RESEARCH PLAN

A. SIGNIFICANCE

A.1. Problem to be addressed. An estimated 7% to 12% of people exposed to a traumatic event will develop Posttraumatic Stress Disorder (PTSD)[1,2], a stress-induced illness characterized by excessive, inappropriate fear responses (e.g., hypervigilance, flashbacks)[3]. The burden of PTSD on this population and on society is great, given those with the disorder are at increased risk for suicidality[4], experience loss of productivity[5], require more health care resources[6], are more likely to develop medical illness[7], and have dysfunction in occupational[8], interpersonal[8-11], and parental/family domains[12,13]. PTSD symptoms include impairment in fear extinction, as memories of an aversive experience are strongly held in PTSD well after the trauma has occurred. Additionally, PTSD is due in part to decreased activation in the ventromedial prefrontal cortex (vmPFC) and hippocampus (HPC), brain regions involved in fear extinction and recall of extinction learning (i.e., extinction retention)[14-28]. Consequently, a pharmacological agent that restores vmPFC-HPC function has the potential to reduce PTSD symptoms in the meditext of treatment that encompasses fear extinction.

Prolonged Exposure (PE) therapy, a first-line psychotherapy for PTSD[29], utilizes extinction learning principles. It aims to generate new, safe memories by repeatedly exposing the patient to feared objects, memories, images, and situations to integrate disconfirming/corrective information that will diminish trauma-related fears[30]. This cognitive processing may be a critical part of extinction retention over time in PE. Thus, effective exposures are crucial for treatment success. The beneficial effects of exposure therapy have been shown to derive from neurophysiological processes typical of fear extinction. Specifically, significant post-therapy increases in PFC activity in conjunction with decreases in amygdala (AMYG) activity[31]. Even though PE produces clinically meaningful improvements for many PTSD patients there is still substantial room for improvement. Many patients prematurely discontinue PE (e.g., 13-39%;[32-35]). In those that do show improvements in PTSD symptoms 60-72% of patients continue to meet criteria for PTSD after completing PE[36-40], which includes repeated exposures to trauma memories (imaginal exposure) and avoided situations (in vivo exposure) and practice exposures (e.g., listen to tapes of imaginal exposure, carry out in vivo exposure) outside of PE sessions as "homework". *PTSD remains a difficult-to-treat disorder, thus, a medication that enhances the neural and neurochemical substrates of extinction and recall of extinction learning could solve this challenge and improve PTSD treatment outcomes*[41-43].

Exciting new evidence from studies in rodent models of fear suggests that activation of the cannabinoid (CB) system specifically within the AMYG, vmPFC, and HPC may regulate fear extinction and recall of extinction learning. For instance, drugs that block CB type 1 (CB1) receptors or genetic deletion of CB1 receptors within these structures prevent recall of extinction learning, whereas activation of these same receptors, via agonists, such as Δ^9-tetrahydrocannabinol (THC), can promote recall of extinction learning[44-54]. In addition, drugs that increase endogenous CB (eCB) levels during fear extinction not only enhance recall of extinction learning, but also impair the return of extinguished fear in rats (i.e., prevent spontaneous recovery of fear)[44]. Recently, PI Rabinak showed that administration of dronabinol (synthetic THC) prior to fear extinction facilitated the subsequent recall of extinction learning in healthy humans using a similar behavioral design as proposed in the current R61 project[55]. Together, rodent and human data indicate that THC modulates neural correlates of fear extinction and its recall, therefore, a critical next step is to examine the efficacy of THC to facilitate recall of extinction learning through activation of the fear extinction circuitry in individuals with PTSD. *In the proposed project we will test fear extinction circuitry as a pharmacological target for THC's actions in PTSD.*

A.2. What neural circuits mediate fear extinction? Convergent evidence from rat and human work has revealed that discrete, yet anatomically and functionally interconnected brain structures are critical for fear extinction and recall of extinction learning (i.e., AMYG, vmPFC, HPC)[14-28,56-59]. During fear acquisition, sensory information about the conditioned stimulus (CS) and the aversive unconditioned stimulus (US) converge in the AMYG and become associated (i.e., yielding the fear memory) and produce conditioned responses of fear (CRs)[18,56]. In functional magnetic resonance imaging (fMRI) studies AMYG activation has been correlated with physiological fear responses (e.g., skin conductance response [SCR]) during conditioning in human participants [LaBar et al[60]: $r = 0.88$, $p < 0.03$; Phelps et al[20]: $r = 0.64$, $p < 0.05$; Phelps et al[61]: $r = 0.59$, $p < 0.05$]. Notably, the AMYG also seems to be involved in extinction learning[19,57,58]. Prefrontal brain regions that interconnect with the AMYG, particularly the vmPFC, are important for the recall of extinction learning and consequent attenuation of fear CRs perhaps by inhibiting AMYG output neurons[20-25,59]. In humans, vmPFC activation during recall of

extinction learning and vmPFC thickness both correlate with magnitude of extinction recall [activation: Milad et al[26]: $r = 0.66$, $p < 0.01$; thickness: Milad et al[62]: $r = 0.52$, $p < 0.03$; Hartley et al: $r = 0.83$, $p < 0.003$] . In addition, the magnitude of task-dependent functional coupling between the AMYG and vmPFC has been shown to be negatively correlated with self-reported intensity of negative affect during regulation of negative affect[63,64]. Similarly, HPC activation is associated with successful recall of extinction learning[26,65] and is positively correlated with vmPFC activation during extinction recall in humans [Milad et al[26]: $r = 0.94$, $p < 0.0001$]. Interestingly, increased AMYG-HPC functional connectivity has been attributed to the persistence of memories for emotionally arousing events in humans[66-70]. *These lines of convergent evidence suggest activation in these brain regions and how they interact with one another are quantifiable brain-based indices of fear extinction and recall of extinction learning in humans.*

A.3. What brain regions are involved in the pathophysiology of PTSD? Dysfunction in fear circuitry has been a consistent finding in PTSD neuroimaging studies and plays a role in maintaining trauma memories. Using a Pavlovian fear conditioning paradigm, Milad and colleagues showed a PTSD group exhibited reduced vmPFC and HPC activity, along with greater psychophysiological reactivity (SCR, a commonly used index of arousal) when attempting to recall extinction learning (i.e., 24 hr post-extinction learning). Thus, signifying impaired retrieval in extinction learning to the previously extinguished conditioned stimulus (CS+E) compared to trauma-exposed controls without PTSD (TEC)[71]. Similarly, PTSD patients show impaired extinction recall with increased SCRs and AMYG activity to the CS+ (i.e. CS that was paired with US)[72]. Of note, the relevance of testing recall of extinction learning pertains to the phenomenon of spontaneous recovery in which previously extinguished fears diminish over time[73]. Other neuroimaging studies of emotion processing have shown the AMYG, also crucial to generating fear reactions[74], is hyperactive in PTSD in response to trauma-related imagery[75,76], combat-related sounds or smells[77-79], trauma-related photographs or words[80-83], and fearful facial expressions[84-87]. With regard to regulation of emotions, our group has shown prefrontal dysfunction in PTSD in dorsolateral prefrontal cortex (dlPFC) and medial prefrontal cortex (mPFC)[88], regions crucially involved in regulating emotion particularly during willful emotional regulation of negative affect evoked by aversive stimuli[25]. Beyond emotion processing and regulation, exaggerated AMYG reactivity observed in PTSD has been posited to be at least partly due to insufficient top-down regulation from the vmPFC, consequently leading to hyperarousal and deficits in extinction recall as well as inability to suppress attention and responses to trauma-related stimuli[89-92]. For example, exaggerated AMYG reactivity is negatively correlated with responses in the dorsal and vmPFC among individuals with PTSD[76,85]. 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[76,93-98]. *Evidence of aberrant activation in fear circuitry in humans with PTSD indicates a mechanism that directly impacts PE success, since treatment relies on activation of fears[89,99-105].*

A.4. Can THC enhance recall of extinction learning? As mentioned previously rodent models of fear suggests activation of the CB system within the AMYG, vmPFC, and HPC may regulate fear extinction and recall of extinction learning and impair the return of extinguished fear[4948-58]. In line with these findings PI Rabinak et al.[55] demonstrated (using procedures similar to this proposed R61 project) that an acute oral dose of THC administered prior to fear extinction facilitates recall of extinction learning in healthy humans. Participants who received placebo (PBO) during fear extinction exhibited, as expected, spontaneous recovery of fear to the CS+E, whereas THC (7.5mg) attenuated spontaneous recovery of fear during a recall test of extinction learning (Fig 1). THC did not affect within-session fear extinction, but only influenced the ability to successfully recall extinction learning when compared to PBO, suggesting THC affects the ability to maintain and/or successfully retrieve extinction memories. These findings are consistent with pre-clinical studies in rats in which CB activation and/or enhancement can facilitate recall of extinction learning[45,47,49,50,52,53,106] and *provide the first evidence that pharmacological enhancement of recall of extinction learning is feasible in humans using CB system modulators.*

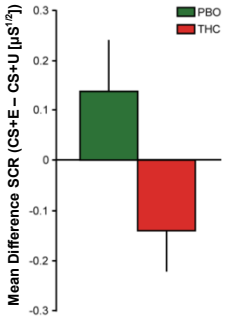

Fig. 1: THC reduces fear recovery during extinction recall; Rabinak et al.[55]

A.5. What neural correlates of fear extinction and recall of extinction learning are a target of CBs? In the brain, eCBs from the postsynaptic cell diffuse in a retrograde fashion to activate presynaptic CB1 receptors, densely localized within AMYG, vmPFC, and HPC[107-110], which then inhibit presynaptic release of neurotransmitters[47,111]. It has been hypothesized that during fear extinction, eCB activation of CB1 receptors in

the AMYG decreases activity in local gamma-aminobutyric acid (GABA) networks, which leads to disinhibition of principal neurons and to extinction of fear CRs[47,51]. Interestingly, intra-basolateral AMYG infusion of CB1 agonists enhances recall of extinction learning (via effects on memory consolidation)[112]. On the other hand, activation of CB1 receptors within the vmPFC during fear extinction induces neuronal plasticity within the vmPFC, and subsequently increases inhibition of brain areas involved in the expression of conditioned fear responses (e.g. AMYG)[49]. In addition, HPC CB1 receptor activation enhances glutamatergic neurotransmission, which may support long-term storage of fear extinction (via memory consolidation)[46].

Studies in PI Rabinak's lab using fMRI found that oral THC (vs. PBO) modulates mPFC-AMYG activation and connectivity during emotional processing[110]. Although not directly related to fear extinction, these data show that THC effects on brain response to threat can be localized to fear-related AMYG-mPFC circuitry. PI Rabinak also investigated CB effects on the underlying neural circuitry involved in healthy volunteers' recall of extinction learning; the fMRI results suggest THC administration during fear extinction subsequently increases vmPFC and HPC activation to the CS+E vs. non-extinguished conditioned stimulus (CS+U) during a recall test of extinction learning relative to PBO[113] (Fig. 2). Collectively, these exciting findings suggest that *activating CB1 receptors within select neural circuitry can enhance efficacy of fear extinction and recall of extinction learning. These findings lead to our proposed translational investigation in patients with PTSD, who exhibit impaired recall of extinction learning and associated aberrant vmPFC-HPC function.*

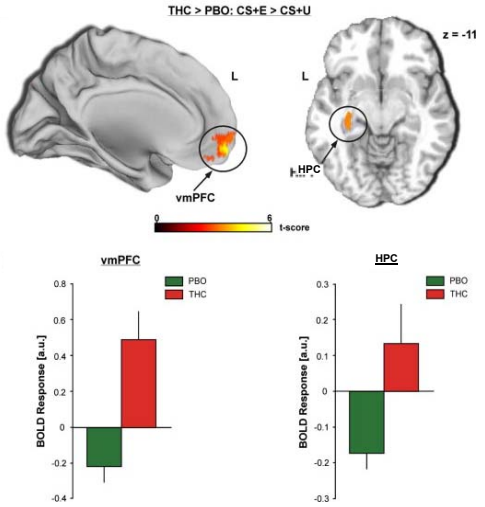

Fig. 2: THC increases vmPFC and HPC activation during recall of extinction learning recall; Rabinak et al.[113]

A.6. Can cannabinoids facilitate fear extinction in PTSD? As noted, extinction retention deficits and vmPFC-HPC dysfunction have been observed in patients with PTSD, and enhancing CB transmission helps extinction recall. Thus, the CB system, which modulates brain activity in regions involved in recall of extinction learning, is a promising mechanism for improving learning that occurs in therapy and may increase the efficacy and durability of PE in treating PTSD (e.g., shortening treatment while strengthening and prolonging gains). Preliminary data from PI Rabinak's K01 suggests an acute dose of THC (7.5mg) given prior to fear extinction in PTSD participants facilitates recall of extinction learning to a CS+E (Fig. 3). *Therefore, the primary goal of the proposed project is to test the hypotheses that (1) administration of an exogenous CB1 agonist will 'rescue' deficits in recall of extinction learning in PTSD patients and (2) these effects will be mediated by increased activation of vmPFC and/or HPC.*

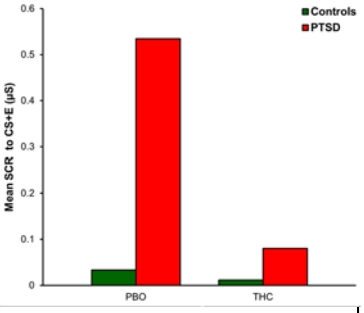

Fig. 3: THC facilitates recall of extinction learning in PTSD

B. INNOVATION

This project is innovative in several ways. First, it is *translational* as the aims, predictions, and study design are propelled by compelling preclinical and clinical neuroscience findings showing that the CB system regulates recall of extinction learning. Second, it is *interdisciplinary* – strategically combining multiple lines of investigation—psychopharmacology, neuroimaging, psychophysiology, and a psychosocial intervention trial (often carried out separately). Third, it tests multiple layers of 'target engagement' including behavioral and neural correlates (recall of extinction learning). Studies using other "cognitive enhancers", such as D-cycloserine, have reported mixed results regarding their efficacy in facilitating exposure therapy[114,115]. However, recent evidence suggests that dose and dose timing differences are largely responsible for these mixed findings[115,116]. Additionally, there are a number of different pharmacological pathways to access the circuits that underlie fear extinction and specific classes of compounds, like THC, may be superior to others in their ability to facilitate fear extinction[117]. Dose and dose timing will be evaluated in the R61 phase

C. APPROACH

C.1. Preliminary Studies. The data here establish the capacity of the research team to carry out: 1) fMRI studies of fear extinction and THC challenge in humans; and 2) extinction studies in PTSD.

