Cannabinoids as Therapeutic Agents for Post-Traumatic Stress Disorder (PTSD)

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By

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Abstract

Post-Traumatic Stress Disorder (PTSD) is a debilitating psychiatric disorder and is the only one in which a traumatic event needs to occur to have a complete diagnosis. The average lifetime prevalence of PTSD in Canada is estimated at 9.2%. The female to male ratio of PTSD incidence is 2.8, which is likely due to the types and severity of trauma and the sex/gender differences in responding to traumatic events. Cannabidiol (CBD) is a non-psychoactive cannabinoid from the *Cannabis* plant. CBD has demonstrated therapeutic potentials for PTSD through anti-inflammatory and anxiolytic effects. Evidence suggests the pharmacological effects of CBD differ between males and females. This study aims to assess sex differences with respect to CBD-associated therapeutic effects on anxiety-like behaviours, cognitive function as well as cortisol and cytokine levels in a rat model of PTSD. The present study evaluated sex differences in Sprague Dawley rats when treated with orally administered CBD oil. Animals were subjected to chronic and unpredictable stress, with two separate predator exposures over 30 days. Female rats performed better than males in behavioural tests when exposed to stress. Exposure to *Cannabis* oil was anxiolytic in males but produced negative effects in female rats. Neurological differences contribute to vastly different behavioural responses in male and female rats; however, physical symptoms from chronic stress are highly similar. Further studies are necessary to determine molecular mechanisms related to stress exposure and the action of CBD.

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I would also like to thank HARAMBE for providing me guidelines for ethical animal research and providing clear guidelines for animal experiments.

Abbreviations List

2-AG	2-arachidonoyl glycerol
AEA	Anandamide
ARRIVE	Animal Research: Reporting of In Vivo Experiments
BNST	Bed Nucleus of Stria Terminalis
CAPS	Cognitive-Affective Processing System
CB1R	Cannabinoid 1 Receptor
CB2R	Cannabinoid 2 Receptor
CBD	Cannabidiol
CRF	Corticotrophin Releasing Factor
ECS	Endogenous Cannabinoid System
EPM	Elevated Plus Maze
HPA	Hypothalamic Pituitary Axis
IL	Interleukin
mPFC	Medial Pre-Frontal Cortex
OFT	Open Field Test
PBS	Phosphate Buffer Solution
PFA	Paraformaldehyde
PTSD	Post-Traumatic Stress Disorder
SD	Sprague Dawley
SUD	Substance Use Disorder
THC	Δ^9-Tetrahydrocannabinol
VTA	Ventral Tegmental Area

WPE	Whole Plant Extract

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Chapter 1: Introduction to cannabinoids as potential therapeutics in Post-Traumatic Stress Disorder (PTSD) and other neurological disorders.

This chapter has been published as a Review in the *Journal of Clinical Neurophysiology.* It will serve as a literature review for the project, as well as an introduction to how *Cannabis* has been used in animal and clinical models as potential therapeutic agents for neurological disorders like PTSD, alcohol and opioid abuse. This chapter will highlight the specific brain areas involved in PTSD and Substance Use Disorder (SUD) and the relationship between them and discuss current and emerging treatments of *Cannabis* for PTSD and SUD. All figures and text have been published with permission from the journal.

Cohen, Jacob[*,†]; Wei, Zelan[*]; Phang, Jonathan[*]; Laprairie, Robert B.[‡,§]; Zhang, Yanbo[*,†]
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1.1. Background

Post-Traumatic Stress Disorder (PTSD) and substance use disorders (SUD) have high comorbidity and pose significant difficulties for patients and treatment challenges for clinicians (Flanagan et al., 2016). The American Veteran's Association (VA) estimates that 27-76% of veterans with PTSD had concurrent SUD (Trivedi et al., 2015). One in three VA patients diagnosed with a SUD will also meet the criteria for PTSD diagnosis (Seal et al., 2011). Comorbid PTSD and SUD are associated with greater clinical distress, poorer treatment responses and poorer quality of life (Marx et al., 2009; Straus et al., 2019), which pose significant challenges to patients, families, clinicians, and the health care system (Back et al., 2009; Hawkins et al., 2012). Seventy percent of female respondents in drug and alcohol rehabilitation facilities have a history of sexual abuse, which is known to produce a high prevalence of PTSD (Liebschutz et al., 2002). Studies have found that PTSD and SUD share neurocircuit pathways and pathophysiology in the brain (Cohen et al., 2020) (Figure. 1.1).

Figure 1.1 Brain Areas Critical to Both PTSD and SUD.

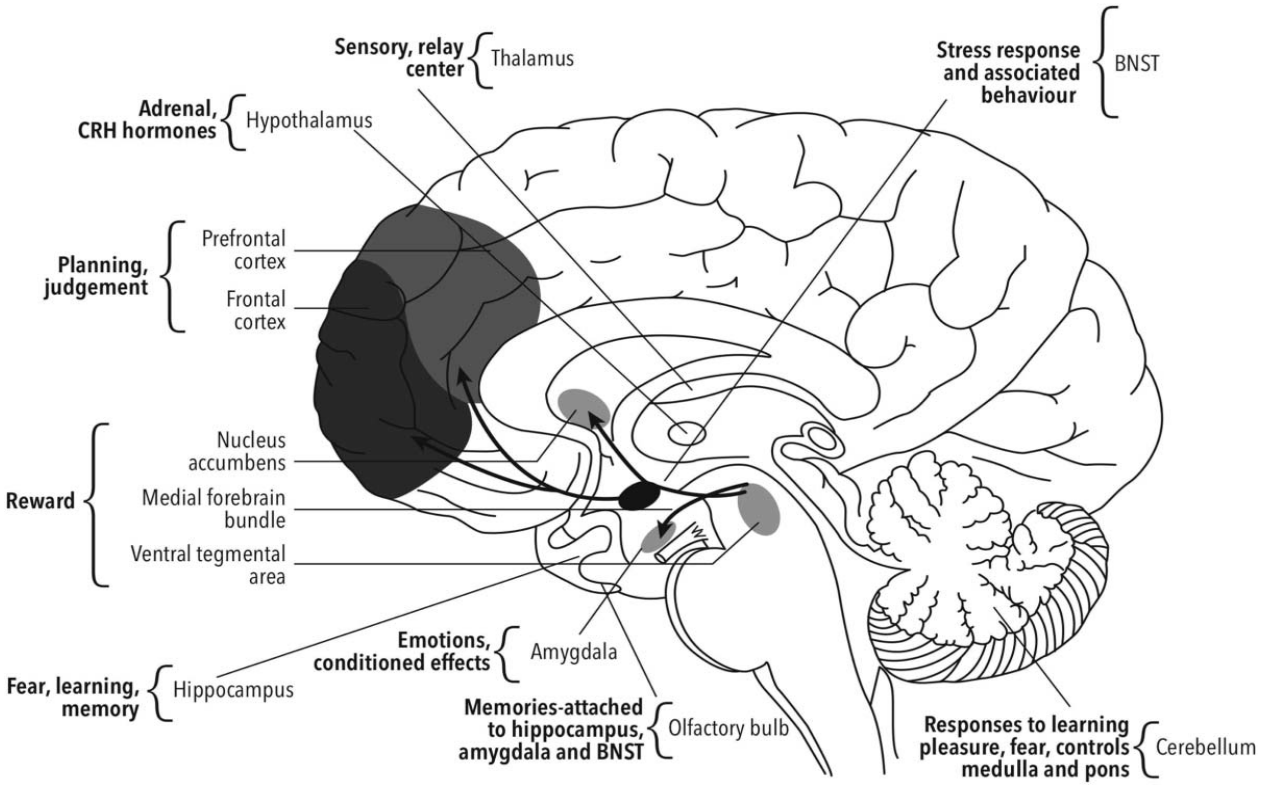

Figure 1.1 Brain areas critical to both PTSD and SUD. Areas showing neural connections responsible for common pathological aspects to both disorders. Cohen, Jacob[*,†]; Wei, Zelan[*]; Phang, Jonathan[*]; Laprairie, Robert B.[‡,§]; Zhang, Yanbo[*,†] Cannabinoids as an Emerging Therapy for Posttraumatic Stress Disorder and Substance Use Disorders, Journal of Clinical Neurophysiology: January 2020 - Volume 37 - Issue 1 - p 28-34 doi: 10.1097/WNP.0000000000000612

Cannabis has been reported anecdotally as a coping tool for patients with PTSD. Preliminary legalization data from Colorado and Oregon indicate *Cannabis* use may reduce the use of more harmful drugs, such as opioids (Livingston et al., 2017). Rigorous clinical studies of *Cannabis* could establish whether *Cannabis*-based medicines can be integrated into treatment regimens for both PTSD and substance use disorder patients opioid use when prescribed for chronic pain, and *Cannabis* use is associated with a decrease in opioid-related deaths (Livingston et al., 2017). This evidence indicates that *Cannabis* and other cannabinoid-related medicines can be promising interventions for PTSD and SUD. Further investigation of each of these areas and investigating the connection between PTSD and SUD may chart a course for well-defined prevention and treatment plans for both disorders. The human endocannabinoid system (ECS) is comprised of endogenous CB1 and CB2 receptors, their endogenous cannabinoid ligands (anandamide and 2-arachidonoylglycerol, 2-AG), and the enzymes that synthesize and degrade those cannabinoids (Battistella et al., 2014; Hill et al., 2018). The ECS plays vital roles in the regulation of mood, pain perception, appetite, learning and memory, and inflammation and has received attention recently for modulation of physiologic and behavioural aspects of many pathologies, including PTSD and SUD (Back et al., 2009; Hasin et al., 2013). The rate of *Cannabis* use is reportedly high in individuals who have PTSD (Alexander, 2012; Back et al., 2009). *Cannabis* may be considered by some as a form of self-medication for PTSD due to the reported anxiolytic, mood-stabilizing, and insomnia-reducing effects (Hawkins et al., 2012). Preliminary clinical data has shown that *Cannabis* may reduce opioid use when prescribed for chronic pain, and *Cannabis* use is associated with a decrease in opioid-related deaths (Fischer et al., 2016). This evidence indicates that *Cannabis* and other cannabinoid-related medicines can be promising interventions for PTSD and SUD. This review will highlight the specific brain areas involved in

PTSD and SUD and the relationship between them and discuss current and emerging treatments of *Cannabis* for PTSD and SUD.

1.2. Post-Traumatic Stress Disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder and is the only disorder in which a traumatic event needs to occur to have a complete diagnosis (Hasin et al., 2013). The Diagnostic and Statistical Manual of Mental Disorders (5th ed.) defines the criteria that must be met to be diagnosed with a psychiatric condition. The criteria state that the patient:

1. Must be exposed to a traumatic stressor. This may be direct, indirect or witnessing a traumatic event such as accidental or violent death.

2. Recurrent, involuntary, and intrusive memories. This may be experienced through flashbacks, intense nightmares, severe distress when exposed to a reminder of the trauma, and severe physiologic reaction when faced with a reminder. There may also be dissociative actions, including loss of consciousness.

3. Avoidance. The patient will exert effort to avoid stimuli and environments related to the trauma they experienced.

4. Negative alteration in cognition and mood. Patients may be unable to recall details of the traumatic event, have persistent negative feelings about themselves or their surroundings, diminished interest in daily activities, inability to feel happy, and feelings of isolation.

5. Hyperarousal to normal stimuli. The patient may have an exaggerated startle response, may be extremely irritable or aggressive, and may have sleep disturbances.

