing pictures from the International Affective Picture System (IAPS)[131]. The protocol involves two task conditions of interest, "Maintain" and "Reappraise," which alternate across blocks in a counterbalanced order. During the Maintain task, participants are instructed to attend to, be aware of, and experience naturally (without trying to change or alter) the emotional state elicited by the pictures; they are told to maintain the evoked affect for the entire task block. During the Reappraise task, participants are instructed to voluntarily decrease the intensity of their negative affect by using the cognitive strategy of reappraisal[132,133], adapted for fMRI[32,129,130,134] they are told to reinterpret the content of the picture so that it no longer elicits a negative response. Extensive instruction on the cognitive strategy of reappraisal will be provided to participants prior to the initiation of the experiment, and understanding of the task will be confirmed prior to scanning by reviewing examples of subject-generated strategies. Subjects will be instructed to not look away from the picture stimuli, close their eyes, or engage in about random thoughts during either the Maintain or Reappraise blocks. For training in Reappraisal, two well validated examples will be provided to facilitate understanding of the strategy: 1) transforming the depicted scenario into less negative or positive terms (e.g., women crying outside of a church could be interpreted as expressing tears of joy from wedding); and 2) rationalizing or objectifying the content of the pictures (e.g., a woman with facial bruises could be translated as an actor wearing makeup rather than a victim of domestic abuse). These types of reappraisal strategies have been found to be successful in volitional regulation of the negative emotions evoked by aversive IAPS pictures. We will provide these examples for illustrative purposes, but will also explain that no single type of reinterpretation will be applicable to every picture, and they will be instructed to use the most effective reframing strategy for each picture. Our experience with the ERT is that it is an easy-to-understand and easy-to-implement set of instructions after training. Prior to each block of pictures, the instruction to "Maintain" or "Reappraise" appears at the center the screen. Immediately following each Maintain and Reappraise block, a black screen with a rating scale will appear asking participants to rate the intensity of their negative affect on a 5-point scale (1=least negative/neutral, 5=extremely negative) via button response by pressing the button 1-5 times. These ratings will be recorded.

*Implicit Memory Recall Test:* Extinction recall may be contextually confounded by the "drug-state" during training, which would enhance spontaneous recovery (rather than enhance recall as predicted). To address this

issue, a neutral memory test sensitive to encoding-retrieval state differences will be administered during Visit 3. Following the design of Duarte et al.[135], participants will be shown a series of black and white line drawings of single objects and will be asked to decide if the object would fit into a shoebox or not, and next day (Visit 4) testing will be done using implicit cued recall and remember/know procedures to determine whether THC during encoding produces a shift from recollection to familiarity-based memory processing in accordance with state-dependent effects.

*Resting State Task:* During this task subjects will look at a white fixation cross on a black background for approximately 10 minutes and will be instructed to relax, keep their eyes open and on the fixation cross and clear their mind. This task allows us to investigate brain activity and drug effects independent of task-based activation.

During times when no experimental events are scheduled, subjects will be free to engage in recreational activities (e.g. read). The session will end approximately 4 hours after the drug is ingested (duration of psychoactive effects is 4 to 6 hours). However, subjects may be asked to remain in the laboratory if they still exhibit observable signs of intoxication or self-rated "high." Subjects agree in the consent form not to take any other drugs, or to operate a motor vehicle for at least 12 after the experimental session. Subjects are instructed not to drive and should arrange alternate transportation through a family member/friend or a taxi will be arranged to take them home.

*C.3.3.1. Study Group Assignment:* Participants will be randomly assigned to one of two groups: dronabinol (Marinol®; 7.5; Solvay Pharmaceuticals, Marietta, GA) or placebo. This dose of THC was chosen because it is the lowest effective dose that has been previously shown to modulate amygdala activity to emotional stimuli and produce behavioral and subjective effects[91,94,136]. In addition, this dose has been shown to facilitate extinction recall and modulate prefrontal and hippocampal activation in humans[95,137]. Study medication dronabinol or placebo will be dispensed to participants by PI Rabinak. Research staff associated with the study will give the drug or placebo to participants assigned at Visit 3 with Paul Kilgore, MD, MPH, a board-certified physician, or his physician designate, present on-site and/or available by pager. Dronabinol is administered only by the oral route and will be placed in opaque capsules with dextrose filler. Placebo capsules will be identical in appearance but will contain only dextrose. All capsules will be administered to participants in double-blind conditions and participants assigned to receive dronabinol (half of the participants) will only receive dronabinol on one occasion. Based on what we know about the time course of THC's peak subjective effects and peak plasma levels of THC[91,94,136-138], we will begin extinction learning approximately 120 minutes after ingestion of either the THC or PBO capsule.