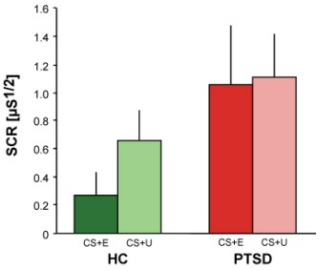

Fig. 4: SCR in healthy controls and PTSD during recall of extinction learning

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

Milad and colleagues have developed a fear conditioning-extinction paradigm in which PTSD patients (vs. trauma-exposed non-PTSD controls) show less activation in vmPFC and HPC and increased arousal (i.e., increased SCRs) during extinction recall to the CS+E vs. CS+U[71]. Similarly, PI Rabinak has pilot data showing fear recovery (increased SCR to the CS+E=CS+U) in PTSD patients (n=14) compared to healthy controls (HCs; n=15) during an extinction recall test 24 hr after extinction learning (Fig. 4).

THC Facilitates Recall of Extinction Learning via Increased Recruitment of the vmPFC-HPC

We have validated that our Pavlovian fear conditioning paradigm is effective in the context of acute pharmacological manipulation[55,113] (Figs. 1, 2, 5, & 6). PI Rabinak demonstrated that THC facilitated retention of extinguished fear responses and prevented spontaneous recovery of fear in healthy humans[55] (Fig. 1). In a separate fMRI study using a similar design as the one proposed, PI Rabinak showed that THC (vs. PBO) during fear extinction subsequently increases vmPFC-HPC activation to the CS+E (vs. the CS+U) during recall of extinction memory[113] (Fig. 2). Additionally, preliminary data suggest THC administration during fear extinction subsequently increases vmPFC-HPC functional coupling (psycho-physiological interaction; PPI) to the CS+E (vs. CS+U) during recall of extinction memory (vs. PBO; Fig. 5).

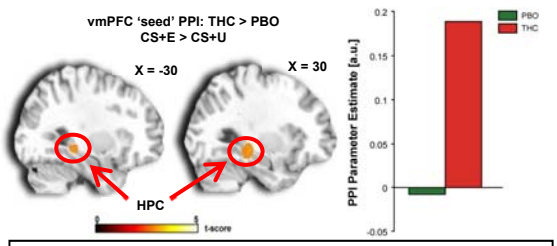

Fig. 5: THC increases vmPFC-HPC functional connectivity at extinction recall

We have also demonstrated THC facilitates extinction processes in health participants (THC n=18, PBO n=22) as signified by less SCR (Fig. 6A) and greater bilateral HPC activation during fear extinction ("EXT") and recall of extinction learning 24 hr ("REC1") *and* one-week ("REC2") post-extinction learning (Fig. 6B and C).

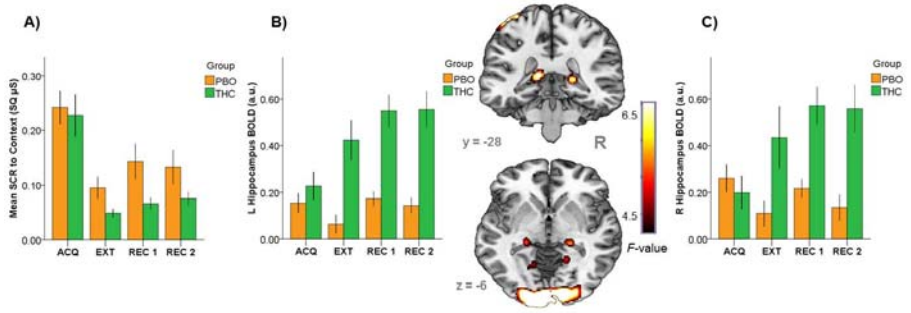

Fig. 6: Effects of THC and PBO on SCR and HPC response during fear acquisition, fear extinction, and recall post 24 hr and 1 week after extinction learning.

C.2. Overall Study Design: The R61 study will employ a randomized, double-blind, PBO-controlled, between-subjects design, coupling a standard Pavlovian fear conditioning paradigm during fMRI with an acute pharmacological challenge using oral dronabinol (THC) or PBO *prior to fear extinction* in PTSD patients and test recall of extinction learning 24 hr (proximal memory) and 1 week later (distal memory) when spontaneous recovery is expected to be robust. 78 PTSD participants will be randomized to 1 of 3 conditions: single doses of 5mg or 10mg of THC, or PBO, using covariate adaptive randomization[118-122], in which a new participant is sequentially allocated to a treatment condition by taking into account baseline characteristics (e.g., CAPS-5, age, sex, race) and previous assignments of participants[123], with 26 PTSD participants per condition. However, because randomization does not guarantee comparability across groups, the groups will be compared to ensure reasonable similarity in the distributions of their baseline characteristics. The imbalance in covariate levels will be measured by a χ^2 goodness-of-fit test (for categorical covariates) or t-test (for quantitative covariates. Variables with significant group differences ($p<0.05$) will be included as covariates in primary analyses. Additionally, 26 healthy trauma-exposed controls (TEC; demographically matched) will be included and tested at comparable time points to separate the impact of trauma from the impact of PTSD. The TEC group will not receive THC, any drug-related monitoring, or undergo blood draws but will otherwise complete all phases of the

experiment. With the conservative anticipation that about 25% will fail to complete all parts of the study, we estimate about 20 PTSD subjects in each condition (60 PTSD participants in total) and 20 TEC participants in total. Participants will be recruited from the Detroit community and mental health clinics (**see 'Protection of Human Participants'**).

Key inclusion criteria: All participants must be age 18-60, able to consent to the study, generally medically and neurologically healthy, and endorse exposure to a Criterion A stressor (defined by clinician-administered PTSD scale for DSM-5; CAPS-5). In addition, PTSD candidates must have significant PTSD severity as indicated by a clinician-administered PTSD scale for DSM-5 (CAPS-5) diagnosis and/or score of ≥ 25 regardless of trauma type as effect size of PE has been shown to be similar across trauma types (i.e., combat/terror, childhood sexual abuse, rape, mixed[124]). TECs must be free of any history of PTSD symptoms (CAPS-5 score < 25 and no diagnosis). **Key exclusion criteria:** Participants in either group with the following are excluded from the study: a) clinically significant medical or neurologic condition; b) MRI contraindications (metal objects in body or claustrophobia); c) positive urine pregnancy test or self-reported current pregnancy identified during screening procedures; d) pervasive development disorder history; e) traumatic brain injury (TBI) with current cognitive impairment related to TBI; f) exclusively left-handedness; g) current severe alcohol or substance use; h) risk of harm to self or others that requires immediate intervention; i) lack of fluency in English; j) current or past diagnosis of bipolar, schizophrenia, or psychotic disorder; or k) presence of contraindications, current or past allergic or adverse reaction, or known sensitivity to cannabinoid-like substances. In addition, PTSD participants with the following will be excluded: a) comorbid mood or anxiety disorder that is *primary* to PTSD (i.e. when treatment is indicated for symptoms beyond PTSD such as major depressive disorder, which does not involved exposure to fears[125]); b) concomitant treatment with medication that has level 1 evidence indicating severe drug-drug interactions with dronabinol that is taken daily. (**see c'Protection of Human Participants'**). TEC's with current diagnoses of any obsessive-compulsive, trauma/stressor-related, depressive, or anxiety disorder will be excluded.

C.2.1. Pavlovian Fear Conditioning Paradigm: This fMRI protocol uses an event-related design developed and validated in Dr. Rabinak's lab[113] (Fig. 5A). During fear conditioning (Context A, 'garden'), two conditioned stimuli (CS+s: e.g. blue and red messenger bags) will be paired with the unconditioned stimulus (US: animated snake striking at participant when bag is opened; Fig. 5B) at a partial reinforcement rate of 60%. One CS+ will be extinguished during the subsequent extinction phase (CS+E; blue) whereas the other will not (CS+U; red). A third CS (e.g. yellow messenger bag) will be presented during conditioning *but never paired* with the US (CS-; empty bag when opened). The US will co-terminate with the CS presentation. Approximately, twenty-four hours after fear acquisition, but no more than 1 week later, participants will undergo extinction learning. Based on the known time course of THC's peak subjective effects and peak plasma levels of THC[55,110,126-128], we will begin extinction learning ≈120 min after ingestion of either THC or PBO capsule. During extinction learning (Context B, 'art gallery'), participants will receive of the CS+E (no snake when bag is opened) and CS-. To assess extinction retention, we will conduct a proximal extinction recall test approximately 24 hr after extinction learning, but no more than 1 week later, (Extinction Recall Visit 4 and distal extinction recall test approximately 1 week after the first

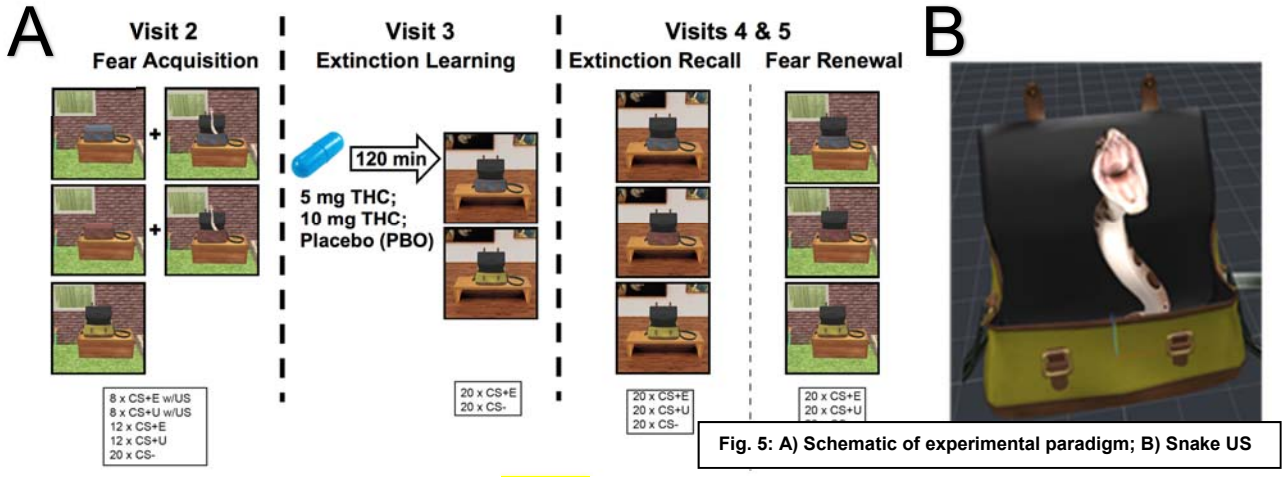

Fig. 5: A) Schematic of experimental paradigm; B) Snake US

extinction recall test, but no more than 3 weeks later (Extinction Recall Visit 5), in which participants will be presented with the CS+E, CS+U, and CS- (no snake) in Context B. Contrasting CS+E and CS+U during extinction recall in Context B will allow us to assess physiological and brain responses specific to recall of extinction learning and independent from recall of fear acquisition. To test for contextual renewal of fear (context shift from the extinction context [B] to the conditioning context [A]), we will conduct a proximal fear renewal test approximately 24 hr after extinction learning, but no more than 1 week later (Fear Renewal Visit 4) and a distal fear renewal test approximately 1 week later, but no more than 3 weeks later (Fear Renewal Visit 5), in which participants will be presented with the CS+E, CS+U, and CS- (no snake) in Context A. Stimulus parameters will be similar to those conducted in our previous studies[113]. During all sessions, our primary measures of conditioned fear will be SCR magnitude and US expectancy ratings for each CS trial. Extinction Learning (Visit 3) and Extinction Recall/Fear Renewal Visits 4 & 5 will be conducted in the MRI scanner with simultaneous recording of SCR and US expectancy ratings, except in the event a participant is unable to be scanned, in which case these visits will be conducted outside the scanner in the PI's research laboratory. Fear acquisition (Visit 2) will be conducted outside of the scanner in the PI's research laboratory. During all sessions, our primary measures of conditioned fear will be SCR magnitude and US expectancy ratings for each CS trial[129].

Genotyping: DNA extraction and genotyping of the rs324420 variant will be completed in Dr. Burghardt's laboratory (see letter). If the participants signs the DNA consent form, DNA will be extracted from buccal swabs using Quiagen buccal DNA kits.

C.2.2. Psychological Measures: Along with standardized assessments of symptom severity and mood/ emotional state (**see 'Protection of Human Participants'**), participants will complete measures at multiple time points (e.g., before and after fear extinction), of perceived success (e.g., reduction Subjective Units of Distress Scale; "SUDS"[130]) in addition to measures that evaluate temporal changes in mood/anxiety level, such as, the state subscale of the Spielberger Trait/State Anxiety Inventory[131]. Subjective drug effects will be conducted throughout the extinction session (Visit 3), when THC/PBO is ingested (**see 'Protection of Human Participants'**).