6. Criterion 2-5 must be present for longer than one month.

7. The symptoms must cause significant distress or have an impact on daily functions in the life of the patient.

8. The disturbance(s) must not be due to medication, use of an illicit substance or other disorder

1.3. Epidemiology

The average lifetime prevalence of PTSD in Canada is estimated at 9.2%; in the USA, the prevalence is 6.8% (Flanagan et al., 2016). Rates for any anxiety disorder tend to be higher for women than men (Kessler et al., 1994), and PTSD incidence has been shown to be particularly skewed toward women 2.80:1 (Pesce et al., 2016). Comparing the gender incidence of PTSD is difficult; however, exposure to different types of violence and severe trauma may differ among genders (Pesce et al., 2016). For example, men are nine times more likely than women to be exposed to gang violence (Vaillancourt, 2008). More men see combat during military service as they make up 85% of the Canadian Armed Forces, Regular Force and Primary Reserve as of January 2014. However, women in Canada are ten times more likely than men to be victims of sexual assault (Breiding et al., 2014). Violent and sexual assault, especially rape, yield the highest prevalence of PTSD. Rape as a primary traumatic stressor is estimated to account for approximately 50% to 60% of all PTSD cases (Hingson et al., 2009). In Canada, there were 21,500 reported sexual assaults in 2015 alone, with many sexual assaults remaining unreported (Allen. M, 2018). Seventy percent of female respondents in drug and alcohol rehabilitation facilities have a history of sexual abuse (Liebschutz et al., 2002). PTSD has a major social and economic impact on the community and health care system. The US VA spent 3.7 billion USD in caring for

diagnosed PTSD patients between 2004 and 2009 (Hingson et al., 2009). Statistics given by the Canadian Department of National Defence state that there are 697,000 veterans in Canada; 2,200 Canadian veterans are homeless, possibly as the result of an undiagnosed and/or untreated mental illness (Pearson et al., 2019).

1.4. Neuropathology

A few brain areas are known to be of significance in the pathophysiology of PTSD, although the exact mechanisms are not fully understood. Areas of the brain involved in fear, learning and memory, emotions, and cognition are known to be affected. A key brain region in the regulation of fear, learning, and emotional processing is the amygdala (Lebow and Chen, 2016). The amygdala is made up of sub-nuclei that each contribute to the processing of sensory information such as sounds, smells, sights or any other incoming stimuli (Krusemark et al., 2013). Glutamate receptors responsible for neuronal excitation N-methyl-D-aspartate receptors (NMDARs) in the basolateral amygdala and, when activated, consolidate fearful memories in the basolateral amygdala through a process known as long-term potentiation (LTP) (Ferguson, 2001; Fischer et al., 2016). When basolateral amygdala NMDAR's are inhibited by NMDAR antagonists, fear and reconsolidation of fearful memories decrease, and memory extinction rates increase (Ferguson, 2001; Fischer et al., 2016). Extinction, the ability to dissociate from fear, also involves the inhibitory neurotransmitter g-aminobutyric acid (GABA) transmission within the amygdala (Ferguson, 2001; Fischer et al., 2016) (Hill et al., 2018). Amygdalar circuitry and neurotransmission in PTSD and other pathologic conditions are still not fully understood. The amygdala does not act alone in regulating fear and learning. The hippocampus, in conjunction with the amygdala, gives us the context associated with learned fear.

The medial prefrontal cortex (mPFC) is critical for the gating of fear extinction (Pellicciari et al., 2013). Lesions to the mPFC increase fearful responses in animals after exposure to stressful conditions (Borghans and Homberg, 2015; Pellicciari et al., 2013; Rakofsky et al., 2012). The mPFC is also known to be responsible for cognition, executive function, and decision making. The bed nucleus stria terminalis (BNST) is divided into 11 to 13 separate nuclei (Lebow and Chen, 2016). The BNST is also twice as large in men relative to women (Flanagan et al., 2016). Visual information passes through the BNST to be encoded by higher-order centers (Flanagan et al., 2016). The function of all nuclei and the difference in males and females are yet to be explained (Flanagan et al., 2016). The BNST has a wide variety of receptors crucial to mood and behaviours, including thirst, aggression, arousal, and bonding (Flanagan et al., 2016). The BNST is also connected to areas that play key roles in regulating mood and sleep, such as; the ventral tegmental area (VTA), amygdala, insular cortex, and hypothalamus (Krusemark et al., 2013; Lebow and Chen, 2016). This explains why human memories and emotions have deep connections to scents, as well as visual stimuli.

1.5. Substance Use Disorder (SUD)

Addiction is defined by the compulsion to seek and consume mood-altering substances, inability to control intake, and emotional states such as anxiety, dysphoria, or even life-threatening physical symptoms when access to the drug is limited or denied (Flanagan et al., 2016; Koob and Volkow, 2016). Many substances such as alcohol, amphetamines, cocaine, and opioid drugs have profound effects directly or indirectly on the VTA, an area highly associated with reward (Flanagan et al., 2016; Koob and Volkow, 2016). The VTA shares major connections with the BNST, basolateral amygdala, mPFC and hippocampus described earlier in relation to PTSD (Fischer et al., 2016). The BNST may affect the propensity for drug addiction by regulating

withdrawal anxiety in the absence of the drug, further contributing to drug-seeking, preoccupation states, and ultimately reuse and relapse (Flanagan et al., 2016; Koob and Volkow, 2016; Lebow and Chen, 2016). The molecular and behavioural importance of the BNST in addiction and SUD still needs to be established in animals and humans.

Figure 2. Description of Physical and Cognitive Symptoms of PTSD and SUD.

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Figure 1.2. Description of physical and cognitive symptoms of how PTSD and SUD may become interconnected and create a feed-forward cycle of disruption to various body systems.

Cohen, Jacob[*,†]; Wei, Zelan[*]; Phang, Jonathan[*]; Laprairie, Robert B.[‡,§]; Zhang, Yanbo[*,†] Cannabinoids as an Emerging Therapy for Posttraumatic Stress Disorder and Substance Use Disorders, Journal of Clinical Neurophysiology: January 2020 - Volume 37 - Issue 1 - p 28-34 doi: 10.1097/WNP.0000000000000612

Approximately 15-35% of VA veteran patients have chronic pain, and 20% have some form of SUD, including binge alcohol consumption (Liebschutz et al., 2002). Data from the VA and a survey from Canadian Forces members deployed to Afghanistan reported that roughly 20% of veterans experience some type of addiction comorbid with their PTSD diagnosis (Canada, 2019; Seal et al., 2011). PTSD, SUD, and chronic pain share many key brain pathways involved in executive function, such as judgment, reward, emotions, stress, and learning. Opioid- and ethanol-dependent central effects are centred in the VTA, BNST, and nucleus accumbens, all of which regulate mood and reward and are important for both PTSD and SUD (Lebow and Chen, 2016). Cannabis has demonstrable clinical efficacy in chronic pain and preliminary efficacy as an anxiolytic and opioid-sparing medicine. If Cannabis can help cope with anxiety (Dahan et al., 2014; Tzadok et al., 2016) and chronic pain, it has the potential to be a method of harm reduction in alcohol use disorders.

1.6. Substance Use Disorder (SUD) and PTSD

One of the most consumed and abused substances worldwide is alcohol. Alcohol is a potent substance with a high level of abuse and potential for addiction. Over 86% of people aged above 18 in the USA reported alcohol consumption in their lifetime (Battistella et al., 2014; Hill et al., 2018). The cost in the USA in the year 2010 for over-consumption of alcohol was USD 249 billion. The cost associated with alcohol abuse in Canada was estimated at roughly USD 15 billion annually(Rehm et al., 2007). Alcohol acts mainly as a g-aminobutyric acid $(GABA)_A$ receptor agonist in the central nervous system (Sawicka and Tracy, 2017). In the VTA, $GABA_A$ positive allosteric activation of the $GABA_A$ Cl channel by alcohol indirectly promotes the mass release of dopamine, the neurotransmitter that drives motivation, reward, and addiction (Liebschutz et al., 2002). The disinhibitory effects of alcohol (e.g., risky behaviours, poor judgment) are thought to

be mediated by alcohol acting in the mPFC (Liebschutz et al., 2002). Patients with PTSD may use alcohol as a coping mechanism for PTSD symptoms (Back et al., 2009).

Twenty percent of veterans treated for PTSD are also diagnosed with a SUD (Back et al., 2009). Over 33% of veterans seeking care for SUD are diagnosed with PTSD. Over 10% of Iraq and Afghanistan veterans develop substance abuse problems; binge drinking is one of the main problems (Back et al., 2009; Fong, 2006). Alcohol abuse may temporarily help PTSD patients with sleep disturbances or help them escape from intrusive thoughts and feelings and/or physical and emotional pain associated with the traumatic experiences (Back et al., 2009). Although alcohol may offer temporary solutions, long term use of alcohol has been shown to have an array of adverse effects including impaired memory and cognition, increased agitation, and withdrawal symptoms including tachycardia, nausea, fever, and life-threatening delirium tremens (Borghans and Homberg, 2015). Avoiding facing the underlying issues in PTSD many also lengthen the duration of symptoms, perpetuating the avoidance associated with the trauma (Back et al., 2009). Cannabis may directly reduce PTSD-associated anxiety and pain and decrease self-medicated alcohol use. Besides, Cannabis can be a harm reduction substitute for alcohol to decrease alcohol-related physical and psychological damages. Chronic pain is defined by the American Center for Disease Control as experiencing pain every day for three months or longer beyond the time of normal healing (Hoots et al., 2019). Chronic pain can lead to comorbid conditions such as impairments in memory, cognition, sleep disturbances, and an overall reduction in the quality of daily life (Dahan et al., 2014). The VA estimates that 15% to 35% of the patients with chronic pain also have PTSD, whereas only 2% of people who do not have chronic pain have PTSD (Trivedi et al., 2015).

1.7. Opioids

The Center for Disease Control estimates that across the USA, 20% of patients seeing a physician for noncancer-related pain symptoms will be prescribed opioids. Although highly effective, opioids are known to be highly addictive and to cause opioid-induced hyperalgesia due to the sensitization of opioid receptors and NMDAR-related changes in neuronal plasticity (Hoet al.t al, 2019). This means that the patient will feel more pain and require more of the drug to suppress that pain (Dahan et al., 2014). To help curb this phenomenon, many clinicians are prescribing opioids with fewer side effects than morphine, such as buprenorphine, for prolonged use as they are less addictive and generally have fewer side effects (Ducharme et al., 2012). Unfortunately, buprenorphine, like all other opioids, can cause severe respiratory depression and death when combined with alcohol (Sawicka and Tracy, 2017). In Canada, there were nearly 2,900 opioid-related deaths in 2016 alone, at a rate of 7.8 deaths per 100,000 (Fischer et al., 2016). Sativex, a combination of synthetic Δ9-tetrahydrocannabinol (THC) and CB, D is recommended by the Canadian Cancer Society to treat pain, nausea, and vomiting associated with cancer and chemotherapy treatments (Crippa et al., 2018). Recently, a limited number of clinical trials have shown that Cannabis use may reduce opioid consumption for chronic pain conditions, and preliminary data from Oregon, a state that legalized Cannabis, has shown a decrease in opioid-related deaths (Livingston et al., 2017).