*C.3.3.2. Mood & Drug Effect Questionnaires:* Standardized questionnaires will be used to assess mood states and subjective drug effects throughout Visit 3, when the drug/placebo is administered. These questionnaires are sensitive to the effects of a variety of psychoactive drugs on affective state/mood and drug liking[139,140]: (1) Visual Analog Scales (VAS); and (2) Drug Effects Questionnaire (DEQ); (3) Addiction Research Center Inventory (ARCI); (4) Spielberger Trait/State Anxiety Inventory (STAI); see below for details. In addition, physiologic measures (HR, BP) will be collected at regular intervals throughout Visit 3. At completion of the extinction session, participants will complete the End of Session Questionnaire (ESQ) which asks them to identify the substance they thought they received (e.g., THC or placebo), and to rate how much they liked its effects. They will be also asked to comment on any unusual effects they experienced, and whether they would take the substance again. These psychological and physiological measures are collected immediately before capsule ingestion (Time 0), and 30, 60, 120, 180, and 240 minutes afterwards, unless otherwise specified (e.g., DEQ will also be collected at 90 and 150min, ESQ only at 240min). Because the effects of the drugs may have a lingering effect, measures of the effects of the drug (DEQ) and review of adverse events will also be collected for exploratory purposes at two additional times: 8 hours after drug administration and the day (24 hours later) following their participation in the extinction session.

Primary subjective outcome measures will include the VAS ratings of how much participants "feel" the drug effect, and the ARCI M Scale. Secondary measures will include ratings on other self-report scales.

A. Visual Analog Scales (VAS): The VAS consists of visual analog scales used to describe current drug effects. This particular form consists of seven 100-mm horizontal lines each labeled with an adjective ("stimulated," "high," "anxious," "sedated," "down," "hungry," and "nauseated"). The left end of each line is labeled "not at all" and the right "extremely." Participants are instructed to place a mark on each line indicating how they feel at the moment.

B. Addiction Research Center Inventory (ARCI): The 53-item ARCI is a true-false questionnaire with five empirically derived scales that are sensitive to the effects of a variety of classes of abused drugs. The scales are the MBG (Morphine-Benzedrine Group), a general measure of drug-induced euphoria; the A

(Amphetamine) scale, a measure specific for dose-related effects of d-amphetamine; the BG (Benzedrine Group), an amphetamine scale consisting mainly of items relating to intellectual efficiency and energy; the PCAG (Pentobarbital-Chlorpromazine Group), a measure of sedation; the LSD (Lysergic Acid), a measure of dysphoria and somatic symptoms; and the M scale, a measure of marijuana's effects.

C. Drug Effects Questionnaire (DEQ): The DEQ consists of five questions concerning current drug effects. Participants indicate whether they: i) are currently feeling any drug effects; ii) are high; iii) dislike any of the effects; iv) like any of the effects; and v) want more of the drug; on a scale from "not at all" to "extremely".

D. The End of Session Questionnaire (ESQ): Participants are asked to identify the substance they received (e.g., THC or placebo), and to rate how much they liked its effects. They are also allowed to comment on any unusual effects they experienced, and whether they would take the substance again.

E. Spielberger Trait/State Anxiety Inventory (STAI): This questionnaire is used to assess the level of both state and trait anxiety in an individual.

All of the questionnaires that will be administered to participants are standardized questionnaires that have been extensively used in research and are valid and reliable.

*C.3.4. Extinction Memory Recall Test, Fear Renewal Test, & Debriefing (Visit 4):* To assess extinction retention, we will conduct an Extinction Recall test 24 hours after the Extinction Learning phase, in which participants will be presented with the CS+E, CS+U, and CS- (no US will be delivered) in Context B. Contrasting CS+E and CS+U during extinction recall in Context B will allow us to assess physiological and brain responses that are specific from extinction recall and are independent from recall of conditioning. To test for renewal of fear (context shift from extinction Context B to conditioning A), we will conduct a Fear Renewal test 24 hours after the Extinction Learning phase, in which subjects will be presented with the CS+E, CS+U, and CS- (no US will be delivered) in Context A. At the end of the experimental session the purpose of the study will be explained to participants. This session will take place in the fMRI scanner at Wayne State University.

