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Research Article

The Effects of Full Spectrum Hemp Oil on Extinction of Stress-Enhanced Fear Learning: A Rat Model of PTSD

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Abstract

Despite overwhelming evidence for more effective treatments, there are only 2 FDA-approved drugs for Post-Traumatic Stress Disorder (PTSD). Indeed, roughly 50% of PTSD patients experience relief from conventional pharmaceuticals and only one third experience full remission. The *Cannabis* plant is a promising novel treatment for PTSD as the endocannabinoid system plays a role in stress, emotion, cognition and fear memory consolidation, retrieval, reconsolidation and extinction. Cannabidiol (CBD), one of the most studied phytocannabinoids in the *Cannabis* plant, has anxiolytic effects, however, the effects of full spectrum hemp oil that contains all of the active compounds from the cannabis plant with less than 0.3% THC has not been studied. Therefore, the present study examined the effects of full spectrum hemp oil on fear extinction following stress-enhanced fear learning (SEFL), a rodent model of PTSD. Adult male rats were assigned to either a no-trauma control (no-shock) or a trauma (15 shocks) group, and to either a control (peanut butter) or hemp oil (dissolved in peanut butter) group and tested for fear expression across five extinction trials. Compared to the no-trauma group, SEFL produced robust freezing in the trauma group in the original context, in a novel context following a single shock and during the first four extinction trials. However, within the trauma group there were no significant differences in freezing between the hemp oil and vehicle treated rats across the 5 extinction trials. These results suggest that full spectrum hemp oil did not significantly affect extinction of SEFL.

Introduction

Background

Post-Traumatic Stress Disorder (PTSD) is a chronic psychopathology characterized by debilitating fluctuations in emotions, mood, cognition and social skills [1]. Currently, the DSM-V-TR classifies PTSD as a trauma and stress-related disorder characterized by four main symptom clusters: Intrusions (nightmares) and disassociations (flashbacks), Avoidance (of the trauma or memory), Negative changes in mood or cognition, and Hyperarousal and reactivity [2]. These four symptom clusters are often associated with or driven by traumatic memories, with a hallmark feature of PTSD being an inability to extinguish memories associated with the traumatic event. Rates of PTSD in the general population are 8-12% but are higher in specific subpopulations including 15% of assault survivors, 45% of physically and/or sexually abused women and 50% of physically

and/or sexually abused children [1,3-6]. There is also an increased risk of suicidal ideation, suicide attempts and suicide completion in people suffering from PTSD compared to the general population [1,7]. Thus, there is a critical need for research aimed at identifying novel and effective pharmacotherapies for treating PTSD.

Despite the need, there are currently only 2 FDA-approved medications for treating PTSD, the selective serotonin reuptake inhibitors (SSRIs) sertraline and paroxetine [8-10]. If these are ineffective or have undesirable side effects, additional off label medications include other SSRIs (e.g., fluoxetine), selective norepinephrine reuptake inhibitors (e.g., venlafaxine) and/or antiepileptic drugs (e.g., topiramate [9]. Additionally, beta adrenergic antagonists can treat hyperarousal and have been shown to enhance fear extinction in patients with PTSD [11]. Unfortunately, SSRIs only help 50% of PTSD patients and less than 30% of patients experience full remission even after years of treatment [12-14]. While antidepressants may help with mood and anxiety in some patients there is no approved treatment for the cognitive symptomology of PTSD [14]. Psychotherapy for PTSD includes cognitive behavioral therapy, and eye movement desensitization and reprocessing [9]. However, deficits in extinction learning in patients with PTSD reduce the effectiveness of psychotherapy; therefore, to improve PTSD treatment, future research targeting novel extinction-enhancing pharmacotherapies that can be used in conjunction with psychotherapy is desperately needed [1,15].

Cannabis and the endocannabinoid system

Cannabis is a plant containing over 100 cannabinoids that are non-psychoactive and non-addictive including the most well-known, cannabidiol [16,17]. The infamous and main psychoactive compound in cannabis is $\Delta 9$ tetrahydrocannabinol [16]. Two cannabinoid receptors, CB_1 and CB_2, are believed to be primarily responsible for the pharmacological actions of cannabis [1,18]. Most of cannabis' known effects are produced via the CB_1 receptor, which is densely expressed in the brain [1,18], particularly in areas that regulate stress, emotions, learning, anxiety-like behaviors and fear learning, including the prefrontal cortex, hippocampus and amygdala [1,19,20]. Blocking or deleting CB_1 receptors impairs extinction of fear learning and induces an anxious-like phenotype in rodents [1,21]. CB_1 receptor agonists decrease the reconsolidation of fear memories while enhancing fear extinction in rodents [11].