1.8. Psychotherapeutic Treatments

Treatment with CBT was much more effective in preventing chronic PTSD in study participants than escitalopram, a common SSRI. However, it is both costly and time-consuming to see a private counsellor or therapist for 4 to 12 sessions and then possible follow-up sessions. In

addition, a positive patient–counsellor relationship is essential for psychotherapy to be effective (Shalev et al., 2012).

1.9. Pharmacological Treatments

The VA and the Canadian Psychiatric Association recommend Selective Serotonin Reuptake Inhibitors (SSRIs) as the first-line pharmacotherapeutic treatment for PTSD (Alexander, 2012; Sharma et al., 2016). The VA data suggest a 60% post-efficacy in SSRI treatment for veterans with PTSD (Alexander, 2012). A 12-week double-masked trial with 208 PTSD patients found that 39% of PTSD patients discontinued treatment with the SSRI sertraline compared with 27% in the placebo group (Shalev et al., 2012). Discontinuation may be common among military members because of the cultural stigma involved with seeking treatment (Sharma et al., 2016). There are also significant side effects with SSRI treatment, including decreased libido, extreme changes in weight, metabolic function, sleep disruption, anorgasmia and orgasmic delay (Alexander, 2012; Fischer et al., 2016; Sharma et al., 2016). This may further contribute to symptoms of depression and add stress to relationships (Dückers et al., 2016). Patients using SSRIs spend less time in REM and slow-wave sleep, thereby having poor quality of sleep (Alexander, 2012; Dückers et al., 2016; Roberts et al., 2015). Nightmares are also more frequent, but the mechanism is not yet understood (Fischer et al., 2016). There may be a risk of suicidal thoughts with a higher risk in children and adolescents than in adults (Fischer et al., 2016). Although SSRI treatment has the potential to yield good results and is a relatively safe option for the patient, it takes 3 to 4 weeks to kick in in patients suffering from acute phases of PTSD (Fischer et al., 2016; Sharma et al., 2016; Suliman et al., 2015). More work needs to be done to educate the public about SSRI use better and reduce the social stigma associated with medication regimens.

Current treatments for PTSD include psychotherapy and pharmacotherapy; however, neither achieves rapid or complete success. Cognitive behavioural therapy (CBT) is a form of psychotherapy that has shown some promise in the treatment of PTSD. During sessions of CBT, patients undergo exposure, cognitive restructuring, and anxiety management techniques (Wang et al., 2007). Sessions typically occur a few weeks or months after the occurrence of the trauma, and patients generally undergo between 4 and 12 sessions. The most extensive study for the effectiveness of CBT was the Jerusalem Trauma Outreach and Prevention Study done by Shalev et al. (Shalev et al., 2012). Two hundred forty-two patients with early-stage PTSD (recently traumatized patients) were recruited and received either CBT, escitalopram (an SSRI-type antidepressant medication), or placebo. Patients with PTSD comorbid with SUD suffer from greater impairment and are resistant to treatment, which poses serious treatment challenges for clinicians (Back et al., 2009; Flanagan et al., 2016). Growing evidence supports an integrated model where patients receive treatment for PTSD and SUD simultaneously. This is preferred over the sequential model that requires abstinence before PTSD treatment (Roberts et al., 2015).

1.10. Evidence for *Cannabis* Use

Currently, there are no pharmacological treatments that target both PTSD and SUD, and researchers are looking at Cannabis as a possibility. The human ECS is comprised of endogenous CB1 and CB2 receptors, their endogenous cannabinoid ligands (anandamide and 2-arachidonoylglycerol), and the enzymes that synthesize and degrade those cannabinoids (Battistella et al., 2014; Hill et al., 2018). The ECS plays vital roles in the regulation of mood, pain perception, appetite, learning and memory, and inflammation and has received attention recently for modulation of physiologic and behavioural aspects of many pathologies, including PTSD and SUD (Back et al., 2009).

Research on the ECS is still in its infancy, and there have been very few and limited studies on humans (Hill et al., 2018). Loflin et al. (Loflin et al., 2017) found that veterans who used Cannabis medicinally for the primary purpose of treating PTSD symptoms reported significantly more symptoms of arousal after being asked about their combat trauma experiences compared with veterans who were recreational users. Although this may suggest that individuals using Cannabis for their PTSD symptoms are doing so in an attempt to minimize their response to traumatic triggers, the authors also concede that these individuals could be self-medicating at a higher degree because they represent a more traumatized population. Similarly, Bonn-Miller et al. found that individuals with probable PTSD used Cannabis to reduce the severity of negative-effect symptoms which include increased frequency of negative emotional states (e.g., fear, guilt, and sadness), diminished interest or participation in significant activities, socially withdrawn behaviour, and persistent reduction in expression of positive emotions, again as a form of coping (Bonn-Miller et al., 2014). Cannabis Use Disorder criteria are similar to the criteria for SUD (Hasin et al., 2013).

Individuals with an already existing Cannabis Use Disorder at baseline were less likely to experience improvement in PTSD symptoms over time compared to those without a Cannabis Use Disorder diagnosis, as evidenced by lower levels of change in total PTSD symptoms, PTSD avoidance-numbing symptoms, and PTSD hyperarousal symptoms (Bonn-Miller et al., 2014). Conversely, (Ruglass et al., 2017) concluded in their study of 126 patients that there was no significant positive or negative association between Cannabis use and end-of-treatment PTSD symptom severity. It is important to note that with this particular study, the length of treatment was 14 weeks, with end-of-treatment being defined as the period immediately following the 14-week treatment period. PTSD symptom severity was measured at baseline and follow-ups using Cognitive-Affective Processing System (CAPS), and no significant changes were found in these

CAPS scores. Interestingly, their study also revealed a crossover effect in which higher Cannabis use was associated with greater PTSD symptom severity earlier on in the treatment process, but lower weekly PTSD symptom severity at the end of the 14-week treatment.

1.11. CBD and Δ^9-THC

The Cannabis plant contains over 140 compounds, which are considered exogenous cannabinoids. The two compounds to gain the most attention by far are Δ9-THC and CBD (Battistella et al., 2014; Hill et al., 2018; Koob and Volkow, 2016). Cannabis is widely used worldwide as a recreational drug. Tetrahydrocannabinol is the primary psychoactive and intoxicating component of the Cannabis plant and contributes to the "high" that recreational users seek (Battistella et al., 2014; Flanagan et al., 2016; Hasin et al., 2013; Hill et al., 2018; Lebow and Chen, 2016). Studies have linked long- term THC consumption to poor learning and memory, and grey matter atrophy (Battistella et al., 2014; Hill et al., 2018).

CBD, however, is non-psychoactive and is therefore not believed to have the same addictive potential and adverse effects seen with THC, although overall safety is yet to be evaluated for both short and long-term use (Hill et al., 2018; Livingston et al., 2017). CBD has shown great promise in animal models of PTSD (Battistella et al., 2014; Flanagan et al., 2016; Hill et al., 2018). CBD has many receptor targets including negative allosteric modulation of CB1 (i.e., indirect inhibition), allosteric modulation of the v and m opioid receptors, partial agonism of the 5-HT1A receptors, partial agonism of CB2R, and many others (Hill et al., 2018; Laprairie et al., 2015). CB1 receptors are mostly concentrated on presynaptic terminals in the CNS (Hasin et al., 2013; Krusemark et al., 2013). CB1 receptors play a role in sleep through the connection to the hypothalamic-pituitary axis (Krusemark et al., 2013). CBD can act on CB2 receptors to modulate

inflammation by suppressing activation of glial cells, cytokine release, and chemoattractant from dendrites (Battistella et al., 2014; Hill et al., 2018; Laprairie et al., 2015). In animal models, CBD can modulate structures within the cortex such as the mPFC and amygdala, limiting fearful memory consolidation and potentiate extinction of fearful memories (Flanagan et al., 2016; Hill et al., 2018; Lebow and Chen, 2016). All of the effects mentioned can benefit patients afflicted by PTSD and SUD alike.

Cannabinoids, including both THC and CBD, are known to interact with major factors of drug-metabolizing enzymes, including CYP 450, which metabolizes nearly half of all known drugs and supplements. Other major families of the CYP 450 family include CYP 3A4 and CYP 2C9, which are responsible for the metabolism of many anti-epileptic drugs and can be severely impacted by natural supplements like St. John's Wort, and grapefruit juice. CYP 2C19 metabolizes many anxiety medications and has interactions with cannabinoids (Tzadok et al., 2016). Although believed by many to be safe for recreational and medicinal use, Cannabis can have profound effects on medications or other supplements commonly consumed. Poor knowledge of full interactions has the potential to yield negative outcomes for recreational and medicinal users and establishing safety thresholds with respect to each compound should be a priority.

With new technology and legalization of Cannabis in Canada, larger-scale clinical trials to determine the safety and efficacy of all cannabinoid products should be established. Methods of regulating patient access across different provinces also need to be set in place. Currently, the legalization of Cannabis in Oregon has correlated with a decrease in opioid-related deaths (Livingston et al., 2017). Moreover, the Israeli Ministry of Health findings suggest that low-dose

THC may benefit patients with severe PTSD (maximum 1:1 ratio of THC: CBD) (Dahan et al., 2014; “Medical Cannabis, Ministry of Health,” 2018.).

1.12. Summary

The neurocircuitry and molecular changes occurring in PTSD and SUD are still not fully understood. Quantifying and qualifying changes in certain areas of the brain and the underlying mechanisms remains a major challenge. The gender difference in PTSD also needs to be fully explained. Current pharmacological treatments for both PTSD and SUD have limited success rates and ultimately continue the cycle of drug dependency. More research needs to be completed to determine safe and effective pharmacological treatments for these disorders. Fully elucidating the role of the ECS and the exploration of Cannabis-based medicines in clinical trials may be helpful for PTSD and relief of SUD symptoms. In cases of trauma, a regimen of appropriate psychological aftercare needs to be completed, which should include a monitoring program and effective psychological counselling. Integration of Cannabis along with psychotherapy regiments such as CBT may prove useful in treating PTSD comorbid with SUDs. Cannabis has the potential to be a method of harm reduction in alcohol use disorders and can deliver rapid relief of symptoms compared with traditional pharmaceutical options. Addiction and PTSD share many pathologic conditions with respect to their neurocircuitry. If a clear and definite link can be established between the etiology of these psychiatric illnesses, successful treatments can be within reach.

Chapter 2. Male and Female Sprague Dawley Rats Administered Whole Plant Extract CBD Oil

This chapter has adapted for a research article in preparation for the journal European Neuropsychopharmacology and will describe the main experiments completed during the project. The abstract was published in European Neuropsychopharmacology.

2.1. Background

Many factors can contribute to the sex and gender differences seen in the prevalence of PTSD between men and women, including diverse biological and cultural differences. Many biological factors have been found to contribute to these differences. Corticotrophin releasing factor (CRF) is known to be critical in the regulation of the hypothalamic-pituitary axis (HPA), and psychiatric disorders such as PTSD. CRF is reported to be higher in females than males in some brain regions such as the amygdala, but males have higher levels in the medial prefrontal cortex (mPFC). These differences may affect anxiety, depression, and related disorders in animal models of PTSD and human patients experiencing PTSD (Harris et al., 2013; Yehuda, 2009).

Intrusive memories are also thought to be regulated by adrenal hormones and different glucocorticoids. (Ney et al., 2019) Fear extinction, which is a component of memory consolidation, has been shown to be improved in patients with PTSD via exogenous glucocorticoid administration, as well as exogenous cannabinoid administration; however, overall effectiveness is not supported (Katzman et al., 2014).