*C.4. fMRI:* All MR scanning will be performed on a 3.0 Tesla Siemens Verio whole-body human MRI scanner with an industry leading 32-channel head coil for superior image quality. A high-resolution structural image will provide precise anatomical localization. Whole-brain fMRI blood oxygen-level dependent (BOLD)-related signal measures will be acquired to measure task- and drug-related effects and to minimize susceptibility artifact (signal loss) at the medial temporal lobe (including the AMYG and the vmPFC)[141-143].

*C.4.1. fMRI Analysis:* Functional data will be processed and analyzed using conventional methods (GLM, event-related design, random effects) with Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London; www.fil.ion.ucl.ac.uk/spm)[144-147], previously described in in our pharmacological fMRI studies[91,94,148,149] and extinction recall studies[102,105]. Analysis will implement 2 complementary approaches[94]: 1) a hypothesis-driven anatomically-focused region of interest (ROI)-based analysis; and 2) exploratory whole-brain voxel-wise analysis. Based on our hypotheses, our two main ROIs are vmPFC and HPC; the AMYG, or at least different sub-regions within AMYG, are involved in fear acquisition[26,36,150] and extinction[25], and thus we identify the AMYG as an ROI *a priori.* These anatomically-defined atlas-based ROIs will be based on anatomical landmarks from human atlases[151-153]. In addition, vmPFC and HPC activation reported by Milad et al.[70] will be used to create a 5 mm radius spherical ROI around peak coordinates to examine data in this study for replication. Extracted BOLD percent signal change (PSC) from each ROI, confined to anatomically-based masks, will be subjected to a repeated measures group (PTSD, TEC, HC) x drug (THC, PBO) x stimulus (CS+E, CS+U) omnibus ANOVA during Extinction Recall. We will test for significant ($p < 0.05$) group x drug x stimulus; all significant main effects and interactions will be followed up by post hoc t-tests with an alpha level of 0.05, two-tailed. If appropriate, we will also include age, gender, and other variables (e.g., mood effects) as additional potential covariates in a separate ANCOVA. For the second approach (whole-brain, voxel-wise), we will employ the same ANOVA approach across the entire brain to search for effects in other areas in an exploratory search to generate hypotheses for subsequent study. Statistical maps for the whole brain analysis will be created using a threshold of $p < 0.001$ with a cluster threshold of 10 voxels. Within the ROIs, a small volume threshold ($p < 0.05$, corrected for multiple comparisons) will be used.

*The context-dependent renewal of fear is another factor that can potentially affect the efficacy of exposure therapy. Therefore, the analysis will also test whether THC can induce generalization of extinction across extinction recall (ABB) and renewal (ABA) contexts.* We will conduct the same statistical tests on extracted PSC from each ROI, confined to anatomically-based masks, during the Fear Renewal test, as described above for the Extinction Recall test.

We will also look at correlations between BOLD responses in the AMYG, vmPFC, and HPC and the extinction retention index (see below) during Extinction Recall and Fear Renewal tests. In addition, we will implement psychophysiological interaction analyses (PPI)[40,154,155] to measure task-dependent functional connectivity among the AMYG, vmPFC, and HPC. Separate connectivity analyses will be performed with each group x drug condition (PTSD-THC, PTSD-PBO, HC-THC, and HC-PBO), using vmPFC and HPC as the seed regions followed by between-group connectivity analyses to assess the effect of THC on functional coupling of the vmPFC, AMYG and HPC during Extinction Recall and Fear Renewal.

*C.5. Psychophysiology:* The primary assessment of fear learning will be a change in SCR, which will be recorded by electrodes attached to the middle phalanges of the second and third digits of the nondominant hand. AcqKnowledge software (BIOPAC Systems) will be used for off-line analysis. We will also obtain subjective US expectancy to the CS+E compared to the CS+U[103].