Additionally, CBD has been shown to produce anxiolytic-like effects by binding to both CB_1 and CB_2 receptors [1,22]. These data suggest that CB_1 and CB_2 regulate stress, anxiety, cognition and learning processes, highlighting these receptors as potential targets for treating PTSD [1,19,20,22].

Indeed, several recent clinical studies recording real-time cannabis use reported reductions in feelings of irritability/agitation and stress, along with increases in mood nearly immediately following cannabis consumption [23-25]. These data suggest that cannabis may be used to treat several symptoms associated with PTSD, however, one remaining unanswered question is does cannabis facilitate the hallmark feature of PTSD: extinction of memories associated with the traumatic event. To address this knowledge gap, the present study utilized the stress-enhanced fear learning (SEFL) model to examine the effects of full spectrum hemp oil on extinction of fear memory in adult male rats. We hypothesized that administering full spectrum hemp oil prior to extinction sessions would facilitate extinction of fear memory.

Materials and methods

Animals

Subjects were experimentally naïve male Long-Evans hooded rats (N=35, PND 110-111 at the time of trauma exposure) born and reared in the Logan Hall Animal Research Facility at the University of New Mexico. Following weaning (~PND 21), rats were pair housed in standard home cages (21.6 x 45.7 x 17.8 cm) in a temperature-controlled colony room (~22°C) with a reverse 12:12-h light-dark cycle (lights off at 10 am); 3 days prior to the start of the experiment rats were single housed for the remainder of the experiment. Food and water were available *ad libitum* in the rat's home cages. Experimental and husbandry procedures followed the Guide for the Care and Use of Laboratory Animals Committee [26] and were approved by the University of New Mexico's Institutional Animal Care and Use Committee.

Prior to the start of behavioral testing, rats were handled for 10 days (60-90 seconds each day) to adapt to experimenter handling stress (see Figure 1 for the experimental timeline). Next, rats were habituated to eating peanut butter for 15 days, which was used as the vehicle for cannabis administration. Finally, rats were randomly assigned to one of four experimental groups: no-trauma/vehicle, no-trauma/hemp oil, trauma/vehicle and trauma/hemp oil.
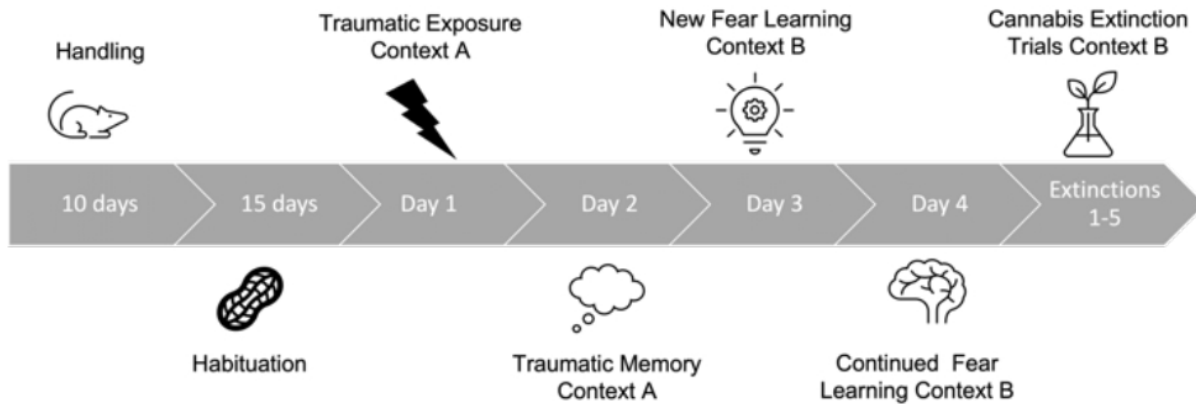
Figure 1: Experimental timeline. The experimental design consisted of 10 days of handling, 15 days of habituation to the peanut butter vehicle, 1 day of trauma exposure (context A), 1 day of traumatic memory testing (context A), 1 day of new fear learning (context B), 1 day of new fear memory testing (context B) and 5 extinction trials (context B), each separated by 1 week.