C.2.3. THC Concentration: Plasma concentrations of THC, 11-OH-THC (THC's active metabolite), and THC-COOH (THC's inactive metabolite) will be quantified in PTSD participants at 9 time points: 1) before receiving THC or PBO on Visit 3; 2) at approximately 30 min intervals after receiving THC or PBO on Visit 3 (i.e., at 30, 60, & 90 min), immediately before going in the MRI scanner, soon after coming out of the MRI scanner; and once before the end of the visit; 3) before recall of extinction learning on Visit 4; and 4) before recall of extinction learning approximately a week later (but no more than 3 weeks later; Visit 5). Blood will be obtained via venipuncture using a 22 or 23g straight needle or a 20-22g shielded IV catheter (MRI compatible) which is appropriate for a longer indwelling time. At each blood draw time point approximately 3mL of blood will be drawn from a vein in the participant's arm or hand by a certified phlebotomist on the research team. Blood concentration levels will be determined using ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS)[132]. Based on previous studies we expect peak plasma THC concentration will occur ≈120 min post-administration[55,110,126-128]; therefore, we will use these samples to confirm timing of peak concentration levels and if necessary adjust the timing between ingestion of THC/PBO and fear extinction to coincide with peak levels. In the event that we are unable to obtain adequate blood samples from a participant this will be noted in the participant file and all other study procedures will proceed.

C.2.4. fMRI: MR scanning at WSU will be performed on a 3.0 Tesla Siemens Magnetom Verio System using an industry-leading 32-channel head-coil for superior image quality. We will use a multi-band EPI sequence [similar to: TR = 2000ms; TE = 30ms; Flip = 73°; 128x128 matrix; FOV = 256 mm; 66 slices; 2.0 x 2.0 x 2.0 mm voxels], which we are currently using in PI Rabinak's K01 project. 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)[133-135].

C.2.5. General Statistical Approach: All analyses will use a conventional alpha level of 0.05 and all tests will be two-tailed. To evaluate the main effects, Analysis of Variance will be used for neuroimaging (i.e., BOLD percent signal change (PSC)) and peripheral measures of fear (e.g., SCR, US expectancy, SUDS, psychological/mood measures). We will also include covariates such as gender and childhood trauma/adversity

if found unbalanced and correlated (r>0.03) with the outcome variable (e.g., MANCOVA, ANCOVA). If significant, multivariate analysis will be followed by univariate analysis, which if significant, will be followed-up with simple effects analysis (e.g., independent t-tests, paired t-tests). In addition to omnibus tests, planned comparisons will be used to test specific hypotheses such as THC dose (vs. PBO) on fear extinction in PTSD, as well as comparisons between THC doses; if there is no significant difference between THC doses we will examine potential positive trends with increasing dose. To evaluate relationships, Pearson correlations will be used along with scatterplots to examine the nature of the relationships. Hochberg step-down multiplicity adjustment will be used to control for all multiple comparisons[136]. Confidence intervals and effect sizes will be also be calculated.

C.2.6. 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)[137-140], as described in our pharmacological fMRI studies[110,113] and fear conditioning studies[113,141,142]. Analysis will implement 2 complementary approaches[127]: 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[20,60,61] and extinction[19], and thus we identify the AMYG as another *a priori* ROI. These ROIs will be based on anatomical landmarks from human atlases[143-145]. In addition, vmPFC, HPC, and AMYG activation reported by Milad et al.[71] 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-5mg, PTSD-10mg, PTSD-PBO, TEC) x stimulus (CS+E, CS+U) MANCOVA controlling for potential confounds (e.g., childhood trauma/adversity) during Extinction Recall. Statistical tests to directly test group and drug effects are described below ('Summary of Planned Analyses to Test Specific Hypotheses'). For the second approach (whole-brain, voxel-wise), we will employ ANCOVA across the entire brain 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 at least 10 voxels. Within the ROIs, a small volume threshold ($p < 0.05$, corrected for multiple comparisons) will be used.

Context-dependent renewal of fear could also affect the efficacy of PE therapy. Therefore, we will also test whether THC induces 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 examine 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 a generalized psychophysiological interaction analyses (PPI)[64,146,147] to measure task-dependent functional connectivity among the AMYG, vmPFC, and HPC. Separate connectivity analyses will be performed with each group condition (PTSD-5mg, PTSD-10mg, PTSD-PBO, and TEC), using AMYG, 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.2.7. Skin Conductance Response (SCR): Assessment of fear learning will be change in SCR magnitude. The SCR signal will be amplified and recorded with a BIOPAC Systems SCR module connected to a PC. All data will be continuously sampled at 1000 Hz. AcqKnowledge software (BIOPAC Systems) will be used for off-line analysis. *Analysis:* SCR for each CS trial will be calculated as described by Consultant Milad[142]. Extinction recall will be assessed by comparing the mean differential response during the first 4 trials of the CS+E vs. the first 4 trials of the CS+U as described below ('Summary of Planned Analyses to Test Specific Hypotheses'). In addition, we will use the "extinction retention index" (ETI) as described by Consultant Milad[142] to measure the magnitude of extinction retention. The ETI adjusts the SCR during extinction recall for differences in CR magnitude during fear acquisition[100]. Analyses for psychophysiological and behavioral data will be conducted by ANOVA models as above. We will also conduct Pearson correlations between psychophysiological and behavioral data to examine relationships.

C.2.8. Behavioral: We will obtain subjective US expectancy ratings (US expectancy) and level of distress (SUDS), a proxy of fear extinction, to the CS+E vs. the CS+U[129].

C.2.9. Summary of Planned Analyses to Test Specific Hypotheses:

Specific Aim 1 (R61 Primary Target Engagement): Assess effects of THC on recall of extinction learning and vmPFC and/or HPC activation in PTSD patients in a dose-finding study. *Hypothesis 1A: Relative to PBO, THC will dose-dependently increase vmPFC and/or HPC activation to a CS+E (vs. CS+U) during proximal (24 hr post-extinction learning) and/or distal (1 week post-extinction learning) recall of extinction learning in PTSD.* PCS (CS+E vs. CS+U) with ROIs (vmPFC, HPC) will be submitted to MANCOVA with Group (PTSD-5mg, PTSD-10mg, PTSD-PBO, TEC) as between-subjects factor and Recall (24 hr, 1 week) as within-subject factor, controlling for potential confounds. In addition to omnibus tests, planned comparisons will examine dose of THC on prefrontal-limbic engagement during recall of extinction learning in PTSD relative to PBO, and comparisons between THC doses; if there is no significant difference between THC doses we will examine potential positive trends with increasing dose. Similar analyses will be conducted using other variables of interest such as psychological measures, mood state, and drug effects. *Hypothesis 1B: Relative to PBO, THC will dose-dependently decrease peripheral measures of extinction recall ("extinction recall index" (ETI), US expectancy, SUDS) to the CS+E during a proximal and/or a distal test of extinction learning recall in PTSD.* These data will be submitted to MANCOVA with Group (PTSD-5mg, PTSD-10mg, PTSD-PBO, TEC) as between-subjects factor and Recall (24 hr, 1 week) as within-subject factor, controlling for potential confounds. Analyses will be comparable to that used to examine BOLD activation (*Hypothesis 1A*). *Hypothesis 1C: vmPFC and/or HPC activation will correspond with extinction recall success (i.e. higher ETI, lower US expectancy, lower SUDS).* Pearson correlation analyses will be used to correlate measures of BOLD activation (PSC) and peripheral measures (e.g., SCR, ETI, US expectancy) to the CS+E vs. CS+U within and across the two contexts/recall and renewal tests.

R61 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 healthy controls and an *n* of 14-16 PTSD patients provide adequate power to observe: 1) SCR differences and AMYG, vmPFC, HPC signal differences between groups during fear extinction and/or extinction recall; and 2) THC's effects on ETI and AMYG, vmPFC, and HPC activation in healthy controls. Similarly, normalization of corticolimbic responsivity to affective stimuli has been observed following exposure-based therapy in an *n* of 14 anxious patients[148] and following administration of a serotonin selective reuptake inhibitor (SSRI) with an *n* of 21 anxious patients[149]. Given that the effect sizes tested within- and between-groups in previous fMRI studies have ranged between moderate to large (Cohen *d* = 0.7 - 1.5) with the anticipated cohort of 20 participants per group (PTSD-5mg, PTSD-10mg, PTSD-PBO, TEC), who complete the entire protocol with usable fMRI and SCR data, will confer >80% power to detect an effect size 0.72 or higher after necessary multiplicity adjustment.

PROTECTION OF HUMAN PARTICIPANTS

This Human Participants Research meets the definition of a clinical trial.

1. Risks to Human Participants

a. Human Participants Involvement, Characteristics, and Design

Involvement of Human Participants:

The total time commitment estimated per participant is 8.5-13 hr across all study visits. This is broken down below:

Visit 1: Screening & Study Entry: Approximately 3 hours for participants, unless a participant has already completed any of the screening assessments as part of another separate study. In this case, if the overlapping screening assessments were completed no more than 1 month prior to Visit 1 and the participant has signed HIPPA Authorization allowing us to access this prior data, Visit 1 will be approximately 1.5 hr.

Note: Due to the length and rigor of this initial screening process, it may sometimes be necessary for participants to complete screening measures across more than one visit (e.g., schedule conflicts). All screening measures will be completed within 2 weeks of the consent date.

Visit 2: Fear Acquisition: Approximately 1 hr for all participants. Participants are considered enrolled in the study once they have begun Visit 2 (if enrolled in PTSD group, participant will be randomized). Visit 2 will occur within 6 months of Visit 1. If more than 6 months have passed, we will re-do Visit 1 before the participant is able to complete visits 2 – 5.

Visit 3: Fear Extinction: Approximately 5 hr for those receiving a capsule (PTSD groups) or approximately 3 hours for those not receiving a capsule (TEC group)

Visit 4: Fear Extinction Recall and Fear Renewal Test (approximately 24 hr after extinction, but no more than 1 week later): Approximately 1.5 hr

Visit 5: Fear Extinction Recall, Fear Renewal Test, & Debriefing (approximately 1 week after extinction, but no more than 3 weeks later): Approximately 1.5 hr

Nature of Participant Population: Participants of both genders will be included in the study and the age range of participants will be from 18-60. Persons below 18 or above 60 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[150] and complete questionnaires related to their general physical health, current medications, with a detailed section on current and lifetime drug and alcohol use. This study will not involve vulnerable populations.

The number of participants expected to participate is 104: 78 trauma-exposed participants with PTSD and 26 trauma-exposed participants without PTSD (TEC; demographically matched). There will be 3 experimental groups: PTSD participants will be assigned to 5mg of THC (n=26), 10mg of THC (n=26), or a placebo (PBO) (n=26). The 26 TEC participants will not receive THC or PBO. With the conservative anticipation 25% will fail to complete all parts of the study, we estimate 20 PTSD participants in each condition (60 PTSD participants in total) and 20 TEC participants will complete the study for a total of 80 participants. Participants will be recruited from the Detroit community and mental health clinics. We will enroll up to 175 participants to guarantee enough viable data and to account for participant drop out.

Recruitment

Participants will be recruited from several sources including flyers and webpage advertisements on Academica. In addition, we will also recruit patients seeking treatment at the Trauma Recovery Center of Southeast Michigan, Wayne State University (WSU) Physicians Group, North Central Health Center in Detroit, Detroit Wayne Mental Health Authority, St. John Providence Eastwood Clinics, WSU Psychology Clinic, WSU Clinical Research Center

in Detroit. Current staff in the Departments of Psychiatry and Psychology and related clinical departments at WSU will be informed about the study to pass along the information (in flyer format) to their participants. In addition, individuals who participate in the study may earn additional compensation for referring other people to the study. Once a participant contacts the project coordinator/research assistant at WSU, they will explain the study to the participant and schedule the participant to come in for a screening and orientation session (Visit 1) at the Eugene Applebaum College of Pharmacy and Health Science (EACPHS) building.

Retention

Strategies for participant retention will be discussed regularly in weekly research meetings, and will be practiced for optimized retention such as contact with the PI and her research team during the study, phone reminders/confirmation of visits, established contact with the study coordinator, arrangements for transportation (as needed) at each visit, reimbursement for parking/travel at each visit and for time and effort. Also, office hours are flexible (e.g., 7am-7pm during the week with weekend availability). Based on these strategies, we anticipate that drop-out will be minimized to less than 25% of the sample.

Inclusion Criteria

Inclusion criteria across all groups will be: (a) generally medically and neurologically healthy, including no evidence of intellectual disability or serious cognitive impairment that would interfere with task performance; (b) between the ages of 18-60; (c) willing and able to give informed consent; (d) exposure to Criterion A stressor as defined by the CAPS-5 and identified by the LEC-5, regardless of type of trauma (e.g., civilian or combat). *Inclusion criteria for the PTSD group will be:* (a) significant PTSD severity as indicated by a clinician-administered PTSD scale for DSM-5 (CAPS-5) diagnosis and/or score (see below) of ≥ 25 of at least the past month prior to study entry (primary PTSD will be identified as the patient's primary concern). *Inclusion criteria for the TEC group will be:* (a) absence of any history of PTSD symptoms (CAPS-5 < 25 and/or no diagnosis), related to any type of trauma.