*C.5.1. Psychophysiological Analysis:* SCR for each CS trial will be calculated as described by Milad et al.[102]. Extinction recall will be assessed by comparing the mean differential response during the first four trials of the CS+E versus the first four trials of the CS+U. In addition, we will use the "extinction retention index" (ETI) as described by Milad et al.[102] to measure the magnitude of extinction retention. The ETI adjusts the SCR during extinction recall for differences in CR magnitude during acquisition[109]. Analyses will be conducted by ANOVA models as above (follow-up t-tests, $\alpha < 0.05$). In addition, we plan to compare and correlate (using Pearson or Spearman rank Correlational analyses) measures of BOLD activation (PSC) and SCR signal to the CS+E vs. CS+U within and across the two contexts/recall and renewal tests.

*C. 6. Power Analysis:* We have conducted several power calculations in our previously published work, used to determine the sample sizes proposed here. First we have consistently found that an *n* of 14-16 HCs and an *n* of 14-16 PTSD patients provide adequate power to observe: 1) vmPFC and HPC signal differences between groups during extinction recall; and 2) THC's effects on AMYG and vmPFC activation and functional connectivity in HCs. Given that the effect sizes tested within- and between-groups in previous studies have ranged between moderate – large (Cohen d 0.8-1.5). With the anticipated cohort 15-20 subjects per group (PTSD-THC, PTSD-PBO, TEC-THC, TEC-PBO, HC-THC, HC-PBO), who complete the entire protocol with usable fMRI and SCR data, this number will confer >90% power to detect moderate effect sizes expected from our significance testing.

***D. Additional Human Subjects Information***

*D.1. Probable Duration of the Study:* The study is intended to run for 4 years. During this time we expect to collect data from 120 usable subjects.

*D.1.1. Proposed Involvement Per Participant:* The total time commitment estimated per participant is 11 hours across all study visits. This is broken down below:

*Visit 1: Screening and Study Entry:* Approximately 3 hours
*Visit 2: Fear Acquisition:* Approximately 1 hour
*Visit 3: Fear Extinction:* Approximately 5 hours
*Visit 4: Fear Extinction Recall, Fear Renewal Test, and Debriefing:* Approximately 2 hours

*D.2. Location Where Research is to be Conducted:* The informed consent, diagnostic tests, and the fear acquisition behavioral session will be conducted in the research offices located at the EACPHS building. Functional imaging and the extinction learning, extinction recall/fear renewal, and debriefing session will be conducted at the WSU Magnetic Resonance Research Facility (MRRF), located in the basement of Harper University Hospital.

*D.3. Termination Criteria:* Participants may be terminated for any of the following: 1) alcohol or drug or psychoactive medication use that would interfere with dronabinol; 2) non-compliance with study protocol requirements; 3) development of an illness requiring that would exclude participation. Participants will be asked to withdraw from the study if they fail to appear for a session. It is imperative to the study that the sessions are consecutive and failure to appear during one of the sessions makes the data unusable. We will make every effort to ensure that the sessions are accessible (i.e., scheduling sessions during evening and weekend hours).

*D.4. Sources of Materials:* Research materials include the information that is obtained from the subjects from the diagnostic assessments/symptom-related interviews and questionnaires, clinical examinations including

medical history and mental health history data, behavioral data on performance on the behavioral tasks, and MRI, including structural/anatomic and functional raw data. These measurements will be performed solely for the purposes of research. All research materials will be maintained in strict confidentiality.

*D.4.1.Clinical Evaluation/Participant Materials:* The interview forms, paper and pencil questionnaires, and the laboratory assessment forms will be labeled with a participant code (not participant name) that is not readily identifiable to non-study staff. These forms will be kept in a locked cabinet where only official study staff can have access. All information obtained during the course of this study is strictly confidential. Our study staff will not divulge any information about interviews or other tests to non-study staff personnel. Data that may be reported in scientific journals will not include any information that identifies any person as a participant in this study.

*D.4.2. Psychophysiological and fMRI:* Data collected from SCR, US expectancy, and fMRI for each participant will be saved with a research identifier number only and stored in computer files without reference to any personally-identifiable information.

*D.4.3. Confidentiality:* All data from participants will be marked with a research identifier number only and kept in locked cabinets. No data will have participant names on them, except for consent forms, which will be stored separately from other questionnaires in a locked file cabinet. Paper records will be kept in locked file drawers in a locked room, to which only authorized research personnel have access. Confidentiality of participant records is assured by assigning each participant with a research identifier number/code, and such data, as well as behavioral/fMRI, are stored in computer files (except for a single tracking file) without reference to name, hospital registration number, or any other type of personally-identifiable information (e.g., birth date, social security number, etc.).