Drugs

Full-spectrum hemp oil was cultivated by Organic-Energetic Solutions LLC (Albuquerque, NM, USA) and is commercially available under the "LyFeBaak" label. Briefly, the cannabis goes through an extraction procedure where the cannabinoids, terpenes, lipids, chlorophyll and wax compounds are stripped from mature hemp flower ("buds") using an ethanol bath. Once all of the compounds from the plant are extracted, the alcohol solution is poured through a filter to remove any plant material. Next, the alcohol is evaporated, leaving only the cannabis compounds in an ultra-concentrated form. This ultra-concentrate is then combined with medium-chain triglyceride (MCT) oil to increase its bioavailability, for its final retail form.

Rats assigned to the two hemp oil groups received 1 mL or approximately .002mg/kg, of full-spectrum hemp oil [27] dissolved in a peanut butter (.152 g) vehicle; rats in the two control groups received the peanut vehicle. Peanut butter was chosen for the vehicle as it is high in fat and cannabis is fat soluble, thus increasing the bioavailability of the hemp oil.

Stress-Enhanced Fear Learning

The Stress-Enhanced Fear Learning (SEFL) procedures were adapted from an animal model of PTSD [28,29]. While no animal models currently capture all of the human complexities associated with PTSD, the SEFL model captures 3 critical components. First, in this model, the trauma consists of an un-cued, single traumatic event, realistic to the development of PTSD in humans [28-30]. Second, the SEFL model affects new fear learning, shown by the exaggerated fear response to a new context, similar to symptomology seen in PTSD patients [28-30]. Third, this model leads to lasting behavioral changes including resistance to extinction [29]. To our knowledge, no other research has used this model to study the effects of cannabis on extinction of SEFL.

Stress-enhanced fear learning took place in two distinct contexts (A & B) that differed in lighting, flooring, sound and odor [29]. Each context consisted of a larger sound attenuating chamber that housed a smaller fear-conditioning shock chamber (25.4 X 29.21 X 29.21 cm; Coulbourn Instruments LLC; Whitehall, PA). Context A consisted of a white visible light and a fan set at 60 dB for background noise [28,30]. The shock chamber inside context A consisted of smooth steel rod flooring with 27 steel bars (4mm diameter) arranged vertically every .635 cm. 1% acetic acid was used to clean the chamber and underneath pan after each use and 11% coconut extract was used to scent the pan [28-30]. Context B consisted of red lighting and a white noise generator set at 60 dB for background noise [28-30]. The shock chamber inside context B consisted of 2 black plexiglass side walls at a 60° angle to create an A-frame and had heavily textured steel rod flooring

with 18 steel bars (6mm diameter) arranged vertically every 1.27 cm [30]. 5% ammonium hydroxide was used to clean the chamber and underneath pan after each use and was also used to scent the pan [28]. In order to further minimize fear generalization, the two contexts were further differentiated by modes of transportation. Rats were either transported on a cart, in their uncovered home cage to context A, or were transported by hand in a clean, empty covered cage to context B [29].

The SEFL procedure occurred over 4 consecutive days followed by 5 extinction trials; each extinction trial was separated by 1 week (see Figure 1). Day one consisted of the trauma or no-trauma exposure in context A. Trauma rats were placed into the shock box in context A and received 240 seconds of pre-exposure to the environment. After this pre-exposure period, rats received 15 1-second footshocks (1milliamp) randomly over a 90-minute period [28-30]. Rats in the no-trauma group were placed in the shock box in context A for the same amount of time but did not receive any footshocks [28-30]. On day 2, rats were placed back into context A for 8 minutes without receiving any footshocks. Rat behavior was recorded to quantify freezing as the primary index of fear (Blanchard & Blanchard, 1969; Fanselow, 1980). Freezing in context A on day two reflects the fear memory associated with the trauma. On day 3, all rats were placed into the novel context B for a 180-second baseline period; freezing during this baseline period reflects fear generalization [29]. Following the baseline period, all rats received a single 1-second footshock (1milliamp) and remained in the box for 30 seconds to observe the fear response (freezing) to the single shock [29]. On day 4, rats were placed back into context B for 8 minutes; freezing in context B reflects SEFL from context A [29].