Exclusion Criteria

Exclusion criteria across groups will be: (a) exclusively left-handed (i.e., score of -100.00 on Handedness Questionnaire); (b) risk of harm to self or others that requires immediate intervention; (c) presence of contraindications, 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); (d) lack of fluency in English; (e) currently pregnant (positive pregnancy test or self-report), planning pregnancy, or lactating (women); (f) traumatic brain injury (as defined by The American Congress of Rehabilitation as a person who has had a traumatically induced physiological disruption of brain function (i.e., the head being struck, the head striking an object, and/or the brain undergoing an acceleration/deceleration movement (i.e., whiplash) without direct external trauma to the head), as manifested by at least one of the following: any loss of consciousness; any loss of memory for events immediately before or after the injury; any alteration in mental status at the time of the incident; or focal neurological deficits that may or may not be transient); (g) inability to tolerate small, enclosed spaces without anxiety (e.g. claustrophobia), as determined by self-report; (h) presence of ferrous-containing metals within the body (e.g., aneurysm clips, shrapnel/retained particles); (i) current or past diagnosis of any bipolar or related disorder or schizophrenia spectrum and other psychotic disorder as determined by the MINI 7.0.2; (j) current severe alcohol or substance use as determined by the MINI 7.0.2; (k) history of pervasive developmental disorder (e.g., Autism, Asperger syndrome). *Exclusion criteria for the PTSD group will be:* (a) current diagnosis of a mood, anxiety, or other disorder *that is more clinically salient* than PTSD (e.g., MDD, OCD, Panic, GAD); (b) concomitant treatments with medication known to have level 1 evidence for severe drug- drug interactions with dronabinol that are taken daily. *Exclusion criteria for the TEC group will be:* (a) current diagnosis of any obsessive-compulsive and related disorders or trauma- and stressor-related disorders as determined by the MINI 7.0.2 for DSM-5; (b) current diagnosis of any depressive disorder as determined by the MINI 7.0.2 and a score > 22 (indicating very severe depression) on the Hamilton Depression Scale (HAM-D); (c) current diagnosis of any anxiety disorder as determined by the MINI 7.0.2 and a score > 24 (indicating moderate to severe anxiety) on the Hamilton Anxiety Scale (HAM-A).

Termination Criteria
Participants may be terminated for any of the following: (a) participant request to exit or withdraw consent; (b) development of risk of harm to self or others that requires immediate intervention; (c) alcohol, drug, medication use that would interfere with dronabinol or has level 1 evidence for severe drug-drug interaction with dronabinol; (d) development of a systemic, medical, neurologic, or psychiatric illness requiring treatment that would exclude participation; (e) clinical deterioration (see below); (f) non-compliance with study protocol requirements. We will make every effort to ensure that the sessions are accessible (i.e., scheduling sessions during evening and weekend hours). We will attempt to schedule Visits 2-4 on consecutive days, but in the event that this is not possible we will allow no more than 1 week between Visits 2 & 3 and Visits 3 & 4 and no more than 3 weeks between Visits 4 & 5. Of note, if there is more than 1 week between Visit 2 & 3, we will re-do Visit 2 and proceed with the rest of the visits as normal. But once a participant completes Visit 3, Visits 4 & 5 need to be completed within the above timeframe otherwise participation will be terminated. We will make every effort to ensure that appointments are accessible (i.e., scheduling an appointment during evening and weekend hours).

Medical and Neurological Health Determination
All health information collected in the pre-screening and baseline interview (Visit 1) will be reviewed by the PI or Co-I Kilgore, who will indicate whether the participant is medically and neurologically healthy enough to continue in the study. These determinations may be made in person, over the phone, or via e-mail, and will be documented using a Medical Review form.

Emergent Suicidal or Homicidal Risk
The clinician or research staff may remove a participant from the clinical trial if the PI thinks that the participant is at imminent suicidal or homicidal risk. The presence of suicidal ideation is evaluated at the initial assessment. Endorsement of suicidal or homicidal ideation will be addressed in session. If the patient is deemed to have specific suicidal or homicidal ideation, the PI, Co-I Kilgore, or Co-I Tancer will be immediately notified and the participant will meet with the PI and/or Co-Is Kilgore and/or Tancer, or other licensed social worker/psychologist/psychiatrist covering for the PI or Co-Is on the premises, and the police will be alerted.

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 (Visit 1) and at the beginning of Visit 3 to make sure that women are not pregnant. Of note, if the participant is unable to provide a urine sample at Visit 1 (e.g., currently menstruating), we will conduct a urine pregnancy test at the beginning of visit 2. We will also conduct pregnancy tests if the study staff and/or participant have reason to believe they may be 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.

Study Group Assignment
Individuals with PTSD will be randomized to a THC or PBO condition. In our previous studies we have shown that 7.5mg of THC facilitates extinction recall and modulates prefrontal and hippocampal activation in healthy humans volunteers[55,113]. Moreover, PI Rabinak has preliminary data to suggest that 7.5mg of THC may facilitate extinction recall in PTSD, but it is not known whether this is the MED. This is an initial step in translation to be tested in the R61 phase. Therefore, we will have a low 5mg condition and a high 10mg condition should 5mg be too low to detect effects. Of note, a 10mg dose of THC does not typically produce anxiety[151] or strong negative effects on cognition[152]. We aim to identify the MED that facilitates recall of extinction learning. 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 (two-thirds of the PTSD participants) will only receive dronabinol on one occasion.

Study medication, dronabinol or PBO, will be dispensed to participants by PI Rabinak, who possesses a Schedule II-V controlled substance research license through the State of Michigan and the DEA and has experience in the use of dronabinol and PBO in human participant research. A registered pharmacist will handle

the overencapsulation of the required dronabinol and creation of the matching placebo. Dr. Rabinak will store the capsules in her office, room 2136 in the Eugene Applebaum College of Pharmacy, in a locked refrigerated safe. She holds current controlled substance licenses from the State of Michigan and Drug Enforcement Administration allowing her to order, store, and dispense dronabinol from her office.

Dr Rabinak will randomize participants to the study groups: 5 mg dronabinol, 10 mg dronabinol, or placebo. She will dispense one pill from the appropriate container labeled by the pharmacist and put the pill into a separate pill bottle. This new pill bottle will be labeled with the participant specific ID. Dr. Rabinak will be responsible for appropriate documentation of drug orders, inventory, subject-specific drug order forms, dispensing, and disposal records. Research staff associated with the study will give the drug or PBO to participants, and Co-I Paul Kilgore, M.D., a board-certified and licensed physician, or Co-I Manuel Tancer, M.D., a board-certified and licenses psychiatrist, or their physician designate, will be present on-site and/or available by pager/phone.

b. Sources of Materials

Primary Diagnostic Assessments (Table 1)

(1) Mini International Neuropsychiatric Interview Version 6.0.0 (MINI): The MINI is a structured interview for general diagnostic assessment providing an assessment of major psychiatric diagnoses; (2) Clinician-Administered PTSD Scale for DSM-5 (CAPS-5)[154], a validated, gold-standard clinician interview measure of PTSD severity. WSU clinical research staff members will conduct diagnostic interviews using MINI/CAPS instruments. While most interviews will be conducted by one rater, inter-rater reliability will be examined to ensure good reliability (Kappa at least 0.6)[155].

Table 1: R61 Assessment Schedule

			FMRI		
			THC or PBO		
	Screening, Informed Consent, & Study Entry	Fear Acquisition	Fear Extinction	Extinction Recall Test 1	Extinction Recall Test 2
MINI	✓				
CAPS-5	✓				
Standard Health Questionnaire	✓				
Ethnicity/Race Form	✓				
MRI Safety Form	✓				
Gender Identity Questionnaire	✓				
Concomitant Medication Form	✓				
Handedness Questionnaire	✓				
Connor-Davidson Resilience Scale	✓				
Posttraumatic Growth Inventory	✓				
LEC-5	✓				
PCL-5	✓				
PSQI	✓				
STAI**	✓		✓		
HAM-D	✓				
HAM-A	✓				
BDI-II	✓				
C-SSRS	✓				
WHODAS 2.0	✓				
CTQ	✓				
ERQ	✓				
ACQ	✓				
US Expectancy		✓	✓	✓	✓
SUDS		✓	✓	✓	✓
VAS**			✓		
DEQ**			✓	✓	

ARCI**			✓		
ESQ**			✓		
Blood Draw Safety Questionnaire**			✓		
Debriefing Interview					✓
*****These measures are NOT completed by the TEC group at Visit 3, as they do not ingest THC/PBO***					

Secondary Measures (Table 1)
(1) Standard Health Questionnaire; (2) Ethnicity/Racial Category Form; (3) MRI Safety Form; (4) Gender Identity Questionnaire; (5) Concomitant Medication Form. This form will be reviewed and updated (if applicable) by the participant at each study visit; (6) Handedness Questionnaire; (7) Connor-Davidson Resilience Scale (CD-RISC); (8) Posttraumatic Growth Inventory (PTGI); (9) Life Events Checklist for DSM-5 (LEC-5)[156]: The LEC-5 is a self-report measure designed to screen for potentially traumatic events in a person's lifetime; (10) PTSD Checklist for DSM-5 (PCL-5)[157]: The PCL-5 is a 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD. It is used for monitoring symptom change during and after treatment, screening individuals with PTSD, and for making a provisional PTSD diagnosis; (11) Pittsburgh Sleep Quality Index (PSQI)[158]: The PSQI is a self-rated questionnaire which assess sleep quality and disturbances over a 1-month time interval; (12) Spielberger State-Trait Anxiety Inventory (STAI)[159]: The STAI is a measure of trait and state anxiety; (13) Hamilton Depression Scale (HAM-D)[160]: The HAM-D is used to determine a patient's level of depression before, during, and after treatment; (14) Hamilton Anxiety Scale (HAM-A)[161]: The HAM-A is a questionnaire used by clinicians to rate the severity of a patient's anxiety; (15) Beck Depression Inventory (BDI-II)[162-165]: The BDI-II is self-report questionnaire used to measure severity of depression; (16) Columbia-Suicide Severity Rating Scale (C-SSRS)[166] is a standardized 8 point clinician-administered suicidal rating system designed to track suicidal adverse events across a treatment trial and covering the wide spectrum of suicidality; (17) World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0)[167]: This is an assessment of global functioning and impairment; (18) Childhood Trauma Questionnaire (CTQ)[168,169]: The CTQ is a 28-item self-report measure that provides brief, reliable, and valid screening for histories of abuse and neglect. It inquiries about five types of maltreatment—emotional, physical and sexual abuse and emotional and physical neglect. (19) Emotion Regulation Questionnaire (ERQ)[170]: The ERQ is designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression; (20) Attentional Control Questionnaire (ACQ)[171]: The ACQ is a self-report questionnaire designed to measure individual differences to major components of attention: attention focusing and attention shifting. The ability to regulate emotions and focus on the task at hand (e.g., attend to fear-cues) is expected to factor into PE therapy; therefore, the ERQ and ACQ are for exploratory purposes.

Additional Secondary Measures (Table 1)
In addition to standardized assessments of symptom severity and mood/emotional state, the following measures will be completed at various time points within a session (e.g., before, during, and after) on days in which fear acquisition and/or fear extinction and extinction recall/fear renewal tests occurs: (1) US expectancy; (2) Subjective Units of Distress Scale (0=absolutely calm; 100=worst distress), frequently used in exposure-based therapy, which will provide a measure of subjective fear.

THC/PBO Day Measures (Table 1)
The following standardized assessments will be collected throughout the session when THC/PBO is ingested to assess mood states and subjective drug effects. These questionnaires are sensitive to the effects of a variety of psychoactive drugs on affective state/mood and drug liking[172,173]: (1) Visual Analog Scales (VAS): This questionnaire consists of visual analog scales used to describe current medication effects. This particular form consists of twenty 100-mm horizontal lines each labeled with an adjective ("clear- headed," "anxious," "stimulated," "tired," "calm," "drowsy," "peaceful," "nervous," "hungry," "energetic," "uneasy," "relaxed," "alert," "content," "focused," "dreamy," "restless," "nauseous" "worn out," and "jittery"). 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; (2) Drug Effects Questionnaire (DEQ)[174]: This questionnaire assesses the extent to which participants "feel any substance effect(s)," "feel high," "like the effects," "dislike the effects," and "want more of the substance" using 100 mm visual analog scales. 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 that moment; (3) Addiction Research Center Inventory (ARCI)[175,176]: 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; (4) Spielberger State-Trait Anxiety Inventory (STAI)[131] (state subscale): This state subscale is used to assess the level of state (current/in the moment) anxiety in an individual; (5) At completion of drug session, participants will complete the End of Session Questionnaire (ESQ) which asks them to indicate the extent to which they think they felt a drug effect, the extent to which they think they received THC or PBO, 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. In addition, physiologic measures (heart rate, blood pressure) will be collected at regular intervals throughout the drug session. (6) The Blood Draw Safety Questionnaire will be completed by the participant at the beginning of the THC/PBO session (Visit 3) and reviewed prior to blood collection at all subsequent visits.

These psychological and physiological measures are collected 1) before receiving THC or PBO on Visit 3; 2) at approximately 30 min intervals after receiving THC or PBO on Visit 3 (i.e., at 30, 60, & 90 min), immediately before going in the MRI scanner, soon after coming out of the MRI scanner; and once before the end of the visit unless otherwise specified (ESQ will be collected only at the end of the drug session; DEQ will also be collected before recall of extinction learning on Visits 4 & 5). 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: approximately 8 hr after drug administration.

Debriefing Session Measures
At the final extinction memory recall session (Visit 5), a short Debriefing Interview will be conducted with the participant. The participant will be asked about what they remember from each session and to provide feedback about their experience in the study. The study aims and measures will be explained in detail to the participant and any questions they have will be answered.

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.

Psychophysiological
Skin conductance recordings (SCR) will be collected, if able, during fear acquisition, fear extinction, and extinction memory recall sessions (i.e., Visits 2-5).