A more recent approach to novel PTSD treatments is through the (ECS) which is involved in many of the key mechanisms underlying PTSD etiology, such as regulation of mood, appetite, and sleep (Hill et al., 2017). Males appear to have higher blood levels of 2-AG than females (Fanelli et al., 2017). Higher CB1 receptor densities are reported in brain regions associated with PTSD in males than females, such as the amygdala, mPFC, cerebellum, and limbic areas, as well as in key parts of endocannabinoid-regulated areas of stress (Morena et al., 2016). Lower levels of endocannabinoid signalling have been associated with PTSD vulnerability (Hill et al., 2013;

Wilker et al., 2016), this may provide a mechanistic explanation for why females are at greatest risk of PTSD.

Sex differences in cannabinoids are understudied. Despite the limitations, studies have concluded that significant sex differences occur in brain areas important for PTSD and Substance Use Disorder (SUD). Animal studies have found that female rats, but not male rats experience a higher level of negative feedback of the HPA axis, resulting in lower basal levels of cortisol (Bangasser and Wiersielis, 2018). Males have also responded more poorly to behavioural tests when exposed to acute and chronic stress than females. This is thought to be because estrogen has demonstrated some neuroprotective effects (Colom-Lapetina et al., 2019). Animal studies, however, do not have large numbers, making it difficult to rely on the data because of low statistical power. Human studies of PTSD in military settings have found that males are more likely to suffer comorbid SUD than females (Hawkins et al., 2012; Loflin et al., 2017) (Feldner et al., 2007). More studies are needed outside of military settings to understand the difference that military service may have, as opposed to a private setting.

Some theories that have attempted to explain PTSD pathophysiology and have been studied include fear extinction, which is the ability to return to normal levels of fear after being exposed to a traumatic event. Impaired fear extinction is thought to be an explanation for heightened arousal, and avoidance behaviours experienced in PTSD. Memory consolidation is the long-term storage of memories. PTSD leads to dysregulation of memory consolidation in the form of fragmented, and intrusive memories, a hallmark of PTSD. The sympathetic nervous system with brain and body connections is common to the dysfunction seen in both fear extinction and memory consolidation. Factors that can compound the issues include moral injury and survivor guilt.

Canadian Association for Mental Health (CAMH) defines moral injury as "Any events, action or inaction transgressing our moral/ethical beliefs, expectations and standards can set the stage for moral injury." Although this is more common in military and EMS, it can happen equally with civilians. Survivors' guilt is a form of moral injury in which there is taking responsibility for all aspects of a traumatic event, whether accurate or not.

Korem and Akirav used a footshock model to deliver a conditioned stimulus (CS) and situational reminders (SR) to test if cannabinoids could help fear extinction or not in a rat model. Although the model demonstrated impaired fear extinction, poorer synaptic plasticity, and higher startle responses, the model was only eight days long, which cannot be considered chronic. The model is also known to enhance depressive symptoms because the animal cannot escape while the shocks are being delivered at random and are more of "learned helplessness" (Korem and Akirav, 2014).

The Cohen model of PTSD (Cohen et al., 2020) used soiled cat litter and placed rats atop the soiled litter for 10 minutes. This model using Predatory Scent Stress (PSS) is highly analogous to human PTSD because it is a random exposure to intensive trauma (Bangasser and Wiersielis, 2018; Borghans and Homberg, 2015). The scent is highly critical to the response of traumatic responses due to olfactory connections to fear circuits. Behavioural testing and other analyses were completed after seven days. Cohen's model, however, only contains a single 10-minute exposure to the trauma. Cohen uses cut-off behavioural criteria to divide the animals into the minimum, median, and extreme responses. Behavioural responses in the median and extreme groups were disrupted, and changes to cortisol response and circulating levels were noticed (Cohen et al., 2018).

Zoladz and colleagues expanded on Cohen's model by introducing social stress in the form of cage changes every day, and the addition of exposure to live cats while restrained in a plexiglass tube that was isolated from the scent (Zoladz et al., 2012). The model lasted for 21 days, and then behavioural changes were observed. This model is considered the most translational because of the social and traumatic stress, as well as the changes that were noticed on a metabolic as well as behavioural level. Similar to humans, rats exposed to social and predatory stress gained less weight and had a significant impact on the adrenal gland, thymus gland and body weight (Zoladz et al., 2012).

Zolads, Akirav, and H. Cohen labs have the most validated models because most parallels can be drawn with human PTSD symptoms. Some loss of translation occurs in these animal models due to the lack of ability to accurately measure criteria needed to diagnose PTSD, such as intrusive and recurring memories. Only Zolads has come close to what can be considered a chronic model (>30 days). However, sex differences have not been addressed.

In the present study, the behavioural effects of a daily oral whole *Cannabis* extract containing 24.54 mg/mL CBD and 1.15 mg/mL Δ^9-tetrahydrocannabinol (THC) in peanut butter were investigated in male and female rats exposed to predator and psychosocial stress (PPS) as a model of PTSD. Behavioural changes were assessed using the open field test (OFT) and elevated plus maze (EPM), as well as measures of body weight, adrenal and thymus gland size, corticosterone levels, and cytokine levels in rats in order to further elucidate the systemic effects of CBD treatment, PPS, and potential sex differences. Our model sought to improve on the previous models described above to create a truly chronic PTSD timeline by making the exposure to trauma last for 30 days before testing. Another improvement we attempted to include in our

model was not allowing rats to touch the soiled cat litter, thereby having wet litter stick to their fur. Although some evidence has shown that female rat behaviours in the EPM and OFT are less affected by stress (Klinger et al., 2019), we expected that chronic stress might have an impact on behavioural performance and would have an impact similar to males with respect to the metabolic and cortisol changes. We hypothesized that *Cannabis* would acts as an anxiolytic equally in male and female rats and would potentially reduce metabolic disruption via reduction of inflammation.

Here, we assessed whether a 1:25 THC: CBD mg/mL *Cannabis* oil had anxiolytic properties in our model of PTSD. We also wanted to determine if there were sex differences within our PTSD model and between male and female rats when allowed to orally self-administer a 1:25 THC: CBD ratio *Cannabis* oil in peanut butter. We hypothesized that male and female rats would respond similarly to chronic exposures to stress with respect to metabolic values such as weight gain, and physical appearance, but possibly that females would perform better in the EPM and OFT with respect to behavioural results based on results by (Colom-Lapetina et al., 2019; Klinger et al., 2019). We hypothesized that the *Cannabis* oil would reduce modelled behaviours of anxiety in our rodent model of PTSD to both male and female rats.

2.2. Materials and Methods

2.2.1. Animals

A total of 64 male and 48 female Sprague-Dawley rats weighing 200–250 g (2–2.5 months old) were purchased from Charles River (Montreal, Canada). Rats were habituated to housing conditions for a minimum of 7 days, with constant temperature, 12 h light/dark cycle, and *ad libitum* access to food and water. Animals were handled once daily. All testing was performed during the light cycle (07:00-19:00). All treatment and testing procedures were approved by the

Animal Review Ethics Board at the University of Saskatchewan, Canada. In consideration of the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, Cohen's power analysis was completed to determine the number of animals needed to conserve statistical power while keeping animal numbers at a minimum. (Kilkenny et al., 2010) Our calculations using the formula $[z^2 \times p(1-p)/e^2]/(1+[z^2 \times p(1-p)/e^2N])$ showed that at minimum 43 animals would be needed, and 64 was ideal.

2.2.2. Drugs

Whole plant extract *Cannabis* oil supplied by a Licensed Canadian Producer of *Cannabis* was self-administered by rats. The oil contained 1.15 mg/mL of THC and 24.54 mg/mL of CBD. Oil was extracted via supercritical CO_2 extraction, using palm oil as a carrier substance. Peanut butter was used as the vehicle and placed inside a cage. Cages were divided during administration to ensure each rat received a single dose using a plastic wall that could not be climbed over. The material was cleaned with a Prevail solution between use. A dosage of 10 mg/kg CBD was calculated by the weight of rats and the volume of *Cannabis* oil in 1 mL based on previous studies that optimized for anxiolytic effects (Mechoulam et al. 2015). During the acclimatization period, all animals were given peanut butter to become habituated. Animals were weighed on day 1, and every 5 days until day 30.

2.2.3. Experimental design

We divided 64 males evenly into 4 groups, and 48 females were divided evenly into 4 groups. Due to facility constraints, male and female rat cohorts had to be run at different time points. The female rat cohort was run exactly one year after the male cohort. Although not ideal, no facility could accommodate a simultaneous male and female group with cat litter exposure.

Choosing to run exactly one year later was to eliminate any differences caused by seasonal or lunar deviations. To assess the effects of *Cannabis* oil treatment in rats exposed to PPS, two experiments were conducted. In the male cohort (N=64), the long-term behavioural effects of 10 mg/kg whole plant extract (WPE) CBD oil and PSS exposure were evaluated based on physical appearance (hair loss, porphyrin staining, hair standing) elevated plus maze (EPM), Open Field Test (OFT), aggressive behaviour (biting, darting), adrenal gland atrophy, thymus hypertrophy, and reduction in basal cortisol levels. Animals were exposed to a psychosocial stressor in the form of random cage changes daily for 30 days. Animals were exposed to predator stress in the form of soiled cat litter for 1 h during the light cycle (0700-1900) on day 2 and again on day 11 during the night cycle (1900-0700). Animals were separated from cat litter by a metal grate. The metal grate prevented the cat litter from sticking to the fur and served as a conditional stimulus for 2nd exposure (Milad and Quirk, 2012; Zuj et al., 2016).

Euthanasia was performed using 5% isoflurane in 1 L/min O_2 to induce anesthesia, and 3% isoflurane to maintain anesthesia. Animals sacrificed for fresh tissue were decapitated, and organs were collected immediately. Tissues were stored at -80ºC. Animals euthanized for immunochemistry were perfused with cold 1X phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA), and then decapitated. Organs were collected immediately after. Organs were stored in 4% PFA at 4ºC for later analysis. Brain tissues for immunochemistry were stored for 24 hours in 4% PFA at 4ºC, then replaced with PBS + 0.05% sodium azide for longer storage. Due to COVID-19 related difficulties, these results could not be included in the Thesis. Blood was collected at the time of euthanasia and centrifuged within 3 h at 10,000 *x*g for 15 min at 4ºC. The serum was collected from the supernatant and frozen (-80ºC). Identical procedures and methods were used for the female cohort exactly one year later.

Figure 2.1. Timeline of Experiments

Figure 3. PPS animals began random cage switches on day 1. On Day 2, during the light hours (07:00-19:00) and day 11 during the dark hours (19:00-07:00), PPS animals were exposed to soiled cat litter. Controls were exposed to cat litter wet with water. The experiment was ended on day 30. On days 33-36, animals were tested in the EPM and OFT, then sacrificed.

2.2.4. Elevated plus-maze

The elevated plus-maze is a plus-shaped platform with 2 opposing open and 2 opposing closed arms (File et al., 1993). Rats were placed on the central platform facing an open arm and allowed to explore the maze for 5 min. The behaviours assessed were duration in open and closed arms and on the central platform, number of open and closed arm entries, and total exploration (entries into all arms). During each interval between the animal trials, the arena was cleaned with Prevail disinfectant and anti-odour solution. All data were tracked and assessed by ANY-Maze software version 6.2.