Extinction commenced one week after day 4 and occurred once a week for 5 weeks. Prior to each extinction trial, rats were pretreated with their assigned drug (vehicle or hemp oil) 2 hours prior to being placed in context B for an 8-minute extinction session to examine the effects of full spectrum hemp oil on the extinction of stress-enhanced fear-learning behavior [31].

Data Analysis

Freezing was operationally defined as the lack of movement except for respiration (Blanchard & Blanchard, 1969; Fanselow, 1980). Percent freezing was analyzed on days 2, 3 and 4, as well as all 5 extinction trials by an experimenter blind to group conditionings. Percent freezing was calculated using the formula $[(A1-A2)/A1]*100$ where A1 represents the total time spent in the context and A2 represents the time spent moving in the context. In order to verify that the conditioning procedures produced SEFL, separate independent samples t-tests were run comparing trauma and no-trauma groups on percent freezing across days. First, to confirm that the traumatic memory was intact following

the repeated footshocks, percent freezing was analyzed on day two during re-exposure to context A. In order to see if fear generalization occurred between contexts, we analyzed freezing in the novel context (B) during the baseline period on day 3. Next, following the single shock in context B, freezing was analyzed to examine whether an exaggerated fear response occurred after the single shock on day 3. In order to examine SEFL, we analyzed freezing in context B on day 4. Lastly, to examine the effects of full spectrum hemp oil on fear extinction, we used a repeated measures ANOVA with trauma and hemp oil groups as between subject factors and the 5 extinction trials as within subject factors. Independent sample t-tests were used to further probe significant interactions where appropriate. Levene's Test for equality of variance was performed on each dependent variable, and Cohen's d or Hedge's g (correction) were computed to provide effect sizes for significant results. SPSS 29 was used to perform all statistical analyses with $\alpha = 0.05$ for all comparisons. All data are reported as the mean ± standard error of the mean using Prism 9.0.

Results

Figure 2A indicates that rats in the trauma group froze significantly more in context A on day 2 compared to their no-trauma counterparts indicating that the memory of the traumatic event was intact $[t(1, 23.64) = 15.86, p<.001, g=4.92]$ Importantly, there was no significant difference between groups during the baseline period before the single shock on day 3, demonstrating that context A and B were sufficiently different and no fear generalization between the two contexts occurred [see Figure 2B; $t(1, 22.79) = 2.02, p>.05, g=.63$]. Figure 2C indicates that rats from the trauma group froze significantly more than their no-trauma counterparts $[t(1,33) = 6.82, p<.001, d=2.32]$ following the single 1-second 1-milliamp footshock, suggesting that previous exposure to the traumatic stressor created an exaggerated fear response to the mild stressor. Lastly, the trauma group froze significantly more than their no-trauma counterparts during re-exposure to context B (see Figure 2D), indicating that the exposure to the traumatic experience on day 1 enhanced fear learning to the mild stressor on day 3 $[t(1,33) = 7.88, p<.001, d=2.68]$.

The effects of full spectrum hemp oil on extinction over the course of the 5 trials are shown in Figure 3. The repeated measures ANOVA detected a significant main effect of extinction $[F(4,124) = 20.87, p<.001]$and an extinction by trauma interaction $[F(4,124) = 18.48, p<.001]$. Subsequent t-tests (see Figure 3A) indicate that rats in the trauma group spent more time freezing than the no-trauma controls on extinction days 1 $[t(1,29.18) = 6.45, p<.001, g=2.04]$, 2 $[t(1,33) = 5.04, p<.001, d=1.71]$, 3 $[t(1,33) = 4.28, p<.001, d=1.45]$ and 4 $[t(1,29.09) = 2.88, p<.005, g=.91]$, but day 5 was only a trend $[t(1,33) = 1.58, p=.12, d=.54]$. These data indicate that compared to no-trauma controls, rats in the trauma group showed delayed extinction. The ANOVA failed to detect a