FMRI Data
FMRI data will be collected, if able, during the fear extinction learning and extinction memory recall and fear renewal sessions (i.e., Visits 3-5).

c. Potential Risks

Diagnostic/Assessment Procedures
The diagnostic interviews (and questionnaires) are time consuming and may be boring to some individuals. These are, however, necessary to determine eligibility for the study. In addition, questions about alcohol/drug use, interpersonal relationships, abuse/trauma history, and questions related to history of suicidal and/or homicidal behavior may be considered sensitive by some participants. The collection of such data poses a potential risk of loss of confidentiality around sensitive information such as psychiatric status, history of substance abuse, etc. Participants will also be informed in the consent document that confidentiality will be limited in cases where the participant reveals intentions to harm themselves or others, and the investigator feels that the proper authorities may need to be notified to prevent the occurrence of harm to the participant, or others.

Experienced mental health workers will conduct interviews and will maintain confidentiality. All data from interviews and questionnaires will be numbered to conceal the identity of the participant.

Other Tasks
The virtual snake presented during the fear conditioning procedure will be uncomfortable and aversive and participants will not know that the virtual snake will only be presented during the first session of the experiment and on several but not all trials. Study staff will be present during all tasks and participants may communicate with them always, including when the participant is in the MRI scanner. A medical clinician on this study (e.g. physician) will be available during all behavioral tasks 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.

Blood Draws
Foreseeable risk or discomforts associated with the blood draw include the potential for pain, bruising, or infection at the blood draw site. This is rare and usually temporary. Occasionally nausea, lightheadedness, or fainting may occur.

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. Additional minor and/or rare risks include: (a) discomfort or anxiety from being in the confined space of the MRI scanner; (b) fast imaging sequences, such as those employed in this study, have the potential to induce peripheral nerve stimulation (PNS). PNS can be described as a light touching sensation on the skin surface and may cause mild discomfort, but is not harmful to the participant; (c) risks of hearing damage due to loud noises produced by the scanner; (d) risk that the magnetic resonance image will reveal a minor or significant lesion in the brain, e. g. a tumor, previously unknown to the participant, and requiring additional follow-up; (e) risk of injury from objects accelerated by the strong magnetic field of the magnet, striking the participant; or metallic substances on the skin or foreign bodies implanted deliberately or accidentally in the participant that acquire kinetic or thermal energy from the magnetic or radiofrequency emissions of the MRI, causing tissue injury to the participant; (f) sometimes, participants report a temporary, slight dizziness or light-headedness when they come out of the scanner; (g) potential risk for pregnant women: According to the NIMH Council Workgroup on MRI Research and Practices (September, 2005), “there is no known risk of MR brain scanning of a pregnant woman to the developing fetus for scanning at 4T or less, and no known mechanism of potential risks under normal operating procedures.” Nevertheless, participants should be warned about potential risks not yet discovered.

Discovery and disclosure of incidental finding or abnormality on MRI scans: 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. If an abnormality is found in a participant’s MRI scan, the PI may ask a radiologist at the MR Facility will review the scan data and provide recommendation to the PI if the incidental finding or abnormality is something the participant should follow-up with their primary care physician about. If follow-up is recommended, the PI will contact the participant. The decision as to whether to proceed with further examination and/or treatment lies solely with the participant and his/her primary care physician.

Dronabinol
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. A board-certified psychiatrist and/or physician associated with the study (Co-I Kilgore or Co-I Tancer) or his/her physician designate, will be available during all behavioral tasks and pharmacological challenge days to evaluate and recommend further evaluation and treatment for the emergence of any adverse events/side effects.

Participants taking medications that have level 1 evidence indicating severe drug-drug interaction with dronabinol daily will be excluded. 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 from 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 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[177]. There is no evidence that participation in controlled laboratory studies such as these increases the risk for developing substance use problems[172,173,178], including those conducted in laboratories (with 20+ years of experience) from which the current protocol is based[110,127,128]. In fact, in an open label study in patients with AIDS who received dronabinol for up to five months, no abuse, diversion or systematic change in personality or social functioning were observed despite the inclusion of a substantial number of patients with a history of drug abuse. 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 with the exception of which drug they received. In order to maintain integrity of the data collected, blinding needs to be maintained.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Recruitment

Participants will be recruited from several sources including referrals, flyers and webpage advertisements on Academica. In addition, we will also recruit patients seeking treatment at the Trauma Recovery Center of Southeast Michigan, Wayne State University (WSU) Physicians Group, North Central Health Center in Detroit, Detroit Wayne Mental Health Authority, St. John Providence Eastwood Clinics, WSU Psychology Clinic, and WSU Clinical Research Center. Current staff in the Departments of Psychiatry and Psychology and related clinical departments at WSU will be informed about the study to pass along the information (in flyer format) to their participants. Once a participant contacts the project coordinator/research assistant, they will explain the study to the participant and schedule the participant to come in for a screening and orientation session (Visit 1) at the Eugene Applebaum College of Pharmacy and Health Science (EACPHS) building. A waiver of signed consent will be requested for the phone or online screen to save a high number of ineligible participants from coming in for the initial assessment session (Visit 1). Information collected in the pre-screening will be used at Visit 1 to determine eligibility. During the initial assessment (Visit 1), the participant will be provided with the informed consent form, along with an oral presentation of the material in the consent form by a research staff member. Only after signing the consent form will the participant be evaluated for participation in the study.

Informed Consent

During the initial assessment (Visit 1), the nature of the research project will be described to participants. 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, and exposure to dronabinol and to behavioral tasks, 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 session in which the drug may be administered. Women will agree that

they are not pregnant and not planning to become pregnant and not nursing. Women will be informed that we will administer a urine pregnancy test at Visit 1. Participants will be informed that blood alcohol levels and urine samples to test for pregnancy and drugs will be obtained on Visit 3 prior to receiving the drug. 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. Participants will be informed that they can discontinue participation at any time without penalty.

b. Protection Against Risk

General Considerations

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 (Eugene Applebaum College of Pharmacy & Health Sciences Building and the Magnetic Resonance Research Facility at WSU) located nearby a hospital, where emergency assistance can be obtained; (c) a radiological technician and member of the research team are present during all fMRI sessions; (d) heart rate (HR) and blood pressure (BP) will be monitored regularly during the fMRI sessions; (e) a member of the research team and Co-I Kilgore, a board-certified physician, or Co-I Tancer, a board-certified psychiatrist, or their physician designate will be available on-site or by pager/phone at all pharmacological challenge sessions; (f) participants will agree not to take any other drugs for 24 hours before and 12 hours following the drug session (Visit 3), and compliance will be monitored by breathalyzer and urine tests conducted at the beginning of Visit 3, prior to drug administration; (g) 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; (h) a complete medical history, review of medications and physical symptoms/signs will be performed prior to entry into this study; (i) participants will be informed that they may withdraw by informing the investigators or research staff when they are present at the time of the study or by using one of the phone numbers/locations provided on the consent form. Withdrawal from the study after the clinical and fMRI/psychophysiological data has been collected is also possible through contacting the investigators or research staff using one of the phone number/locations provided on the consent form. Effective screening should exclude participants who would be placed at greater risk. All the testing will be done in the presence of appropriately trained research staff and in a facility specifically designed, equipped, and functioning to support these types of studies. Participants are free to drop out of the study at any time without cost or penalty. Participants will be informed that they do not have to participate if any procedure or questionnaire causes them discomfort.

Confidentiality

All data from participants are 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 are stored separately from other questionnaires in a locked file cabinet. Paper records are 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/psychophysiological data, are stored in password-protected computer files, such as encrypted Microsoft Excel files, (except for a single tracking file) on a password and firewall-protected private lab server as well as in an Electronic Data Capture system (Castor). Data that may be reported in scientific journals will not include any information that identifies any person as a participant in this study.

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 or a private, password and firewall-protected server, where only approved study staff can have access. All information obtained during this study is strictly confidential, except when there is either a danger to self or others. Members of the research teams 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.

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 police will be alerted.

Suicide Risk
If a participant endorses current (e.g., within the past month) suicidal ideation on the C-SSRS (e.g., a score of yes for question 4 and/or 5 from the suicidal ideation section of the C-SSRS), the PI and/or Co-I Kilgore, and/or Co-I Tancer will immediately be notified and the participant may meet with the PI and/or Co-I Kilgore and/or Co-I Tancer, or other licensed social worker/psychologist/psychiatrist covering for the PI on the premises, and the police may be alerted. Determinations about level of risk and appropriate actions to be taken will be made by the PI and/or Co-I Kilgore together with the research staff member who conducted the suicidality assessment. In all cases, the research staff and/or the PI will follow up with the participant and provide mental health resources/referrals as needed. The PI may remove a participant from the protocol if the research team thinks that the participant is at imminent suicidal risk and may be a danger to himself/herself.

Confidentiality of Breathalyzer/Drug & Pregnancy Tests Prior to Scans or Assessment
The results of the breath alcohol test and urine drug and pregnancy tests performed at the beginning of Visit 3 (and Visit 1 for pregnancy test) 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 breath alcohol and urine tests will be performed at the beginning of the visit. If the participant produces a positive pregnancy test at either Visit 1 or Visit 3 or reports becoming pregnant at any time during which they are active in the study, they will be withdrawn from the study at that time. At the beginning of Visit 3, if the participant (a) fails the alcohol breathalyzer test and/or (b) fails the urine drug test without any current medication use (e.g., non-illicit) which explains the positive result, the session will end at that time and the remaining visits will be rescheduled. Visits 3-5 will be rescheduled such that Visit 3 occurs within 1 week of Visit 2, otherwise the participant will need to re-do Visit 2. If the participant cannot be rescheduled or fails the urine drug test for a second time (i.e., at their rescheduled Visit 3) they will be withdrawn from the study. In the event of failed drug screen, follow-up questions will be asked to determine whether the result can be explained by a daily medication and not illicit drug use. This information will be stored in a secure computer database that uses participant codes (rather than names) as identifiers.

Other Tasks
Participants are re-assured that they can withdraw from the study at any time if they do not wish to experience the virtual snake. All participants will be informed that they are able to take a break at any time during any of the behavioral tasks.

Dronabinol
Participants are made aware of the side effects common to dronabinol in the informed consent document and again during their initial screening visit. Participants will be monitored for side effects at the pharmacological challenge visits. Participants are reassured that they can withdraw from the study at any time.

Magnetic Resonance Imaging
To minimize the risk of discomfort and anxiety, and to make the participant as comfortable as possible, we will use custom pads and pillows. The participant can communicate with the machine operator via an intercom and may trigger an audible alarm at any time. Before the participant rolls into the core of the magnet, he or she is always reminded that they are free to stop the study at any time if they become uncomfortable. If they were to experience an anxious reaction, the study would be halted, and study staff would monitor the participant for further/worsening anxiety symptoms. The participant would have the option to meet with the PI or another study psychiatrist, psychologist, or social worker. Participants often find that once outside of the scanner, they experience immediate relief of any anxiety and discomfort. If the study team has any doubts about the relief of anxiety, follow-up telephone calls would be made later that day or 1-3 days after the session to confirm the transient nature of their reaction.

The MRI machine is operated within FDA guidelines so the potential for inducing peripheral nerve stimulation (e.g., experiencing twitching sensation) is low.

All participants are required to wear foam earplugs to reduce the risk of hearing damage.

In the event of an anomalous finding on the MRI, the PI would contact the participant and explain that the participant should contact their primary care provider to obtain a clinical MRI scan (see plan below for more details).

The MRI suite is kept clear of all objects that could be picked up by the magnetic field. MRI personnel are trained in safety procedures, which include training around the materials that cannot be brought into the scanner room. The technician administering the scan is trained to review each participant's MRI safety form to assess suitability of the participant to enter the MR environment. Additionally, participants are screened at multiple points. An initial phone screening is done, in which the MRI is explained and participants are excluded from participation if they have contraindications to an MRI scan. On the day of the scan (Visit 3), the participant completes an MRI safety screening form, which is reviewed by study personnel, as well as the MRI technician. Participants are prompted at two points to remove all metallic objects, soon after they arrive at the MRI center. Participants are given instructions to empty their pockets, remove jewelry, watches, wallets, and they are shown a storage box where belongings can be safely stored. Immediately before entering the scanner room, they are prompted again to ensure that they have removed any items that might interfere with the scan or interact with the magnetic field.

To minimize risks of dizziness or light-headedness, participants are carefully eased out of the scanner, and a technician guides the participant as they arise from the scanner bed, instructing them to rise slowly, ensuring that they have adequate balance.

Participants are informed that pregnancy is a contraindication to receiving a research MRI scan. At the initial phone screening, female participants are asked about pregnancy or the possibility of pregnancy. When the participant is consented for the study, language in the informed consent document mentions this exclusion, again. All women of child bearing age will be given a urine pregnancy test at the screening visit (Visit 1) and beginning of Visit 3 prior to drug administration. Women with a positive pregnancy test will not be permitted to continue in the study.

If a woman is found to be pregnant, the study coordinator will assess the impact of this surprise finding upon the participant. If the participant is particularly distressed, the PI may be notified and will meet with the participant to counsel her about the surprise of this unexpected, stressful news.

Incidental findings arising from assessment**:** Incidental findings discovered during a research scan have the potential to raise significant anxiety in participants, while the nature of a research scan seriously limits the usefulness of the scan for resolving an incidental finding. At the first level, it is critical to make all participants aware of this risk. The following language will be used to warn participants during the informed consent process:

"There is the potential that a magnetic resonance image may reveal an abnormality that is already in your body, such as a cyst or tumor. Many such abnormalities are not clinically significant, but you may need or want to investigate them further. Such a finding might require additional studies, and maybe even treatment, which would not be paid for by the investigators, the sponsor, or Wayne State University."