2.2.5. Open field test

The open field apparatus was constructed of acrylic material and measured 60 cm × 60 cm × 60 cm. The entire apparatus was painted black except for the floor in white. The lines divided the floor into 16 evenly spaced squares (15 × 15 cm). The central part consisted of 4 squares in the center of the apparatus. Each animal was placed in the center of the test apparatus and performed the test for 5 min. During each interval between the phases of experiments, the arena was cleaned

with Prevail disinfectant and anti-odour solution. For each rat, the following behaviours were recorded: time in center, time in perimeter, entries to each zone, time mobile, time immobile, and freezing in each zone. During each interval between the animal trials, the arena was cleaned with Prevail disinfectant and anti-odour solution. All data were tracked and assessed by ANY-Maze software version 6.2.

2.2.6. Physical Appearance, Aggression, Body and Glandular Weights

Rats were assessed for hair loss, porphyrin staining, and hair standing, which are standard measures of rodent anxiety (Lloyd & Wolfenson, 1998). Aggressive behaviours such as biting, and darting were observed throughout the course of the experiment. Animal weight was recorded daily over the 30-day study period and at the time of euthanasia. Adrenal and thymus glands were removed at the time of euthanasia and placed in 4% PFA (Sigma, Montreal, QC) until the time of measurement. Gland weights were normalized to total animal body weight.

2.2.7. Cortisol and Cytokines

Cortisol was measured using a commercially available cortisol enzyme-linked immunosorbent assay according to the manufacturer's instructions (Enzo, Farmingdale, USA) Samples were analyzed in duplicates. Cytokine Multiplex Immunoassay analyzed with a BioPlex 200 Cytokine Array Assay Kit Source: Millipore MILLIPLEX. Analysis performed by Eve Technologies (Calgary, Canada). Cytokines analyzed included: eotaxin, epidermal growth factor (EGF), fractalkine, interferon (IFN)g, interleukin (IL)-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17A, IL-18, IP-10, keratinocyte chemoattractant (KC), tumour necrosis factor (TNF)-a, Leptin, macrophage inflammatory protein (MIP)-1a, MIP-2, RANTES, vascular endothelial growth factor (VEGF).

2.2.8. Statistical Analysis

For the behavioural, cytokine, body weight, glandular, and cortisol levels, the statistical analyses were performed using two-way ANOVA, and in which PPS-exposure (Control vs. PSS-exposure) and treatment with or without CBD (Vehicle vs. CBD) were factors. Separate analyses and male PPS/treatment; female PPS/treatment. were performed with sex as a factor using male control/cbd ; female control/cbd *Post-hoc* Tukey tests were used to examine differences between individual groups. The prevalence of affected rats as a function of the rat group was tested using cross-tabulation and non-parametric Chi-square tests.

2.3. Results

2.3.1. Elevated plus-maze

Male rats not exposed to PPS spent more time in the open arms of the EPM more than PPS male rats on average but did not meet significance. Male rats given CBD spent significantly more time in open arms of the EPM than control rats [$p<0.1$ Tukey; $F(1, 52) = 2.555$] (Figure 2.2A). Male non-PPS rats given CBD travelled greater distances in open arms more than rats given vehicle alone [$p<0.05$; $F(1, 43) = 9.641$], however female PPS rats travelled greater distances than control females [$p<0.01$; $F(1, 44) = 6.611$] (Figure 4B). Male and female PPS rats treated with CBD did not yield any significant improvements in any of the EPM parameters compared with male PPS rats. Surprisingly, female PPS rats treated with CBD spent significantly less time mobile in the open arms compared to female PPS rats [$p<0.05$; $F(1, 46) = 3.475$] (Figure 2.2B). A main sex effect was observed for the EPM between males and females [$p<0.001$; $F(1, 44) = 82.07$]. Female PPS rats treated with CBD spent significantly less time mobile than female PPS rats [$F(1, 50) = 6.259$]. Based on the data, we can see that sex is a major factor in behavioural outcomes in the EPM. CBD has an anxiolytic effect on control males but is anxiogenic when administered to female PPS rats.

Figure 2.2. Elevated Plus Maze

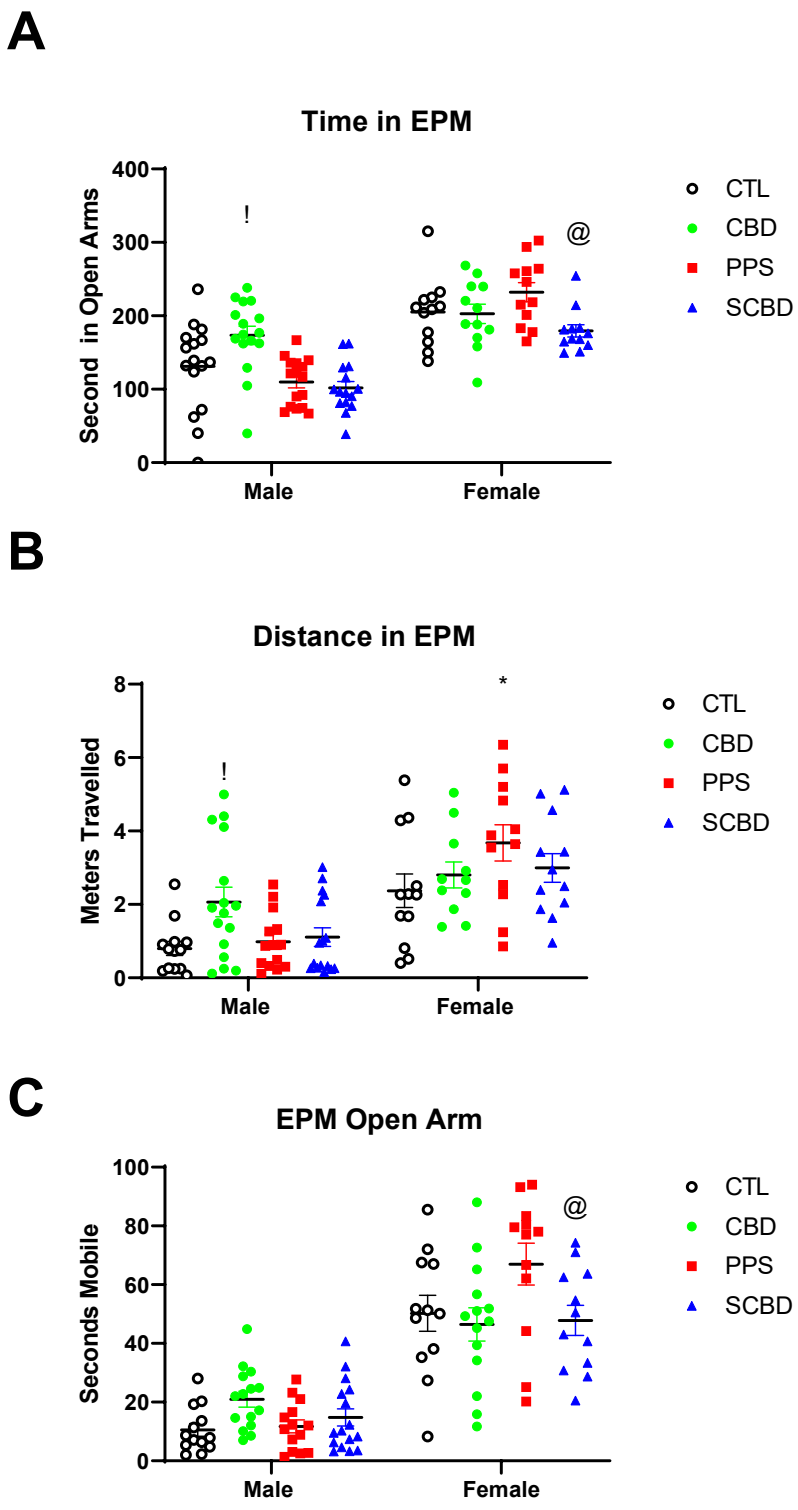


Figure 2.2. Elevated Plus Maze (EPM). N=12-16 per group. **2.2A**: Time (seconds) spent in open arms of the EPM, ! $p<0.1$ Tukey@ $p<0.05$ PPS:SCBD. Male CBD rats spent significantly more time in open arms than male controls. Stressed animals spent less time, but significance was not met. Female PPS rats treated with *Cannabis* spent significantly less time than PPS rats in the open. **2.2B**: Distance Travelled (meters) in open arms of the EPM, ! $p<0.1$ Tukey; * $p<0.05$. Male CBD rats travelled significantly more than Control. Female PPS rats travelled significantly more than female controls. **2.2C**: Time Mobile (seconds) in the Open arms of the EPM, @ $p<0.05$ PPS: SCBD. Female PPS rats treated with CBD spent significantly less time mobile in the open arms than PPS females. ! $p < 0.1$ control *versus* CBD within males, * $p < 0.05$ control *versus* PPS within females as determined by two-way ANOVA followed by Tukey's *post-hoc* test. Data are mean ± S.E.M. within points representing individuals scores.

2.3.2. Open Field Test

Male PPS rats crossed lines significantly less than control male rats [$p<0.1$, D; $F(1, 51) = 3.481$]. Female PPS rats treated with CBD crossed significantly less lines than female PPS rats [$p<0.01$; $F(1, 51) = 12.38$]. There was a significant difference between male and female rats [$p<0.001$; $F(1, 51) = 21.78$]. Male rats treated with CBD travelled more distance than control male rats. Male control rats froze significantly less in the center than PPS male rats [$p<0.1$; $F(1, 80) = 3.350$]. No Significant differences were observed otherwise. A significant interaction between PPS and PPS treated with CBD was observed between males and females [$p<0.05$; $F(1, 51) = 4.169$].