significant interaction between extinction and hemp oil [$F_{(4,124)} = .85$, $p>.05$] and the three-way interaction between extinction, trauma and hemp oil was not significant [$F_{(4, 124)} = .28$, $p>.05$]. These data suggest that full spectrum hemp oil did not facilitate rates of extinction in the trauma (see Figure 3B) or no-trauma (see Figure 3C) groups.
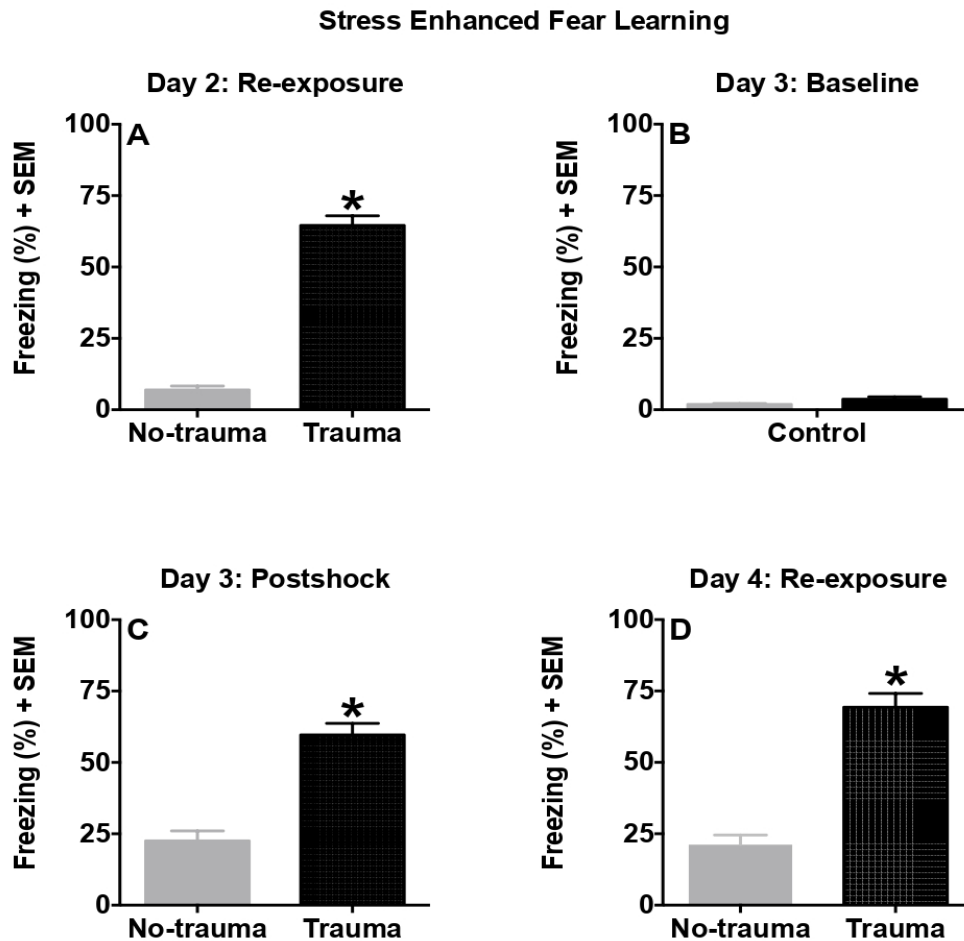