Another important consideration is to make participants aware that they are receiving a research scan, which cannot be used to assess the clinical significance of any finding. The informed consent document will contain language stating the following:

"The types of scans we will use are not very sensitive to many abnormalities. The scanning procedures used for this study cannot be used make medical diagnoses from the scan. That is, even if there is an abnormality in your body, it is likely that it would not be discovered by the people who inspect the images. Therefore, it is likely that any abnormality that you may currently have will not be revealed by the images obtained in this experiment. If you have any current health concerns, you should consult your doctor."

Standard operating procedure at Wayne State University's imaging center does not entail reading of research MRI scans by neuroradiologists. Nevertheless, the experienced technicians and investigators collecting research data do encounter anomalous incidental findings, such as the presence of a large cyst or tumor, and may consult with a radiologist to determine if follow-up should be recommended to the participant. The following protocol is in place to handle these events.

Discovery of a finding: Incidental findings that arise during assessment, such as an abnormal finding in the MRI, will be brought to the immediate attention of the PI.

Gathering additional information: The PI will review the finding, and seek consultation as appropriate.

Informing the participant: If the PI is available while the participant is being scanned, and can assess the finding and decide about informing the participant, this will be done immediately. If the PI is not available for a face-to-face meeting with the participant, study staff are instructed to complete as much of the protocol as is reasonable, without revealing the existence of an anomaly. The intention here is to control the circumstances by which the participant is informed of the anomaly, making sure that the PI is the person who talks to the participant, can answer questions and gauge the emotional reaction of the participant to the news. The participant will be informed by the PI, personally, either through a phone call or a face-to-face meeting. While a face-to-face meeting is preferred, this may not be immediately convenient for the participant, and the PI must weigh the relative benefits of the more personal setting versus anxiety engendered by anticipating a meeting to discuss something the participant did not expect to hear.

Informing the participant's health care provider: If follow-up is recommended, the PI will contact the participant. The decision as to whether to proceed with further examination and/or treatment lies solely with the participant and his/her primary care physician. The PI nor study staff will contact the participant's primary care provider about the incidental finding.

3. Potential Benefit of the Proposed Research to Human Participants 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 costs to participants associated with participation in this study other than transient discomfort.

4. Importance of the Knowledge to be Gained

This R61 study will provide the most directly translational and critical test of THC effects in PTSD, advancing our understanding of the neurobiology of extinction learning, and potentially hastening the development of novel pharmacological modulators of the cannabinoid system to maximize the efficacy of exposure therapy for PTSD. The risks involved in this study are minimal when compared to the benefits gained.

5. Data and Safety Monitoring Plan

Protocol deviations will be defined as noncompliance with the research protocol that does not increase risk or decrease benefit and/or affect the integrity of the data. Protocol deviations may result from the action of the participant, research staff, or MR Facility and MR staff. All protocol deviations will be documented on a participant-specific Protocol Deviation Form and will explain any deviation from the approved protocol. Protocol

deviations will be reported to the IRB and NIMH PO by the PI with the continuing review and annual progress report, respectively.

Adverse Events (AEs) are defined as any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal MRI finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research. Study personnel will ask each participant about the presence/absence of adverse events (AEs) at every study visit and document AE presence/absence. AEs will be documented in a participant-specific AE log, which will include documentation of the following information: a) grading the AE as mild, moderate, or severe; b) determining how likely the AE is related to the participant's participation in the study procedures; c) action taken regarding study participation; d) outcome of the AE; e) whether the AE was expected or unexpected (not listed as a potential side effect/risk in the protocol); and f) whether the AE is determined to be a serious adverse event (SAE).

A serious adverse event (SAE) is any severe 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 existing hospitalization, (d) results in persistent or significant disability, (e) is a congenital anomaly or birth defect, or (f) any other AE that, based on appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. Moderate AEs are defined as any non-serious event which causes discomfort and requires treatment, but does not pose any significant or permanent risk of harm to the participant or require in-patient hospitalization, including social or psychological trauma causing moderate or temporary distress, significant embarrassment, stigmatization of individual or community/ group, disruption of familial/social relationships or nontrivial emotional distress. Moderate AEs will include evaluation in the emergency room not leading to hospitalization. Mild is defined as any non-serious event of less than moderate severity. All SAEs and unexpected AEs will be reported to the PI or Co-Is Kilgore or Tancer as soon as possible within 24 hours of the study staff becoming aware of the S/AE. The PI or Co-I's Tancer or Kilgore will determine whether participation in the study should be altered or terminated for the safety of the participant.

All unexpected problems, defined as any incident, experience, or outcome that is **unexpected**, **related or possibly related** to participation in the research, and suggest that the research places participants are **greater risk of harm than was previously known or recognized** will be reported by the PI to the IRB and NIMH PO as soon as possible, but within 5 business days of the PI becoming aware of the AE. For all AEs and SAEs that are deemed expected and/or unrelated to the study, a summary will be submitted by the PI to the NIMH PO with the annual progress report.

Documentation of protocol deviations will begin at Visit 1 and documentation of AEs will begin once a participant begins Visit 3 and will be collected/documented until the participant completes the study procedures (at the end of Visit 5) or is withdraw from the study (by self or my research staff).

Weekly meetings of the research staff of this study will be conducted that will include review of accrual, consenting procedures, protocol adherence, AEs, and quality control of all data obtained from the study in the previous weeks. Minutes of these meetings will be recorded, signed by the PI, and archived in a Regulatory Folder on the lab server. In addition, to participant-specific protocol deviation forms, protocol deviation will also be documented in a study-wide deviations log to facilitate monitoring and reporting to regulatory authorities. Similarly, AEs will be also documented in a study-wide AE log, which will be used to facilitate safety monitoring and identify AE trends across subjects, as well as overall AE reporting to the IRB and NIMH.

We will conduct a preliminary analysis during the early stages of data collection (once ≈10% of the THC group has completed the study) to assess the efficacy and safety of the 5mg and 10mg doses and submit an amendment to adjust the dosing as needed (e.g. if 10mg shows significant improvement over 5mg, a further

increase in dosing [15mg] would be worthwhile). Our efficacy and safety metrics are based on the following criteria:

1) a. At least a medium effect size (Cohen's $d \geq 0.05$) between THC and PBO conditions in BOLD responses in *a priori* ROIs (vmPFC and/or HPC) and/or peripheral measures (SCR, US expectancy, SUDS) during recall of extinction learning; or

b. Relationship between change in BOLD responses in *a priori* ROIs (vmPFC and/or HPC) and/or change in peripheral measures (SCR, US expectancy, SUDS) during recall of extinction learning in THC (vs. PBO) is of at least a medium effect size ($r \geq 0.30$).

2) There will be no adverse events of more than mild severity that are rated as possibly, probably, or definitely related to acute administration of THC (more than PBO) in PTSD participants.

We will apply O'Brien-Fleming[179] type monitoring bounds as guidelines for the decisions regarding continuation or early termination of the trial. In addition to efficacy comparisons, frequency of AEs along with formal statistical comparisons of the frequency of AEs will be performed.

Reporting responsibility

The PI will have ultimate responsibility for event reporting. Monitoring will occur by the research team, who will then bring all AEs to the attention of the PI. Co-Is Kilgore or Tancer will be consulted for evaluation and gradation of AEs. The PI and the study manager/coordinator will be responsible for overseeing data integrity, safety monitoring, and reporting of adverse events.

Training in Fair, Responsible, and Ethical Conduct of Research

The PI and the research team have undergone training in fair and ethical conduct of research in general and on human participants. The PI and the research team have taken HIPAA training mandated by WSU. An additional consent form consistent with the HIPAA 2003 rules will be used to educate the research participants and obtain informed consent regarding safeguards for the privacy and security of health information.

6. ClinicalTrails.gov Requirements

This project includes an applicable clinical trial, which will require registration in ClinicalTrials.gov. The responsible party is the principal investigator of this project: Christine Rabinak, Ph.D.

Bibliography and References Cited

1 Kessler, R. C. *et al.* Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* **62**, 593-602, doi:10.1001/archpsyc.62.6.593 (2005).

2 Kessler, R. C. *et al.* Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* **51**, 8-19 (1994).

3 APA. *Diagnostic and statistical manual of mental disorders IV-TR*. 4th, text rev. edn, (Amer Psychiatric Pub Inc., 2000).

4 Panagioti, M., Gooding, P. A. & Tarrier, N. A meta-analysis of the association between posttraumatic stress disorder and suicidality: the role of comorbid depression. *Compr Psychiatry* **53**, 915-930, doi:10.1016/j.comppsych.2012.02.009 (2012).

5 Hoge, E. A., Austin, E. D. & Pollack, M. H. Resilience: research evidence and conceptual considerations for posttraumatic stress disorder. *Depress Anxiety* **24**, 139-152, doi:10.1002/da.20175 (2007).

6 Francois, C., Despiegel, N., Maman, K., Saragoussi, D. & Auquier, P. Anxiety disorders, major depressive disorder and the dynamic relationship between these conditions: treatment patterns and cost analysis. *J Med Econ* **13**, 99-109, doi:10.3111/13696991003591321 (2010).

7 Jakupcak, M., Luterek, J., Hunt, S., Conybeare, D. & McFall, M. posttraumatic stress and its relationship to physical health functioning in a sample of Iraq and Afghanistan War veterans seeking

postdeployment VA health care. *J Nerv Ment Dis* **196**, 425-428, doi:10.1097/NMD.0b013e31817108ed (2008).

8 Norman, S. B., Stein, M. B. & Davidson, J. R. Profiling posttraumatic functional impairment. *J Nerv Ment Dis* **195**, 48-53, doi:10.1097/01.nmd.0000252135.25114.02 (2007).

9 Amaya-Jackson, L. *et al.* Functional impairment and utilization of services associated with posttraumatic stress in the community. *J Trauma Stress* **12**, 709-724, doi:10.1023/a:1024781504756 (1999).

10 Dekel, R. & Solomon, Z. Marital relations among former prisoners of war: contribution of posttraumatic stress disorder, aggression, and sexual satisfaction. *J Fam Psychol* **20**, 709-712, doi:10.1037/0893-3200.20.4.709 (2006).

11 Riggs, D. S., Byrne, C. A., Weathers, F. W. & Litz, B. T. The quality of the intimate relationships of male Vietnam veterans: problems associated with posttraumatic stress disorder. *J Trauma Stress* **11**, 87-101, doi:10.1023/a:1024409200155 (1998).

12 Cohen, L. R., Hien, D. A. & Batchelder, S. The impact of cumulative maternal trauma and diagnosis on parenting behavior. *Child Maltreat* **13**, 27-38, doi:10.1177/1077559507310045 (2008).

13 Jordan, B. K. *et al.* Problems in families of male Vietnam veterans with posttraumatic stress disorder. *J Consult Clin Psychol* **60**, 916-926 (1992).

14 Myers, K. M. & Davis, M. Mechanisms of fear extinction. *Mol Psychiatry* **12**, 120-150 (2007).

15 Bouton, M. E., Westbrook, R. F., Corcoran, K. A. & Maren, S. Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biol Psychiatry* **60**, 352-360 (2006).

16 Milad, M. R., Rauch, S. L., Pitman, R. K. & Quirk, G. J. Fear extinction in rats: implications for human brain imaging and anxiety disorders. *Biol Psychol* **73**, 61-71 (2006).

17 Quirk, G. J. & Beer, J. S. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol* **16**, 723-727 (2006).

18 LeDoux, J. E. Emotion circuits in the brain. *Annu Rev Neurosci* **23**, 155-184 (2000).

19 Herry, C. *et al.* Switching on and off fear by distinct neuronal circuits. *Nature* **454**, 600-606, doi:nature07166 [pii]
10.1038/nature07166 [doi] (2008).

20 Phelps, E. A., Delgado, M. R., Nearing, K. I. & LeDoux, J. E. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* **43**, 897-905 (2004).

21 Milad, M. R. & Quirk, G. J. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* **420**, 70-74, doi:10.1038/nature01138 (2002).

22 Quirk, G. J., Garcia, R. & Gonzalez-Lima, F. Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry* **60**, 337-343 (2006).

23 Quirk, G. J., Likhtik, E., Pelletier, J. G. & Pare, D. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J Neurosci* **23**, 8800-8807 (2003).

24 Quirk, G. J. & Mueller, D. Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* **33**, 56-72 (2008).

25 Ochsner, K. N. & Gross, J. J. The cognitive control of emotion. *Trends Cogn Sci* **9**, 242-249, doi:S1364-6613(05)00090-2 [pii]
10.1016/j.tics.2005.03.010 [doi] (2005).

26 Milad, M. R. *et al.* Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry* **62**, 446-454, doi:10.1016/j.biopsych.2006.10.011 (2007).

27 Kalisch, R. *et al.* Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *J Neurosci* **26**, 9503-9511 (2006).

28 Corcoran, K. A., Desmond, T. J., Frey, K. A. & Maren, S. Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *J Neurosci* **25**, 8978-8987 (2005).

29 Difede, J., Olden, M. & Cukor, J. Evidence-based treatment of post-traumatic stress disorder. *Annu Rev Med* **65**, 319-332, doi:10.1146/annurev-med-051812-145438 (2014).

30 Foa, E. B. & Kozak, M. J. Emotional processing of fear: exposure to corrective information. *Psychol Bull* **99**, 20-35 (1986).