Figure 2.3. Open Field Test

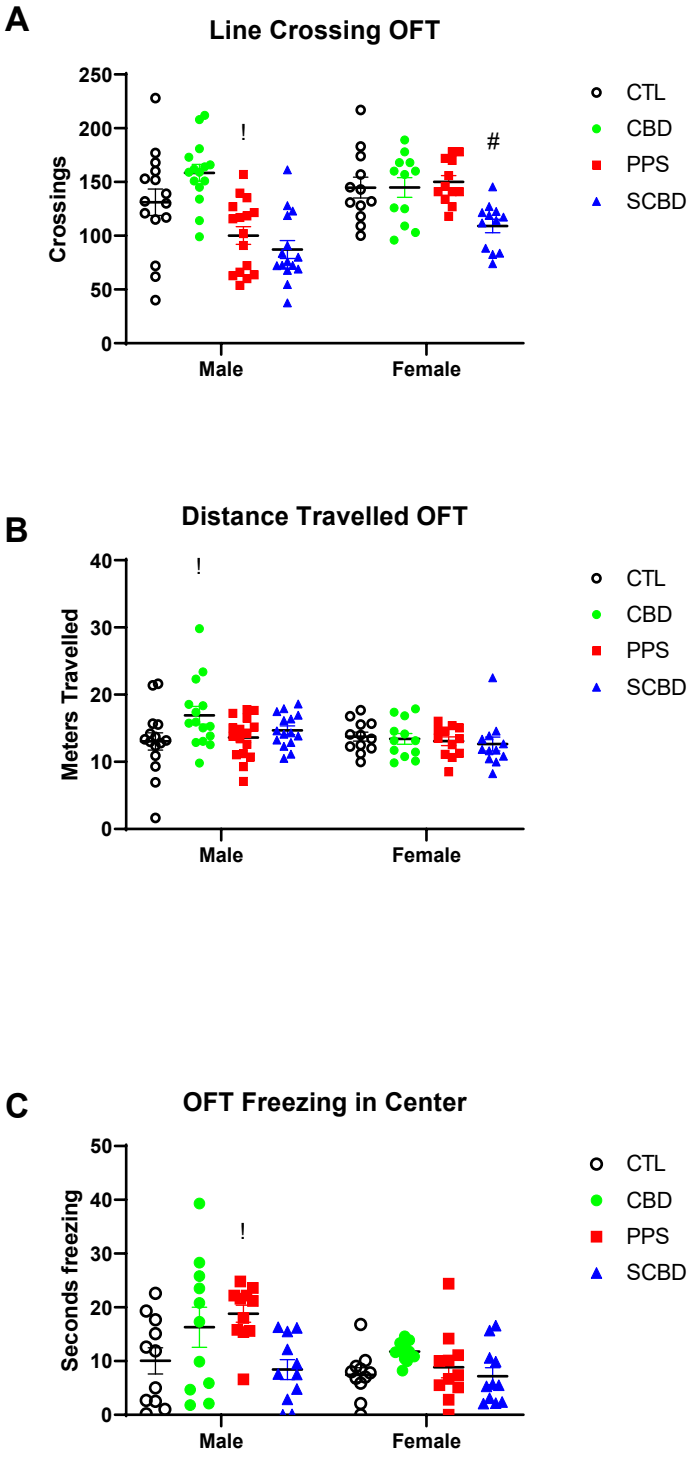


Figure 2.3. Open Field Test (OFT). N=12-16 per group. **2.3A**: Total number of line crossings in the OFT. Post-Hoc Tukey's test revealed a significance of $p<0.1$ $p<0.05$ Fisher's LSD between PPS males and control males. Post-Hoc Tukey's test revealed a significance of $p<0.01$ between SCBD females and PPS females. ! $p<0.1$; # $p<0.01$. Male PPS rats crossed significantly fewer lines than controls. **2.3B**: Significance of $p<0.1$ $p<0.05$ Fisher's LSD between CBD males and control males. ! $p<0.1$. Males treated with CBD travelled more distance if the OFT. There was a trend for male PPS but did not meet significance. **2.3C:** 3-way ANOVA with Tukey's post-hoc comparison showed $p<0.1$ Tukey, $p<0.05$ Fisher LSD. Male PPS rats froze significantly more compared to male controls.

2.3.3. Corticosterone

Analysis of corticosterone levels revealed significant interactions between treatments in males. However, *post-hoc* analyses did not reveal significant differences between individual groups [$p<0.05$; $F(1, 30) = 6.400$]. Female corticosterone baseline levels were different from males. Two-way ANOVA revealed significance between treatment groups in males. However, the post-hoc analysis did not reveal individual significance. [$p<0.05$; $F(1, 29) = 5.640$]. *Post-hoc* analyses revealed that corticosterone levels were significantly lower in females treated with CBD, PPS females, and PPS females treated with CBD relative to control females [$p<0.01$; $F(1, 29) = 5.928$]

Figure 2.4: Male and Female Corticosterone

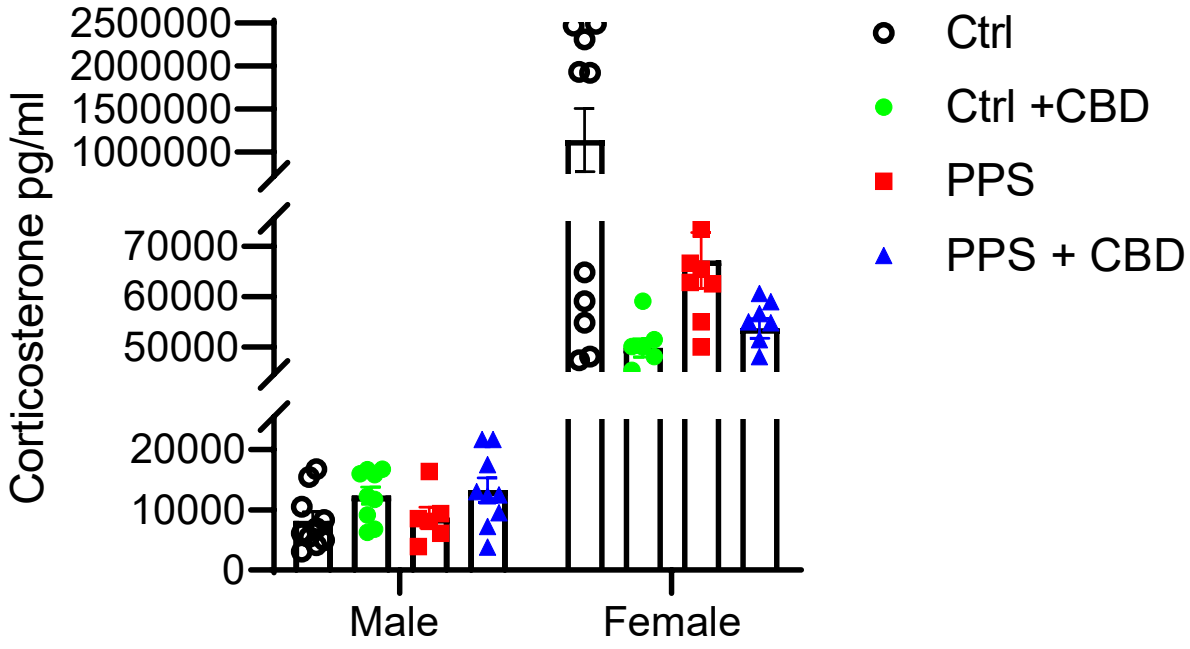
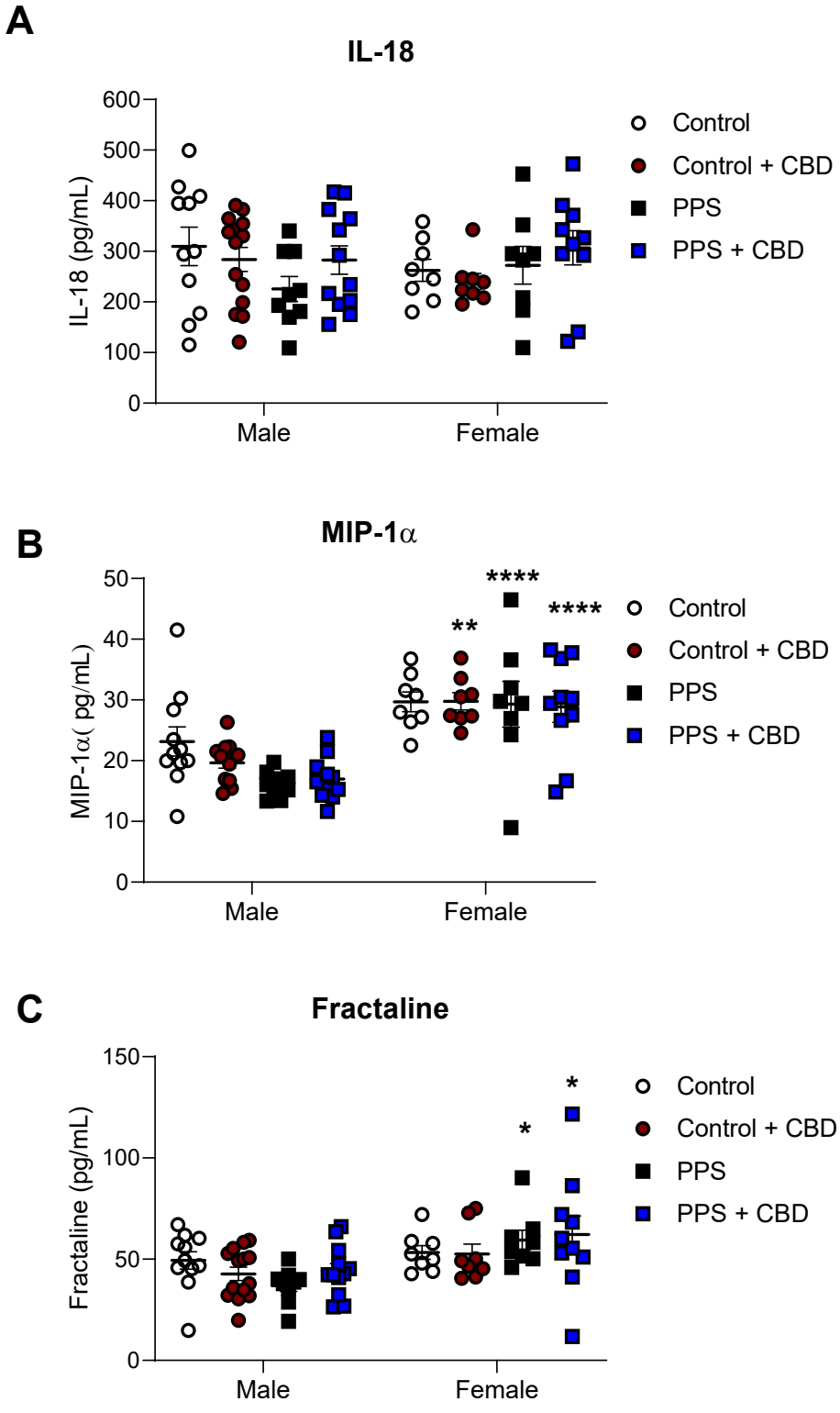


Figure 2.4. Male serum corticosterone values expressed in pg/ml. N=8-12 per group. Male rats administered CBD were more elevated than Control and PPS, but no individual significance was found with *post-hoc* analyses. Female serum corticosterone values expressed in pg/ml. Two-way ANOVA with *post-hoc* Tukey's test revealed a significance of $p<0.01$ between CBD, PPS, and SCBD female rats compared with control females. ** $p<0.01$. CBD seemed to show a trend for an increase in corticosterone that did not meet significance in males. CBD and Stress significantly lowered female corticosterone values.

2.3.4. Cytokines

Male and female rats experience opposite reactions to IL-18 response, although no significance was found **(2.5A)**. Male PPS rats had a significantly lower MIP-1α level compared to male controls [$p<0.05$; $F(1, 76) = 3.811$] (**2.5B**). Male PPS rats also had significantly lower VEGF levels than male controls **2.5E** [$p<0.01$; $F(1, 75) = 6.665$]. Female rats had significantly lower levels of IL-1 α, IL-2 IL-10, and IL-12 compared to males **2.5D, I-K**. Female levels of Fractaline, MIP- α, and RANTES were significantly higher than males **2.5B-C, F**.