Figure 2: Freezing in context A and B on days 1 through 4. (A) Freezing in context A on day 2. The trauma group spent significantly more time freezing than the no-trauma group the day after the trauma (15 footshocks), indicating that the memory of the traumatic experience was intact. (B) Freezing baseline in context B on day 3. Prior to receiving the single footshock in context B, there was no significant difference in time spent freezing between the trauma and no-trauma groups (independent samples t-test, $p>.05$), indicating that there was no fear generalization between the two distinct contexts. (C) Freezing after the single shock in context B on day 3. The trauma group spent significantly more time freezing than the no-trauma group immediately following the single footshock on day 3, indicating an exaggerated fear response. (D) Freezing in context B on day 4. The trauma group spent significantly more time freezing than the no-trauma group the day after the single footshock, indicating enhanced fear learning in the trauma group. Asterisk (*) represents a significant difference compared to no-trauma controls (independent samples t-test, $p<.001$ in each case).
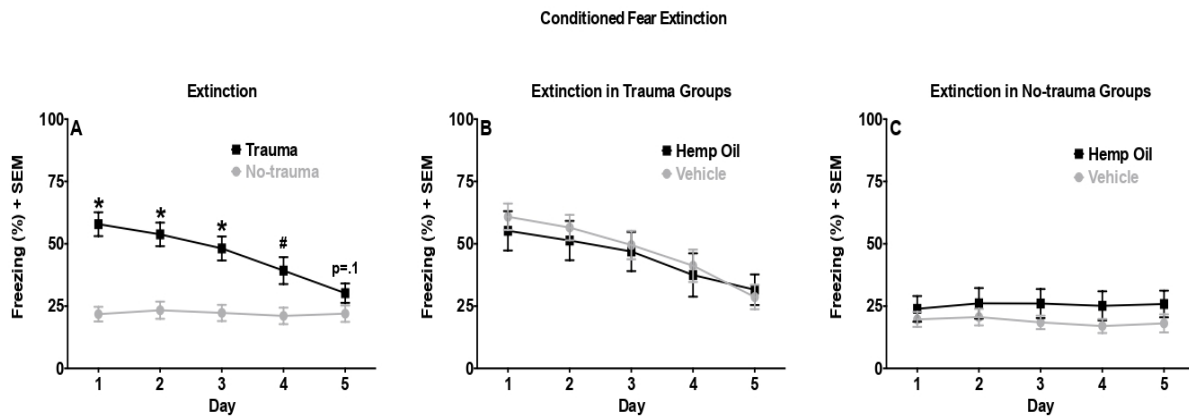
Figure 3: Freezing in context B across the 5 extinction trials. (A) Freezing in trauma and no trauma groups across the 5 extinction trials. The ANOVA indicated a main effect of extinction and a trauma by extinction interaction ($p < .001$ in each case). Subsequent independent sample *t*-tests indicated that rats in the trauma group froze significantly more than rats in the no-trauma group on extinction days 1 through 4 ($p < .005$ in each case), indicating that the trauma group showed resistance to extinction. (B – C) Effects of hemp oil on freezing across the 5 extinction trials in the trauma (B) and no-trauma (C) groups. In contrast, the ANOVA failed to detect a hemp oil by extinction interaction or a hemp oil by trauma by extinction interaction ($p > .05$ in each case), suggesting that full spectrum hemp oil did not facilitate fear extinction in the trauma or the no-trauma groups. Asterisk (*) and pound sign (#) represent significant differences compared to no-trauma controls (*$p < .001$; #$p < .005$).

Discussion

Our results replicate previous reports indicating that the SEFL model is a robust model of PTSD-like symptoms in adult male rats [28-30,32,33]. As expected, the trauma group (15 footshocks) showed higher levels of freezing compared to the no-trauma group (no footshocks) when they were returned to the traumatic context on day 2 (Figure 2A), indicating that the memory of the traumatic stressor was intact. Both groups showed minimal freezing in the novel context during the baseline period on day 3 (Figure 2B), suggesting that the enhanced freezing that occurred in this context following the single footshock (Figure 2C) was due to the mild stressor interacting with the prior trauma (context A) to produce an exaggerated fear response and was not simply the result of fear generalization from the trauma context. In addition to this heightened fear response following the single footshock, trauma rats re-exposed to context B on day 4 (Figure 2D), showed enhanced freezing compared to no-trauma rats indicating a stress-enhanced fear response (i.e., SEFL). It should be noted that this enhanced fear response is not due to generalization (Figure 2B) or increased fear expression (freezing) as the traumatic stressor must come before the mild stressor to increase fear of the novel context [28]. Collectively, the present data along with results from previous studies [28-30,32,33] indicate that the SEFL model produces reliable and long-lasting enhanced fear learning that is consistent with the symptomology seen in PTSD patients, suggesting it is a valid rodent model to probe the neural mechanisms of PTSD.

Contrary to our prediction, results from the present study indicate that full spectrum hemp oil did not facilitate extinction of SEFL across the 5 extinction trials (Figure 3). One possible reason that we failed to detect a significant reduction in freezing is that the hemp oil dose (.002 mg/kg) was insufficient. A study by Franzen and colleagues found that CBD (3.0 and 10 mg/kg) administered before conditioned context exposure reduced freezing in adult female rats. Further, they found that these doses of CBD reduced anxiety-like behavior in the elevated plus-maze (EPM). Interestingly, in an unpublished pilot study conducted in our lab the lower dose of hemp oil (.002 mg/kg) produced similar results to Franzen et al.'s study on anxiety-like behavior in the EPM. However, because the EPM and fear conditioning models reflect different underlying constructs (i.e., anxiety vs. fear), as well as the different procedures between the fear conditioning procedures in the present study and Franzen study (i.e., SEFL vs. standard fear conditioning), a higher hemp oil dose may be required to reduce conditioned freezing in the SEFL model.