31 Hauner, K. K., Mineka, S., Voss, J. L. & Paller, K. A. Exposure therapy triggers lasting reorganization of neural fear processing. *Proc Natl Acad Sci U S A* **109**, 9203-9208, doi:10.1073/pnas.1205242109 (2012).
32 Hembree, E. A. *et al.* Do patients drop out prematurely from exposure therapy for PTSD? *J Trauma Stress* **16**, 555-562, doi:10.1023/B:JOTS.0000004078.93012.7d (2003).
33 Eftekhari, A. *et al.* Effectiveness of national implementation of prolonged exposure therapy in Veterans Affairs care. *JAMA Psychiatry* **70**, 949-955, doi:10.1001/jamapsychiatry.2013.36 (2013).
34 Norton, P. J., Hayes-Skelton, S. A. & Klenck, S. C. What happens in session does not stay in session: changes within exposures predict subsequent improvement and dropout. *J Anxiety Disord* **25**, 654-660, doi:10.1016/j.janxdis.2011.02.006 (2011).
35 Schneier, F. R. *et al.* Combined prolonged exposure therapy and paroxetine for PTSD related to the World Trade Center attack: a randomized controlled trial. *Am J Psychiatry* **169**, 80-88, doi:10.1176/appi.ajp.2011.11020321 (2012).
36 Resick, P. A., Nishith, P., Weaver, T. L., Astin, M. C. & Feuer, C. A. A comparison of cognitive-processing therapy with prolonged exposure and a waiting condition for the treatment of chronic posttraumatic stress disorder in female rape victims. *J Consult Clin Psychol* **70**, 867-879 (2002).
37 Schnurr, P. P. *et al.* Cognitive behavioral therapy for posttraumatic stress disorder in women: a randomized controlled trial. *JAMA* **297**, 820-830, doi:10.1001/jama.297.8.820 (2007).
38 Simmons, A. N., Norman, S. B., Spadoni, A. D. & Strigo, I. A. Neurosubstrates of remission following prolonged exposure therapy in veterans with posttraumatic stress disorder. *Psychother Psychosom* **82**, 382-389, doi:10.1159/000348867 (2013).
39 Simon, N. M. *et al.* Paroxetine CR augmentation for posttraumatic stress disorder refractory to prolonged exposure therapy. *J Clin Psychiatry* **69**, 400-405 (2008).
40 Steenkamp, M. M., Litz, B. T., Hoge, C. W. & Marmar, C. R. Psychotherapy for Military-Related PTSD: A Review of Randomized Clinical Trials. *Jama* **314**, 489-500, doi:10.1001/jama.2015.8370 (2015).
41 Jovanovic, T. & Ressler, K. J. How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *A . J. Psychiatry* **167**, 648-662 (2010).
42 Graham, B. M. & Milad, M. R. The study of fear extinction: implications for anxiety disorders. *Am J Psychiatry* **168**, 1255-1265, doi:10.1176/appi.ajp.2011.11040557 (2011).
43 Milad, M. R. & Quirk, G. J. Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol* **63**, 129-151, doi:10.1146/annurev.psych.121208.131631 (2012).
44 Bitencourt, R. M., Pamplona, F. A. & Takahashi, R. N. Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *European Neuropsychopharmacology* **18**, 849-859 (2008).
45 Chhatwal, J. P., Davis, M., Maguschak, K. A. & Ressler, K. J. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* **30**, 516-524 (2005).
46 de Oliveira Alvares, L., Pasqualini Genro, B., Diehl, F., Molina, V. A. & Quillfeldt, J. A. Opposite action of hippocampal CB1 receptors in memory reconsolidation and extinction. *Neuroscience* **154**, 1648-1655 (2008).
47 Lafenetre, P., Chaouloff, F. & Marsicano, G. The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. *Pharmacol Res* **56**, 367-381 (2007).
48 Lin, H. C., Mao, S. C., Chen, P. S. & Gean, P. W. Chronic cannabinoid administration in vivo compromises extinction of fear memory. *Learn Mem* **15**, 876-884 (2008).
49 Lin, H. C., Mao, S. C., Su, C. L. & Gean, P. W. The role of prefrontal cortex CB1 receptors in the modulation of fear memory. *Cereb Cortex* **19**, 165-175 (2009).
50 Lutz, B. The endocannabinoid system and extinction learning. *Mol Neurobiol* **36**, 92-101 (2007).
51 Marsicano, G. *et al.* The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530-534 (2002).
52 Pamplona, F. A., Bitencourt, R. M. & Takahashi, R. N. Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. *Neurobiol Learn Mem* **90**, 290-293 (2008).

53 Pamplona, F. A., Prediger, R. D., Pandolfo, P. & Takahashi, R. N. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. *Psychopharmacology (Berl)* **188**, 641-649 (2006).
54 Roche, M., O'Connor, E., Diskin, C. & Finn, D. P. The effect of CB(1) receptor antagonism in the right basolateral amygdala on conditioned fear and associated analgesia in rats. *Eur J Neurosci* **26**, 2643-2653 (2007).
55 Rabinak, C. A. *et al.* Cannabinoid facilitation of fear extinction memory recall in humans. *Neuropharmacology* **64**, 396-402, doi:10.1016/j.neuropharm.2012.06.063
S0028-3908(12)00337-1 [pii] (2013).
56 Davis, M. & Whalen, P. J. The amygdala: vigilance and emotion. *Mol Psychiatry* **6**, 13-34 (2001).
57 Amano, T., Unal, C. T. & Pare, D. Synaptic correlates of fear extinction in the amygdala. *Nat Neurosci* **13**, 489-494, doi:nn.2499 [pii]
10.1038/nn.2499 [doi].
58 Pape, H. C. & Pare, D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol Rev* **90**, 419-463, doi:90/2/419 [pii]
10.1152/physrev.00037.2009 [doi] (2010).
59 Pare, D., Quirk, G. J. & Ledoux, J. E. New vistas on amygdala networks in conditioned fear. *J Neurophysiol* **92**, 1-9 (2004).
60 LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E. & Phelps, E. A. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* **20**, 937-945 (1998).
61 Phelps, E. A. *et al.* Activation of the left amygdala to a cognitive representation of fear. *Nat Neurosci* **4**, 437-441 (2001).
62 Milad, M. R. *et al.* Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 10706-10711 (2005).
63 Hartley, C. A., Fischl, B. & Phelps, E. A. Brain structure correlates of individual differences in the acquisition and inhibition of conditioned fear. *Cereb Cortex* **21**, 1954-1962, doi:10.1093/cercor/bhq253 (2011).
64 Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J. & Phan, K. L. Amygdala-frontal connectivity during emotion regulation. *Soc Cogn Affect Neurosci* **2**, 303-312, doi:10.1093/scan/nsm029 [doi] (2007).
65 Kalisch, R. *et al.* Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *J Neurosci* **26**, 9503-9511 (2006).
66 Hamann, S. B., Ely, T. D., Grafton, S. T. & Kilts, C. D. Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nat Neurosci* **2**, 289-293, doi:10.1038/6404 [doi] (1999).
67 Kilpatrick, L. & Cahill, L. Amygdala modulation of parahippocampal and frontal regions during emotionally influenced memory storage. *NeuroImage* **20**, 2091-2099, doi:S1053811903004786 [pii] (2003).
68 Ritchey, M., Dolcos, F. & Cabeza, R. Role of amygdala connectivity in the persistence of emotional memories over time: an event-related FMRI investigation. *Cereb Cortex* **18**, 2494-2504, doi:bhm262 [pii]
10.1093/cercor/bhm262 [doi] (2008).
69 Phelps, E. A. Human emotion and memory: interactions of the amygdala and hippocampal complex. *Curr Opin Neurobiol* **14**, 198-202, doi:10.1016/j.conb.2004.03.015 [doi]
S0959438804000479 [pii] (2004).
70 Murty, V. P., Ritchey, M., Adcock, R. A. & LaBar, K. S. Reprint of: fMRI studies of successful emotional memory encoding: a quantitative meta-analysis. *Neuropsychologia* **49**, 695-705, doi:S0028-3932(11)00093-5 [pii]
10.1016/j.neuropsychologia.2011.02.031 [doi] (2011).
71 Milad, M. R. *et al.* Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry* **66**, 1075-1082, doi:10.1016/j.biopsych.2009.06.026 (2009).

72 Garfinkel, S. N. *et al.* Impaired contextual modulation of memories in PTSD: an fMRI and psychophysiological study of extinction retention and fear renewal. *J Neurosci* **34**, 13435-13443, doi:10.1523/jneurosci.4287-13.2014 (2014).
73 Bouton, M. E. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol Psychiatry* **52**, 976-986 (2002).
74 LeDoux, J. E. Emotion circuits in the brain. *Annual Review of Neuroscience* **23**, 155-184 (2000).
75 Shin, L. M. *et al.* Visual imagery and perception in posttraumatic stress disorder. A positron emission tomographic investigation. *Arch Gen Psychiatry* **54**, 233-241 (1997).
76 Shin, L. M. *et al.* Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. *Arch Gen Psychiatry* **61**, 168-176 (2004).
77 Liberzon, I. *et al.* Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiatry* **45**, 817-826, doi:S0006-3223(98)00246-7 [pii] (1999).
78 Pissiota, A. *et al.* Neurofunctional correlates of posttraumatic stress disorder: a PET symptom provocation study. *Eur Arch Psychiatry Clin Neurosci* **252**, 68-75, doi:10.1007/s004060200014 [doi] (2002).
79 Vermetten, E., Schmahl, C., Southwick, S. M. & Bremner, J. D. Positron tomographic emission study of olfactory induced emotional recall in veterans with and without combat-related posttraumatic stress disorder. *Psychopharmacol Bull* **40**, 8-30 (2007).
80 Driessen, M. *et al.* Posttraumatic stress disorder and fMRI activation patterns of traumatic memory in patients with borderline personality disorder. *Biol Psychiatry* **55**, 603-611, doi:10.1016/j.biopsych.2003.08.018 [doi]
S0006322303009235 [pii] (2004).
81 Morey, R. A. *et al.* The role of trauma-related distractors on neural systems for working memory and emotion processing in posttraumatic stress disorder. *J Psychiatr Res* **43**, 809-817, doi:S0022-3956(08)00242-2 [pii]
10.1016/j.jpsychires.2008.10.014 [doi] (2009).
82 Protopopescu, X. *et al.* Differential time courses and specificity of amygdala activity in posttraumatic stress disorder subjects and normal control subjects. *Biol Psychiatry* **57**, 464-473, doi:S0006-3223(04)01366-6 [pii]
10.1016/j.biopsych.2004.12.026 [doi] (2005).
83 Hendler, T. *et al.* Sensing the invisible: differential sensitivity of visual cortex and amygdala to traumatic context. *NeuroImage* **19**, 587-600, doi:S1053811903001411 [pii] (2003).
84 Rauch, S. L. *et al.* Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* **47**, 769-776, doi:S0006-3223(00)00828-3 [pii] (2000).
85 Shin, L. M. *et al.* A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry* **62**, 273-281, doi:62/3/273 [pii]
10.1001/archpsyc.62.3.273 [doi] (2005).
86 Bryant, R. A. *et al.* Enhanced amygdala and medial prefrontal activation during nonconscious processing of fear in posttraumatic stress disorder: an fMRI study. *Hum Brain Mapp* **29**, 517-523, doi:10.1002/hbm.20415 [doi] (2008).
87 Williams, L. M. *et al.* Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. *NeuroImage* **29**, 347-357, doi:S1053-8119(05)00542-2 [pii]
10.1016/j.neuroimage.2005.03.047 [doi] (2006).
88 Rabinak, C. A. *et al.* Focal and aberrant prefrontal engagement during emotion regulation in veterans with posttraumatic stress disorder. *Depress Anxiety* **31**, 851-861, doi:10.1002/da.22243 (2014).
89 Pitman, R. K., Shin, L. M. & Rauch, S. L. Investigating the pathogenesis of posttraumatic stress disorder with neuroimaging. *J Clin Psychiatry* **62 Suppl 17**, 47-54 (2001).
90 Rauch, S. L., Shin, L. M., Whalen, P. J. & Pitman, R. K. Neuroimaging and the neuroanatomy of PTSD. *CNS Spectrums* **3**, 30-41 (1998).

91 Liberzon, I. & Phan, K. L. Brain-imaging studies of posttraumatic stress disorder. *CNS Spectr* **8**, 641-650 (2003).
92 Rauch, S. L. & Shin, L. M. Functional neuroimaging studies in posttraumatic stress disorder. *Ann N Y Acad Sci* **821**, 83-98 (1997).
93 Bremner, J. D. *et al.* Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. *Am J Psychiatry* **156**, 1787-1795 (1999).
94 Bremner, J. D. *et al.* Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. *Biol Psychiatry* **53**, 879-889, doi:S0006322302018917 [pii] (2003).
95 Shin, L. M. *et al.* Hippocampal function in posttraumatic stress disorder. *Hippocampus* **14**, 292-300, doi:10.1002/hipo.10183 [doi] (2004).
96 Bonne, O. *et al.* Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *Am J Psychiatry* **158**, 1248-1251 (2001).
97 Shin, L. M., Rauch, S. L. & Pitman, R. K. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann N Y Acad Sci* **1071**, 67-79, doi:1071/1/67 [pii]
10.1196/annals.1364.007 [doi] (2006).
98 Werner, N. S. *et al.* Hippocampal function during associative learning in patients with posttraumatic stress disorder. *J Psychiatr Res* **43**, 309-318, doi:S0022-3956(08)00077-0 [pii]
10.1016/j.jpsychires.2008.03.011 [doi] (2009).
99 Rougemont-Bucking, A. *et al.* Altered processing of contextual information during fear extinction in PTSD: an fMRI study. *CNS Neurosci Ther* **17**, 227-236, doi:10.1111/j.1755-5949.2010.00152.x (2011).
100 Milad, M. R. *et al.* Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry* **66**, 1075-1082 (2009).
101 Foa, E. B. Psychosocial treatment of posttraumatic stress disorder. *J Clin Psychiatry* **61 Suppl 5**, 43-48; discussion 49-51 (2000).
102 van Minnen, A. & Hagenaars, M. Fear activation and habituation patterns as early process predictors of response to prolonged exposure treatment in PTSD. *J Trauma Stress* **15**, 359-367 (2002).
103 Charney, D. S. & Deutch, A. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. *Crit Rev Neurobiol* **10**, 419-446 (1996).
104 Orr, S. P. *et al.* De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *J Abnorm Psychol* **109**, 290-298 (2000).
105 Milad, M. R. *et al.* Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. *J Psychiatr Res* **42**, 515-520, doi:10.1016/j.jpsychires.2008.01.017 (2008).
106 Bitencourt, R. M., Pamplona, F. A. & Takahashi, R. N. Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur Neuropsychopharmacol* **18**, 849-859 (2008).
107 Katona, I. *et al.* Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci* **21**, 9506-9518 (2001).
108 Katona, I. *et al.* GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* **100**, 797-804 (2000).
109 Katona, I. *et al.* Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19**, 4544-4558 (1999).
110 Rabinak, C. A., Sripada, C. S., Angstadt, M., de Wit, H. & Phan, K. L. Cannabinoid modulation of subgenual anterior cingulate cortex activation during experience of negative affect. *J Neural Transm* **119**, 701-707, doi:10.1007/s00702-011-0747-x (2012).
111 Chhatwal, J. P. & Ressler, K. J. Modulation of fear and anxiety by the endogenous cannabinoid system. *CNS Spectr* **12**, 211-220 (2007).
112 Campolongo, P. *et al.* Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc Natl Acad Sci U S A* **106**, 4888-4893, doi:10.1073/pnas.0900835106 (2009).