Figure 2.5. Cytokine Serum Battery



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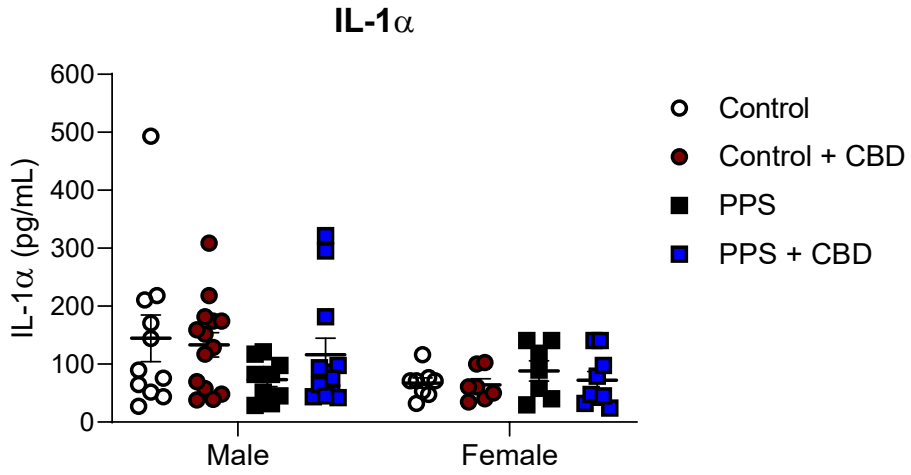
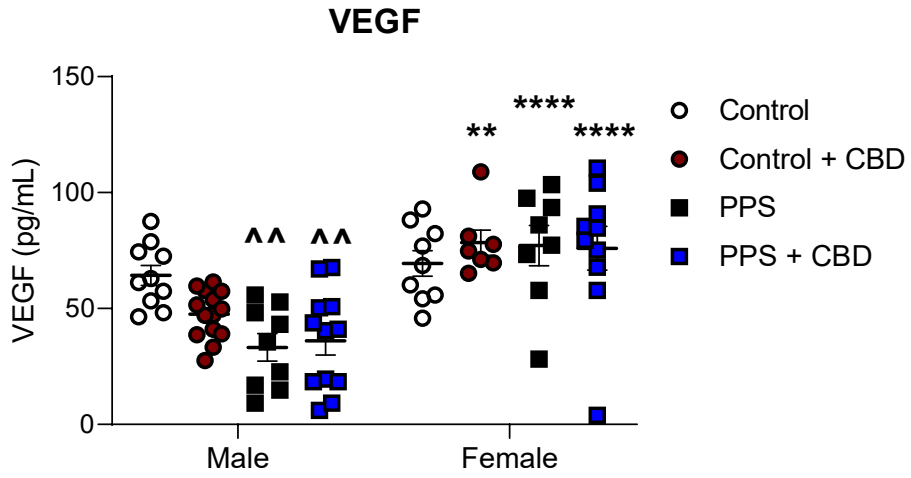
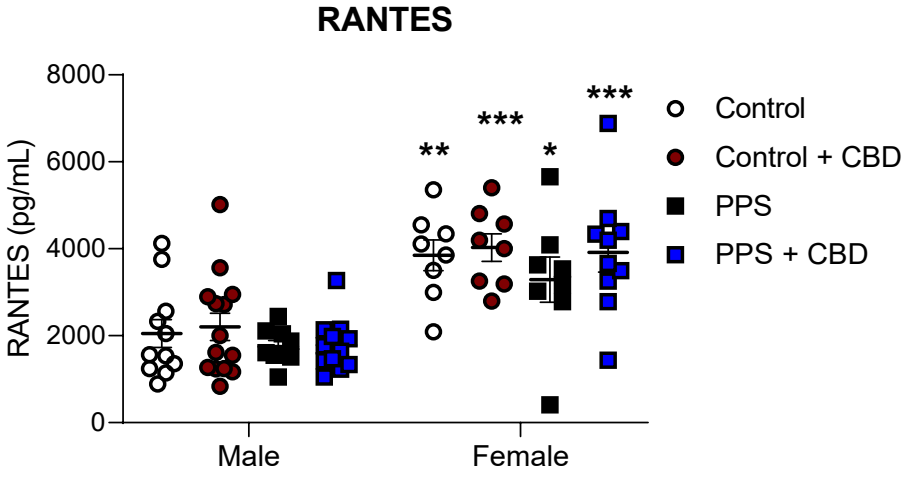

IL-1α
IL-1α (pg/mL)
600
500
400
300
200
100
0
Male
Female
Control
Control + CBD
PPS
PPS + CBD

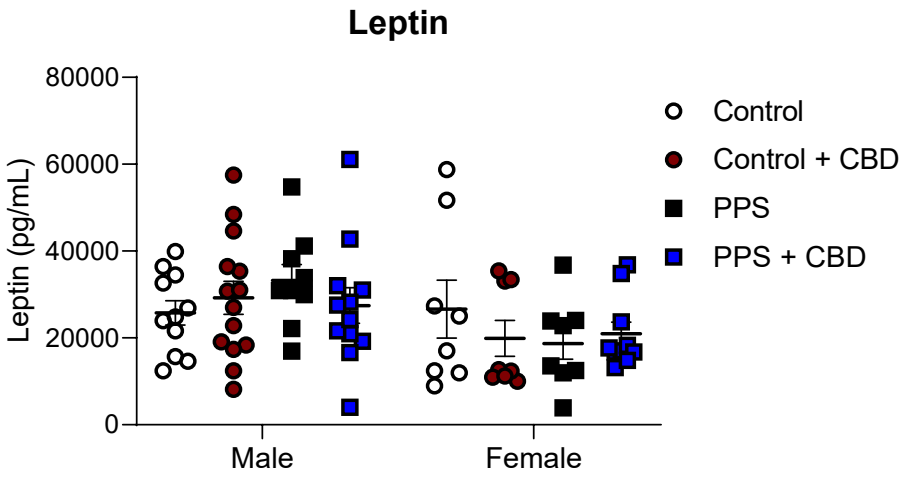
E


VEGF
VEGF (pg/mL)
150
100
50
0
^^
^^
**
****
****
Male
Female
Control
Control + CBD
PPS
PPS + CBD

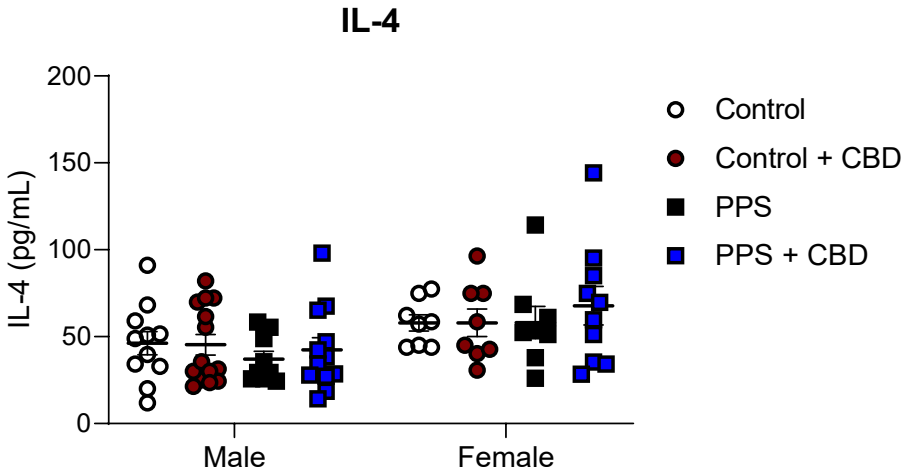
F


RANTES
RANTES (pg/mL)
8000
6000
4000
2000
0
**
***
*
***
Male
Female
Control
Control + CBD
PPS
PPS + CBD

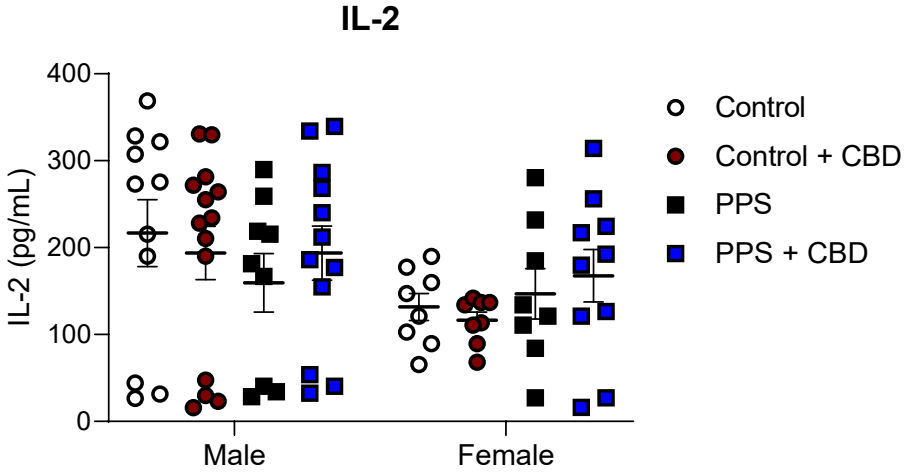
G


Leptin
Leptin (pg/mL)
80000
60000
40000
20000
0
Male
Female
Control
Control + CBD
PPS
PPS + CBD

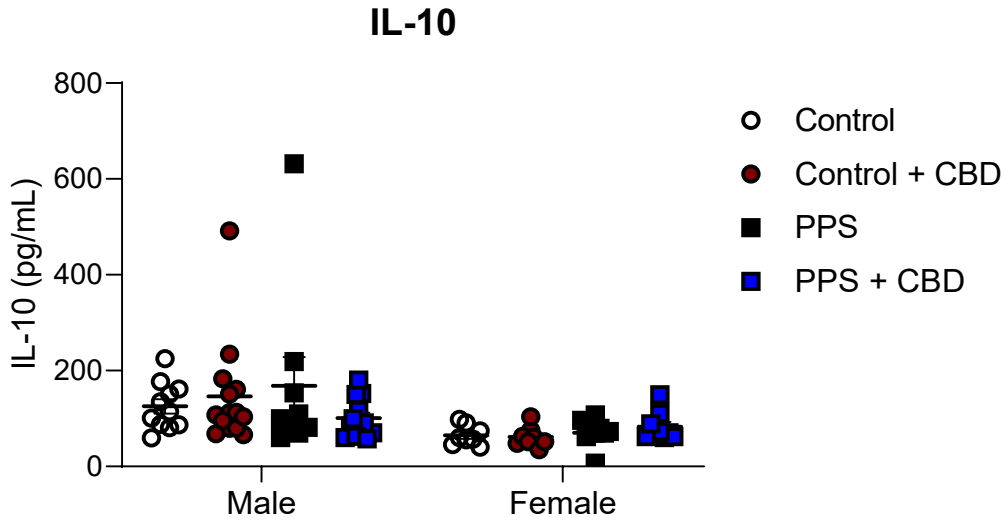
H


IL-4
IL-4 (pg/mL)
200
150
100
50
0
Male
Female
Control
Control + CBD
PPS
PPS + CBD

I


IL-2
IL-2 (pg/mL)
400
300
200
100
0
Male
Female
Control
Control + CBD
PPS
PPS + CBD

J


IL-10
800
600
400
200
0
IL-10 (pg/mL)
Male
Female
Control
Control + CBD
PPS
PPS + CBD

K

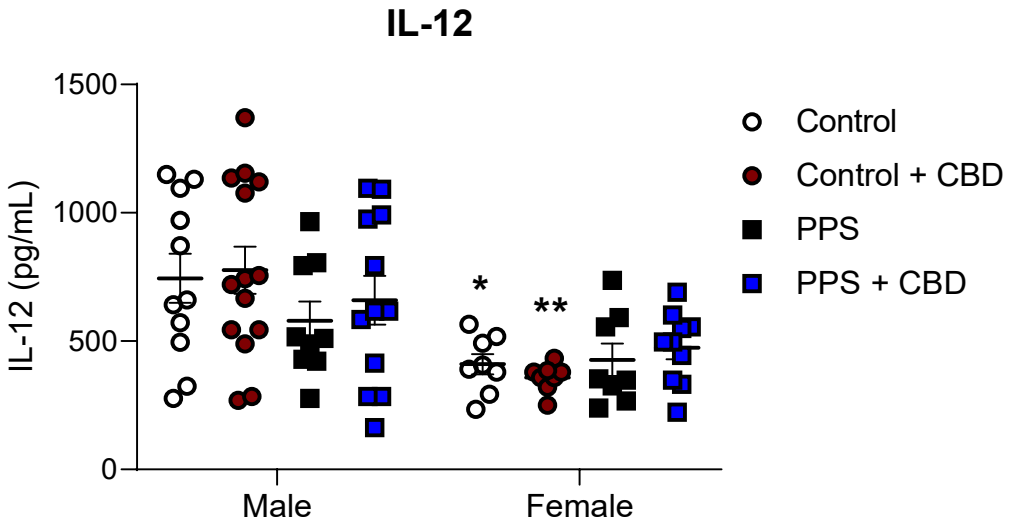

IL-12
1500
1000
500
0
IL-12 (pg/mL)
*
**
Male
Female
Control
Control + CBD
PPS
PPS + CBD