*D.4.4. Limits of Confidentiality on Clinical Information:* Confidentiality is limited, however, where there is either a danger to self or others. If the participant is discovered to be acutely homicidal or suicidal during the evaluation period the participant will be evaluated for hospitalization in a mental health facility (either voluntarily or involuntarily as necessary). If, for whatever reason, the participant is not hospitalized when it is determined that he/she is either homicidal or suicidal (e.g., we receive a phone call from the participant or another person) the police will be alerted to bring the participant to a psychiatric emergency room.

*D.4.5. Confidentiality of Breathalyzer/Drug Tests Prior to Scans or Assessment:* The results of the breathalyzer test and urine drug and pregnancy tests performed before sessions will remain confidential. The only individuals who will have knowledge of the results of these tests are research staff directly working on the project. The breathalyzer and urine tests will be performed at the beginning of the visit. If a participant fails the urine or drug test the first time they may be re-scheduled. If re-scheduled, there will be no documentation that the participants failed this test. If the participant fails the test a second time or refuses to be rescheduled for the visit, then it will be documented that the participant was “ineligible” for the study. This information will be stored in a secure computer database that uses participant codes (rather than names) as identifiers.

*D.5. Potential Risks and Data and Safety Monitoring Plan (DSMP):*

*D.5.1.Data integrity & Confidentiality:* Data entered by the research assistants will be closely monitored by the PI, investigators, and research coordinator and a spot check of data will also be done periodically by same to ensure the accuracy of all recorded data. All subject data is kept in databases that allow for the monitoring of data entry and changes. None of the databases contain identifiers linkable to the volunteers. The code for linking individual subjects with their data is kept in a separate location from their research files, under lock. These are accessible only to the PI, investigators, and personnel authorized by them. Image data collected during the performance of the studies is coded by case number and temporarily stored in firewall-protected servers in the MRRF. After transfer of the data to the image processing laboratory, this is stored in mirrored RAID arrays which are both firewall-protected and isolated from access outside the immediate local network. The RAID arrays are also used to store all other data from the volunteers, and are backed up daily with digital tape. Failure of up to two drives in the RAID array does not result in the loss of data. RAID array drives are hot-swappable, and back-up drives are available in the event that individual drives may fail.

*D.5.2.Safety Monitoring:* All Investigators and all other research personnel with subject or subject data contact will complete human subjects training. Every measure will be taken to protect subjects against even the rarest possible side effects. To protect against, or minimize any possible risks, we adhere to the following procedures: (a) Participants will be carefully screened to exclude those who are physically, neurologically, or psychiatrically at risk (see above); (b) Studies are conducted in laboratory areas (EACPHS building and MRRF) located nearby a hospital, where emergency assistance can be obtained; (c) A radiological technician

and research assistant are present during all fMRI sessions; and a physician associated with the study is available on-site or by pager at all sessions. Heart rate (HR) and blood pressure (BP) will be monitored regularly in the WSU MRRF, where we will employ MR-safe and MR-compatible monitors for heart rate and blood pressure; (d) Participants agree not to take any other drugs for 24 hours before and 12 hours following sessions, and compliance before the sessions is monitored by breathalyzer and urine tests; (e) Participants are instructed not to drive and should arrange alternate transportation through a family member/friend or a taxi will be arranged to take them home during Visit 3 (extinction learning); (f) A complete medical history, review of medications and physical symptoms/signs will be performed prior to entry into this study; (g) Participants with prior or current history of major medical or neurological disorders are excluded from participation. Effective screening should exclude participants who would be placed at greater risk. All of the testing will be done in the presence of medical supervision and in a facility specifically designed, equipped, and functioning to support these types of studies. Participants will be fully informed of all the possible side-effects that could be encountered the study protocol (i.e. dronabinol, MRI, behavioral tasks) and are free to drop out of the study at any time without cost. Participants will be informed that they do not have to participate if any procedure or questionnaire causes them discomfort. Subjects will be encouraged to contact the investigators if they notice any symptoms or untoward side effects. All the volunteers will have direct access to the phone numbers and pagers of the study coordinators, the primary investigator, Dr. Rabinak, and Dr. Kilgore, M.D., a Board-certified physician, or his physician designate, as well as a 24-hour contact number (emergency room services). This information is included in the copy of the consent forms provided to the subjects.