113 Rabinak, C. A. *et al.* Cannabinoid modulation of prefrontal-limbic activation during fear extinction learning and recall in humans. *Neurobiol Learn Mem* **113**, 125-134, doi:10.1016/j.nlm.2013.09.009 (2014).
114 de Kleine, R. A., Rothbaum, B. O. & van Minnen, A. Pharmacological enhancement of exposure-based treatment in PTSD: a qualitative review. *European journal of psychotraumatology* **4**, doi:10.3402/ejpt.v4i0.21626 (2013).
115 Hofmann, S. G. d-CYCLOSERINE FOR TREATING ANXIETY DISORDERS: MAKING GOOD EXPOSURES BETTER AND BAD EXPOSURES WORSE. *Depression and Anxiety* **31**, 175-177, doi:10.1002/da.22257 (2014).
116 Hofmann, S. G., Mundy, E. A. & Curtiss, J. Neuroenhancement of Exposure Therapy in Anxiety Disorders. *AIMS neuroscience* **2**, 123-138, doi:10.3934/Neuroscience.2015.3.123 (2015).
117 Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A. & Ressler, K. J. Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol Ther* **149**, 150-190, doi:10.1016/j.pharmthera.2014.12.004 (2015).
118 Endo, A., Nagatani, F., Hamada, C. & Yoshimura, I. Minimization method for balancing continuous prognostic variables between treatment and control groups using Kullback-Leibler divergence. *Contemporary clinical trials* **27**, 420-431, doi:10.1016/j.cct.2006.05.002 (2006).
119 Kalish, L. A. & Begg, C. B. Treatment allocation methods in clinical trials: a review. *Statistics in medicine* **4**, 129-144 (1985).
120 Lin, Y., Zhu, M. & Su, Z. The pursuit of balance: An overview of covariate-adaptive randomization techniques in clinical trials. *Contemporary clinical trials*, doi:10.1016/j.cct.2015.07.011 (2015).
121 Pocock, S. J. & Simon, R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* **31**, 103-115 (1975).
122 Xiao, L., Huang, Q., Yank, V. & Ma, J. An easily accessible Web-based minimization random allocation system for clinical trials. *Journal of medical Internet research* **15**, e139, doi:10.2196/jmir.2392 (2013).
123 Hu, F., Hu, Y., Ma, Z. & Rosenberger, W. F. Adaptive randomization for balancing over covariates. *Wiley Interdisciplinary Reviews: Computational Statistics* **6**, 288-303, doi:10.1002/wics.1309 (2014).
124 Powers, M. B., Halpern, J. M., Ferenschak, M. P., Gillihan, S. J. & Foa, E. B. A meta-analytic review of prolonged exposure for posttraumatic stress disorder. *Clin Psychol Rev* **30**, 635-641, doi:10.1016/j.cpr.2010.04.007 (2010).
125 Beck, J. S. & Beck, A. T. *Cognitive Behavior Therapy, Second Edition: Basics and Beyond*. (The Guilford Press, 2011).
126 Kirk, J. M. & de Wit, H. Responses to oral delta9-tetrahydrocannabinol in frequent and infrequent marijuana users. *Pharmacol Biochem Behav* **63**, 137-142 (1999).
127 Phan, K. L. *et al.* Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci* **28**, 2313-2319 (2008).
128 Wachtel, S. R., ElSohly, M. A., Ross, S. A., Ambre, J. & de Wit, H. Comparison of the subjective effects of Delta(9)-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology (Berl)* **161**, 331-339 (2002).
129 Dunsmoor, J. E., Bandettini, P. A. & Knight, D. C. Neural correlates of unconditioned response diminution during Pavlovian conditioning. *Neuroimage* **40**, 811-817 (2008).
130 Wolpe, J. & Lazarus, A. A. *Behavior therapy techniques*. (Pergamon Press, 1966).
131 Spielberger, C. *Manual for the State-Trait Anxiety Inventory, STAI (Form Y) ("Self-Evaluation Questionnaire")*. (Consulting Psychologists Press, 1983).
132 Dubois, N., Paccou, A. P., De Backer, B. G. & Charlier, C. J. Validation of the Quantitative Determination of Tetrahydrocannabinol and Its Two Major Metabolites in Plasma by Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry According to the Total Error Approach. *Journal of Analytical Toxicology* **36**, 25-29, doi:10.1093/jat/bkr009 (2012).
133 Noll, D. C., Stenger, V. A., Vazquez, A. L. & Peltier, S. J. in *Medical radiology: Functional mri* (eds C. Moonen & P.A. Bandettini) (Spring-Verlag, 1999).
134 Yip, C. Y., Fessler, J. A. & Noll, D. C. Advanced three-dimensional tailored RF pulse for signal recovery in T2*-weighted functional magnetic resonance imaging. *Magn Reson Med* **56**, 1050-1059 (2006).

135 Yang, Y. *et al.* Simultaneous perfusion and BOLD imaging using reverse spiral scanning at 3T: characterization of functional contrast and susceptibility artifacts. *Magn Reson Med* **48**, 278-289 (2002).
136 Hochberg, Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* **75**, 800-802 (1988).
137 Friston, K. J. *et al.* Event-related fMRI: characterizing differential responses. *NeuroImage* **7**, 30-40, doi:S1053-8119(97)90306-2 [pii]
10.1006/nimg.1997.0306 [doi] (1998).
138 Friston, K. J., Frith, C. D., Frackowiak, R. S. & Turner, R. Characterizing dynamic brain responses with fMRI: a multivariate approach. *NeuroImage* **2**, 166-172, doi:S1053811985710191 [pii] (1995).
139 Friston, K. J. *et al.* Analysis of fMRI time-series revisited. *NeuroImage* **2**, 45-53, doi:S1053-8119(85)71007-5 [pii]
10.1006/nimg.1995.1007 [doi] (1995).
140 Josephs, O., Turner, R. & Friston, K. Event-related f MRI. *Hum Brain Mapp* **5**, 243-248, doi:10.1002/(SICI)1097-0193(1997)5:4<243::AID-HBM7>3.0.CO;2-3 [doi] (1997).
141 Milad, M. R. *et al.* Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proc Natl Acad Sci U S A* **102**, 10706-10711 (2005).
142 Milad, M. R. *et al.* Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry* **62**, 446-454 (2007).
143 Lancaster, J. L. *et al.* Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* **10**, 120-131 (2000).
144 Talairach, J. & Tournoux, P. *Co-Planar Stereotaxic Atlas of the Human Brain*. (Theime, 1988).
145 Mai, J., Asscheuer, J. & Paxinos, G. *Atlas of the Human Brain*. (Academic Press, 1997).
146 Das, P. *et al.* Pathways for fear perception: modulation of amygdala activity by thalamo-cortical systems. *Neuroimage* **26**, 141-148 (2005).
147 Friston, K. J. *et al.* Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage* **6**, 218-229, doi:S1053-8119(97)90291-3 [pii]
10.1006/nimg.1997.0291 [doi] (1997).
148 Klumpp, H., Fitzgerald, D. A. & Phan, K. L. Neural predictors and mechanisms of cognitive behavioral therapy on threat processing in social anxiety disorder. *Prog Neuropsychopharmacol Biol Psychiatry* **45**, 83-91, doi:10.1016/j.pnpbp.2013.05.004 (2013).
149 Godlewska, B. R., Norbury, R., Selvaraj, S., Cowen, P. J. & Harmer, C. J. Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. *Psychol Med* **42**, 2609-2617, doi:10.1017/s0033291712000591 (2012).
150 Sheehan, D. V. *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* **59 Suppl 20**, 22-33;quiz 34-57 (1998).
151 Zuurman, L., Ippel, A. E., Moin, E. & van Gerven, J. M. Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br J Clin Pharmacol* **67**, 5-21, doi:10.1111/j.1365-2125.2008.03329.x (2009).
152 Curran, H. V., Brignell, C., Fletcher, S., Middleton, P. & Henry, J. Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology (Berl)* **164**, 61-70, doi:10.1007/s00213-002-1169-0 (2002).
154 Weathers, F. W. *et al. The Clinician-Adminstered PTSD Scale for DSM-5 (CAPS-5)*, <Interview available from the National Center for PTSD at www.ptsd.va.gov> (2013).
155 Landis, J. R. & Koch, G. G. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* **33**, 363-374 (1977).
156 Weathers, F. W. *et al. The Life Events Checklist for DSM-5 (LEC-5)*, <Instrument available from the National Center for PTSD at www.ptsd.va.gov> (2013).
157 Weathers, F. W. *et al. The PTSD Checklist for DSM-5 (PCL-5)*, <Scale available from the National Center for PTSD at www.ptsd.va.gov> (2013).
158 Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R. & Kupfer, D. J. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* **28**, 193-213 (1989).

159 Spielberger, C., Gorsuch, R., Lushene, R. & al., e. *Manual for the State-Trait Anxiety Inventory: STAI (Form Y).* (Consulting Psychologist Press, 1983).
160 Hamilton, M. A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry* **23**, 56-62 (1960).
161 Hamilton, M. The assessment of anxiety states by rating. *British Journal of Medical Psychology* **32**, 50-55 (1959).
162 Beck, A., Steer, R., Ball, R. & W, R. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *Journal of Personality Assessment* **67**, 588-597 (2996).
163 Beck, A., Ward, C., Mendelson, M., Mock, J. & Erbaugh, J. An inventory for measuring depression. *Arch Gen Psychiatry* **4**, 561-571 (1961).
164 Beck, A. & Steer, R. Internal consistencies of the original and revised Beck Depression Inventory. *Journal of Clinical Psychology* **40**, 1365-1367 (1984).
165 Beck, A., Rial, W. & Rickets, K. Short form of depression inventory: cross-validation. *Psychological Reports* **34**, 1184-1186 (1974).
166 Posner, K., Oquendo, M. A., Gould, M., Stanley, B. & Davies, M. Columbia Classification Algorithm of Suicide Assessment (C-CASA): Classification of suicidal events in the FDA's pediatric suicidal risk analysis of antidepressants. *The American Journal of Psychiatry* **164**, 1035-1043 (2007).
167 TB, Ü., N, K. & S, C. *Measuring Health and Disability: Manual for WHO Disability Assessment Schedule (WHODAS 2.0).* (World Health Organization, 2010).
168 Bernstein, D. P. *et al.* Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl* **27**, 169-190 (2003).
169 Bernstein, D. P. & Fink, L. *Childhood Trauma Questionnaire: A retrospective self-report manual.*, (Psychological Corp., 1998).
170 Gross, J. J. & John, O. P. Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being. *J Pers Soc Psychol* **85**, 348-362 (2003).
171 Derryberry, D. & Reed, M. A. Anxiety-related attentional biases and their regulation by attentional control. *J Abnorm Psychol* **111**, 225-236 (2002).
172 de Wit, H. & Griffiths, R. R. Testing the abuse liability of anxiolytic and hypnotic drugs in humans. *Drug Alcohol Depend* **28**, 83-111 (1991).
173 Fischman, M. W. & Foltin, R. W. Utility of subjective-effects measurements in assessing abuse liability of drugs in humans. *Br J Addict* **86**, 1563-1570 (1991).
174 Morean, M. E. *et al.* The drug effects questionnaire: psychometric support across three drug types. *Psychopharmacology (Berl)* **227**, 177-192, doi:10.1007/s00213-012-2954-z (2013).
175 Martin, W. R., Sloan, J. W., Sapira, J. D. & Jasinski, D. R. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clin Pharmacol Ther* **12**, 245-258 (1971).
176 Chait, L. D., Fischman, M. W. & Schuster, C. R. 'Hangover' effects the morning after marijuana smoking. *Drug Alcohol Depend* **15**, 229-238 (1985).
177 Calhoun, S. R., Galloway, G. P. & Smith, D. E. Abuse potential of dronabinol (Marinol). *Journal of psychoactive drugs* **30**, 187-196, doi:10.1080/02791072.1998.10399689 (1998).
178 Schuster, C. R. Testing and abuse liability of drugs in humans. *NIDA Res Monogr* **92**, 1-6 (1989).
179 O'Brien, P. C. & Fleming, T. R. A multiple testing procedure for clinical trials. *Biometrics* **35**, 549-556 (1979).