A second possible reason we did not detect effects of full spectrum hemp oil on extinction of SEFL might be that hemp oil alters locomotor activity. CBD is thought to have little to no effects on locomotion, but when locomotor activity is altered, CBD is thought to balance out these deficits. For example, one study suggests that CBD increases locomotor activity after a spinal

injury while other studies suggest that CBD decreases locomotor activity in cases of Tardive Dyskinesis as well as from cocaine-induced hyperactivity [34-36]. CBD is also thought to decrease catalepsy and oral movements in patients with Parkinson's Disease [37]. Research on the effects of CBD on spontaneous locomotor activity is mixed. One study concluded that in rodents, CBD does not affect locomotor activity in the rotarod test, however, it decreased locomotor activity in the open field test [38]. This directly contradicts another study that reported CBD did not alter locomotor activity in the open field test [39]. Other studies suggest that CBD does not affect locomotor activity in tests like the light/dark test or the EPM, however, CBD did increase time freezing in both the conditioned emotional response test as well as in fear conditioning models [37,40,41]. The latter result contradicts Franzen et al.'s study that found CBD decreased freezing in a standard contextual fear conditioning model [42]. In our study, the full spectrum hemp oil treated rats did not show altered freezing when compared to their non-hemp oil counterparts, however, the possible effect of CBD on freezing in fear conditioning models may explain why we found no effect of full spectrum hemp oil on conditioned freezing following SEFL.

A third possible reason we did not detect effects of full spectrum hemp oil on extinction of SEFL may be due to what is known as the "entourage effect". The entourage effect occurs when all of the 100 or more compounds in the cannabis plant are combined, including cannabinoids, flavonoids and terpenes, and are hypothesized to work together to produce the desired effects of cannabis [43]. However, Federal laws limit the legal amount of THC in any type of *Cannabis* plant to be equal to or less than 0.3% weight/dried flower [44]. In the present study, we choose to examine the effects full spectrum hemp oil containing less than 0.3% THC as there is a large population of people who likely want the health effects of cannabis without the psychoactive effects of THC and follow the federal legality of hemp oil. In either case, a higher percentage of THC might be needed for the entourage effect to take effect and facilitate fear extinction following SEFL. Indeed, a recent study looking at THC to CBD ratios and their effects on memory found that cannabis use with higher THC content had the greatest effect on memory; therefore, it is possible that a higher THC content would better facilitate the extinction of SEFL [45].

Finally, we may have failed to detect an effect of full spectrum hemp oil on the facilitation of extinction as a result of the extinction protocol that was utilized. For example, hemp oil may have facilitated fear extinction if the trials were run consecutively across days instead of weekly. Additionally, it is possible that more than 5 extinction sessions, either run consecutively or weekly, may be needed for hemp oil to accelerate fear extinction. Future studies are needed to address these possible explanations.

Conclusions

Collectively, the present results indicate that full spectrum hemp oil did not facilitate extinction of SEFL. Given that THC may have greater effects on memory, a broader THC:CBD ratio dose range should be investigated using this experimental design. Previous studies have reported conflicting results with CBD impacting locomotion while other studies report no change [37-41]. Further research needs to be conducted to understand the relationship between CBD and locomotion, as well as full spectrum hemp oil, as this could impact the present results. While we found no effect of full spectrum hemp oil on extinction in males, Franzen et al., [42] found an effect in females suggesting sex may play a significant role in on how cannabis affects the extinction of fear memories. Thus, future research examining the effects of hemp oil on extinction of SEFL in female rats is needed. Lastly, a promising direction for future PTSD research could be to combine cannabis with other pharmacotherapies and/or psychotherapies. This approach might yield a more effective PTSD treatment since monotherapy is rarely successful in the treatment and remission of PTSD in humans. Future research should continue to investigate the link between the endocannabinoid system and its potential treatment for PTSD. Replication and extension of the present results using additional doses and/or cannabis combinations are needed to fully clarify if cannabis represents a viable treatment for PTSD.

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