*D.5.3. Diagnostic/Phenomenological Procedures:* The structured diagnostic interviews and questionnaires may contain questions that concern behaviors and thoughts that may be embarrassing or sensitive in nature. Interviews will be conducted by experienced mental health workers that will maintain confidentiality and all data from interviews and questionnaires will be numbered to help conceal the identity of the participant. All participants will be informed that they are free to take a break at any time during the clinical interviews and questionnaires if they become bored, tired, or otherwise agitated. Participants will also be informed that they can refuse to answer any question that they feel is too personal or distressing.

*D.5.4. Dronabinol related risks:* Dronabinol is associated with some adverse experiences (incidence 1%-10%) including: asthenia, increases in heart rate, palpitations, facial flush, sensory impairment, headache, nausea, vomiting, dry mouth, changes in appetite, easy laughing, euphoria, restlessness, panic attacks, anxiety/nervousness, paranoid reaction, confusion, dizziness, drowsiness, and impairment in coordination. Paul Kilgore, M.D., a Board-certified physician, or his physician designate and will be available during all behavioral tasks in order to evaluate and recommend further evaluation and treatment for the emergence of any adverse events/side effects.

Participants taking psychoactive/psychotropic medications or medications that would interact with dronabinol (drug-drug interactions) will be excluded. Known medications that interact with cannabinoids/THC/dronabinol include: amphetamines, cocaine, other sympathomimetic agents; Atropine, scopolamine, antihistamines, other anticholinergic agents; amitriptyline, amoxapine, desipramine, other tricyclic antidepressants; barbiturates, benzodiazepines, ethanol, lithium, opioids, buspirone, muscle relaxants, other CNS depressants; disulfiram, fluoxetine, antipyrine, barbiturates; theophylline. Also, we will exclude participants with a known sensitivity to the active drug or capsule excipients, including cannabinoid oil, sesame oil, gelatin, glycerin, and titanium dioxide. Pregnant participants will be excluded form participation because there is insufficient data to assure safety of the fetus during dronabinol exposure, as well as during MR scanning. In addition, nursing mothers will be excluded from the study because dronabinol is concentrated in and secreted in human breast milk and is absorbed by the nursing baby (for reference see: http://www.fda.gov/ohrms/dockets/05n0479/05N-0479-emc0004-04.pdf).

Marinol®/dronabinol capsules is one of the psychoactive compounds present in cannabis, and is abusable and controlled [Schedule III (CIII)] under the Controlled Substances Act. Both psychological and physiological dependence have been noted in healthy individuals receiving dronabinol, but addiction is uncommon and has only been seen after prolonged high dose administration. Although any exposure to dronabinol may entail some risk for development of problems of abuse, this is highly unlikely in view of the fact that participants assigned to the dronabinol group will receive only a one-time dose of dronabinol, the careful screening of participants, and the laboratory setting in which studies are conducted. Dronabinol is administered only by the oral route and participants assigned to receive dronabinol (half of the participants) will only receive dronabinol on one occasion. There is no evidence that participation in controlled laboratory studies such as these increases the risk for developing substance use problems[139,140,156], including those conducted in

laboratories (with 20+ years of experience) from which the current protocol is based[91,94,136]. There are low social, legal or psychological risks associated with ingestion of dronabinol as a volunteer in this research study. Because the participants are physically healthy volunteers there are no alternative treatments. Participants will be fully debriefed following the study. During debriefing, any questions participants may have will be answered and participants will be informed whether they received placebo or dronabinol.

*D.5.5. Behavioral Tasks:* The US given during the fear conditioning procedure will be uncomfortable and aversive (e.g. loud noise [103]; a mild electric shock to the wrist[36,101,102,104-110]), however not painful and participants will not know that the US will only be delivered during the first session of the experiment and on several but not all trials. There is little risk to participating in the other behavioral tasks (viewing emotional faces, negative images) other than boredom or mild subjective anxiety. Study staff will be present during all tasks and participants may communicate with them at all times, including when the participant is in the MRI scanner. A medical clinician on this study (e.g. clinical psychiatrist or psychologist) will be available during all behavioral tasks in order to evaluate and recommend treatment for the emergence of any anxiety/panic attack, elevated levels of anxiety, changes in vital signs (heart rate and/or blood pressure), or emotional discomfort.

*D.5.6. Magnetic Resonance Imaging:* Magnetic resonance imaging is non-invasive, widely used, and safe. The potential risks such as static magnetic field, radio-frequency field, magnetic field gradients, and acoustic noise are rarely dangerous or life threatening. Prior to inclusion in the study, the presence of potential MR risks, such as pacemakers, surgical clips, metallic surgical devices, and/or other irremovable ferrous-containing materials will be excluded. There is a minor risk of discomfort or anxiety from being in the confined space of the MRI scanner. If the participant were to experience anxiety/panic, the study would be terminated and the participant would receive counseling from a physician (available during all scans). In addition, follow-up telephone calls would be made within 3 days of an episode to confirm the transient nature of their reaction. Before the participant enters the bore of the MR magnet, he/she is always reminded that he/she is free to stop the study at any time if he/she is uncomfortable. The participant will be able to communicate with the MR technologist/operator and research staff via an intercom and may self-trigger an alarm at any time to stop the scanner and alert the research staff. Our research staff and the MRI Lab staff will provide pads and blankets to make the participant as comfortable as possible. The participant will be able to talk to research staff throughout the study, and will be able let the staff know right away if they want to stop the study and get out of the scanner. The MRI scanner makes loud, vibrating noises. The participant will wear foam earplugs and headphones to reduce the loud noises made by the scanner and prevent any hearing damage. Some studies, like this one, have the potential to cause "peripheral nerve stimulation" (PNS). PNS is a light touching sensation on the skin surface, lasting only for a few seconds. It may cause mild discomfort, but is not harmful to the participant. The MRI machine is operated within FDA guidelines so the potential for inducing PNS is low. Sometimes, participants report a temporary, slight dizziness, light-headedness or nausea during or immediately after the scanning session. If the participant feels dizzy or light-headed, the research staff will have the participant get up slowly from the scanner. Because the strong electromagnetic fields can move metal objects and cause heating, there is a risk that loose objects (jewelry, keys) outside the participant's body could be accelerated by the magnetic field and strike him or her, causing injury. There is also a risk that the magnetic fields could disturb a metal fragment in the participant's body, interfere with an implanted device, such as a pacemaker or neurostimulator, or cause metal (including foil-backed medication patches) on or in the participant's body to heat up, causing the participant harm. The research staff and MRI Laboratory staff keep the environment around the MRI scanner completely free of loose metal objects that could be moved by the magnetic field, and the staff will make sure that the participant has no metal on his or her body that could be affected by the MRI scanner. The research staff will also ask the participant questions and have the participant complete an MRI screening form to make sure that the participant has no metal inside his or her body that would cause him or her harm during the MRI scan. During the formal consent process all participants will be informed about the potential risks of discovering an incidental finding or abnormality on their MRI scan. Many such abnormalities are not clinically significant, but the participant may need or want to investigate them further. If an abnormality is found in a participant's MRI scan, the PI will contact the participant and refer the participant for medical follow-up for the problem if the participant requests, including a referral to a primary care physician. If a participant has a primary care physician, the PI will contact the participant's doctor, at the request and with permission from the participant, to inform him/her of the finding on the MRI scan and to help him/her to get the participant appropriate follow-up. The decision as to whether to proceed with further examination and/or treatment lies solely with the participant and his/her primary care physician and would not be paid for by the investigators, the sponsor, or Wayne State University.

The MRI scanning procedures are experimental, but they follow the guidelines established by the U.S. Food and Drug Administration for MRI scanning. Care will be taken to avoid all known risks associated with MRI. However, this procedure may involve risks that are currently not anticipated. The participants will be constantly monitored for any side effects and will be treated appropriately be physicians and nurses available. The study may be aborted if the participant has any discomfort. The safety of the participants will be continually monitored.

*D.5.7.Ongoing DSMP by PI and Staff:* Weekly meetings of the research staff of this study will be conducted that will include review of accrual, consenting procedures, protocol adherence, adverse events, and quality control of all data obtained from the study in the previous week. Minutes of these meetings will be recorded, signed by the PI, and archived in study's regulatory binder. All changes in protocol design will be reviewed by the IRB at WSU before such changes in protocol design take place.

*D.5.8.Reporting of Adverse Events:* Adverse events (AEs) will be recorded and tracked for this study. AEs will be reported per IRB and NIH/NIMH requirements. An AE is any experience that has taken place during the course of a research project, which, in the opinion of the investigators, was harmful to a subject participating in the research, increased the risks of harm in the research, or had an unfavorable impact on the risk/benefit ratio. AEs will be graded using the mild, moderate, severe terminology as defined:

**Mild** – Noticeable to the subject, does not interfere with the subject's daily activities, usually does not require additional therapy, dose reduction, or discontinuation of the study.

**Moderate** – Interferes with the subject's daily activities, possibly requires additional therapy, but does not require discontinuation of the study.

**Severe** – Severely limits the subject's daily activities and may require discontinuation of the study. This would include all adverse events defined as "Serious" by the IRB. The PI will assign attribution as definitely associated, probably associated, possibly associated, or unrelated. Adverse events will also be recorded as expected or unexpected.

All adverse events (AEs) occurring during the course of the study will be reported to the PI. All AEs will be evaluated, medically treated or referred to medical treatment, and followed until resolved satisfactorily. If deemed necessary by a physician, a participant may be withdrawn from the study. AEs will be evaluated for serious adverse event (SAE) criteria. A Serious Adverse Event (SAE) is any adverse experience occurring during the study that: (a) results in death, (b) is life threatening (e.g., suicidality, homicidality), (c) results in hospitalization or prolongation of hospitalization, or (d) results in persistent disability. If a participant from the study or the PI discontinues a participant's participation due to a SAE, the participant will receive follow-up medical care as necessary. Follow-up care will continue until the participant no longer requires hospitalization, the condition is stabilized with no future change expected, the problem is determined to be unrelated to the drug used in the study, or the participant dies. Finally, if considered related to the trial, unanticipated problems involving risks to subjects or others will be reported to both the IRB and NIH/NIMH.

All SAEs and/or unexpected AEs will be reported to the IRB and NIH/NIMH within 7 days of occurrence or recognition. Fatal or life-threatening adverse events will be reported to the above institutions within 24 hours. Regular annual reviews of protocol activity and all AEs will be submitted to the IRB and NIH/NIMH. Other less serious and expected AEs will also be reported to the above institutions with compliance to their requirements.

*D.5.9.Persons responsible:* At Wayne State University PI Rabinak, Co-Investigators, and the Project Coordinator will be responsible for overseeing data integrity, safety monitoring, and reporting of adverse events.

*D.8. Payment to Subjects:* Participants who complete the study will be paid up to $110 for their participation. Participants are compensated for what they complete based on the following breakdown: $30 for Visit 1; $10 for Visit 2; $50 for Visit 3; $20 for Visit 4

*D.9. Potential Benefit of the Proposed Research to Human Subjects and Others:* There will be no direct benefit to participants for participation in this study, other than; they will be compensated for their time for participation in this study. There will be no cost to participants associated with participation in this study other than transient discomfort.

*D.10. Importance of the Knowledge to be Gained:* The risks involved in this study are minimal when compared to the benefits gained. Benefits to society include the potential for increased knowledge regarding the effects of the cannabinoid system on emotional memory and the brain, which may help us treat anxiety disorders,

such as PTSD. Current knowledge from studies in animal models and healthy humans of aversive learning and memory suggest that THC facilitates the extinction of fear memories after a traumatic event. The use of Marinol®, a synthetic form of THC, in PTSD patients may also provide similar effects and if so, will prompt the development of clinical trials in which dronabinol is used as an adjunct to exposure-based therapies to facilitate treatment in patients with anxiety disorders. Currently Marinol® is prescribed to humans to treat anorexia associated with weight loss in patients with AIDS and nausea and vomiting associated with cancer chemotherapy. We expect that the medication when given only once at the specified dose (7.5 mg) in a closely monitored laboratory setting will be well tolerated by participants without SAEs and carries little risk for development of subsequent problems including abuse/dependence. This expectation is supported by the fact that we have not had any SAEs with this dose and design in our past extinction studies in healthy humans[95,137]. We believe that the risks are minimal and the benefits are substantial.

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