Figure 2.5: Cytokine Serum levels reported in pg/mL, n=8-12 per group. **2.5A**: Serum level of IL-18. **2.5B**: Serum level of MIP-1α, ** $p<0.01$ compared to males within the treatment group, **** $p<0.0001$ compared to males within the treatment group. **2.5C**: Serum level of fractalkine, $p<0.05$ compared to males within the treatment group. **2.5D**: Serum level of IL-1α. **2.5E**: Serum level of VEGF, ** $p<0.01$ compared to males within the treatment group, **** $p<0.0001$ compared to males within the treatment group. ^^ $p<0.01$ compared to male control. **2.5F**: Serum levels of RANTES. ** $p<0.01$ compared to males within the treatment group. *** $p<0.001$ compared to males within the treatment group. **7G**: Serum levels of Leptin. **2.5H**: Serum levels of IL-4. **2.5I**: Serum Levels of IL-.2 **2.5J**: Serum levels of IL-10. **2.5K**: Serum levels of IL-12. All results showed sex differences between males and females, especially in IL family cytokines, VEGF and fractalkine. This shows that there is a major difference in the immune response to stress as well as CBD between males and females.

2.3.5. Adrenal Gland

The female CBD gland to body ratio was significantly higher than the female control ratio. Analyses revealed no other significant interactions between male or female rats. The male PPS adrenal glands ratio was lower than Control but did not meet significance. Female adrenal gland ratio was significantly higher than males [$p<0.001$: $F(1, 34) = 18.39$]. Mixed-effects analysis for sex type 3 analysis revealed the significance of $p<0.0001$ Chi-square for matching [21.63, 1].

Figure 2.6. Adrenal Gland Ratio

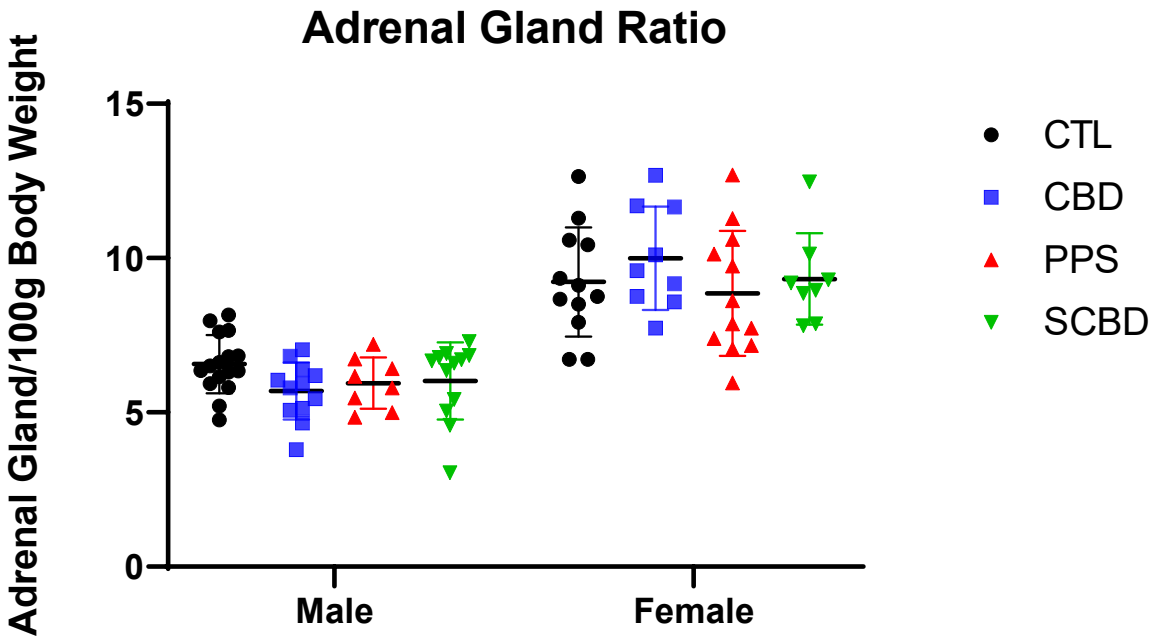


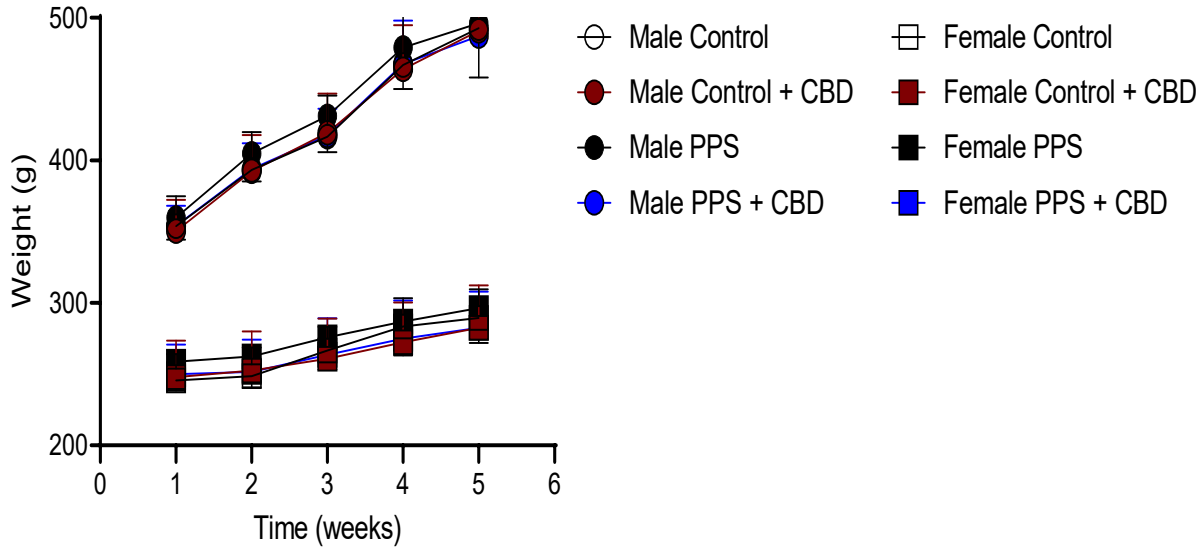
Figure 2.6. Male and female rat adrenal gland weight per 100 mg of body weight. Female rats' adrenal glands were significantly heavier than male rats. Individual post-hoc analyses did not reveal any significance between groups. However, a trend indicated that CBD may raise adrenal gland weights when exposed to PPS.

2.3.6. Body Weight

Weights were taken for males and females every week for 5 weeks. Significance was found between male and female growth rates and total weights [$p<0.0001$; $F(1, 104) = 767.8$]. Male PPS rats fell just beneath significance but had a lower-trending growth rate than control males, however male PPS rats treated with CBD oil grew significantly more than PPS rats [$p<0.01$; $F(3, 45) = 12.22$]. Female rats did not display variance between groups like the males with the exception of female PPS rats treated with CBD oil, which grew significantly less than control females [$p<0.05$; $F(3, 33) = 3.619$].

Figure 2.7. Body Weight and Growth Rate

A



B

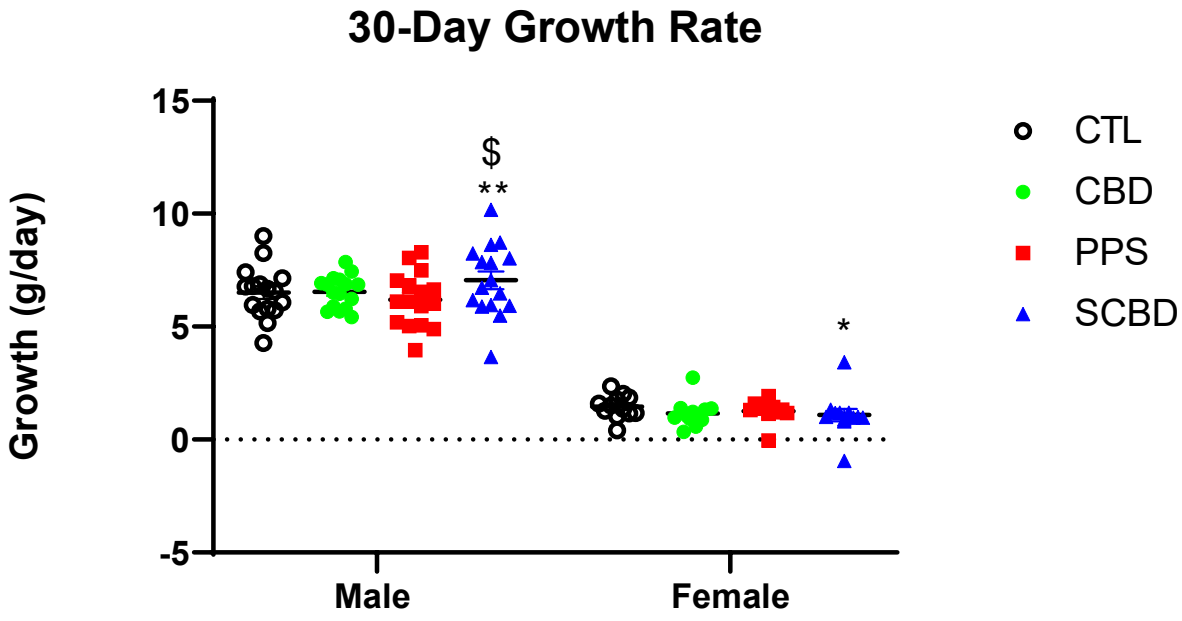


Figure 2.7. A: Weights of rats taken at weekly intervals over a period of 5 weeks. B: ** $p<0.01$ between male control and SCBD rats. $ $p<0.0001$ between PPS males and PPS males treated with CBD oil. * $p<0.05$ between PPS females treated with CBD oil and control females. Although significance was not met, PPS made males and females gain slightly less weight. Male PPS rats given CBD gained more weight, whereas female rats given CBD gained less weight than controls.

2.3.7. Appearance and Aggression

Signs of aggression when exposed to researchers. This included biting, hair standing, and freezing in the corner of the cage when the lid was opened. Thinning hair and porphyrin staining near the neck were considered poor appearance. 25% PPS males, and 12.5% of PPS males treated with CBD displayed these behaviours. 33.3% of PPS females displayed these signs, with one of them experiencing alopecia unrelated to overgrooming. PPS females treated with CBD exhibited worse signs with 41.6% displaying signs, and 25% experiencing alopecia unrelated to overgrooming. Chi-Square test revealed the significance of $p<0.001$ [19.63, 3] N=12-16

2.8. Reactions to Stress

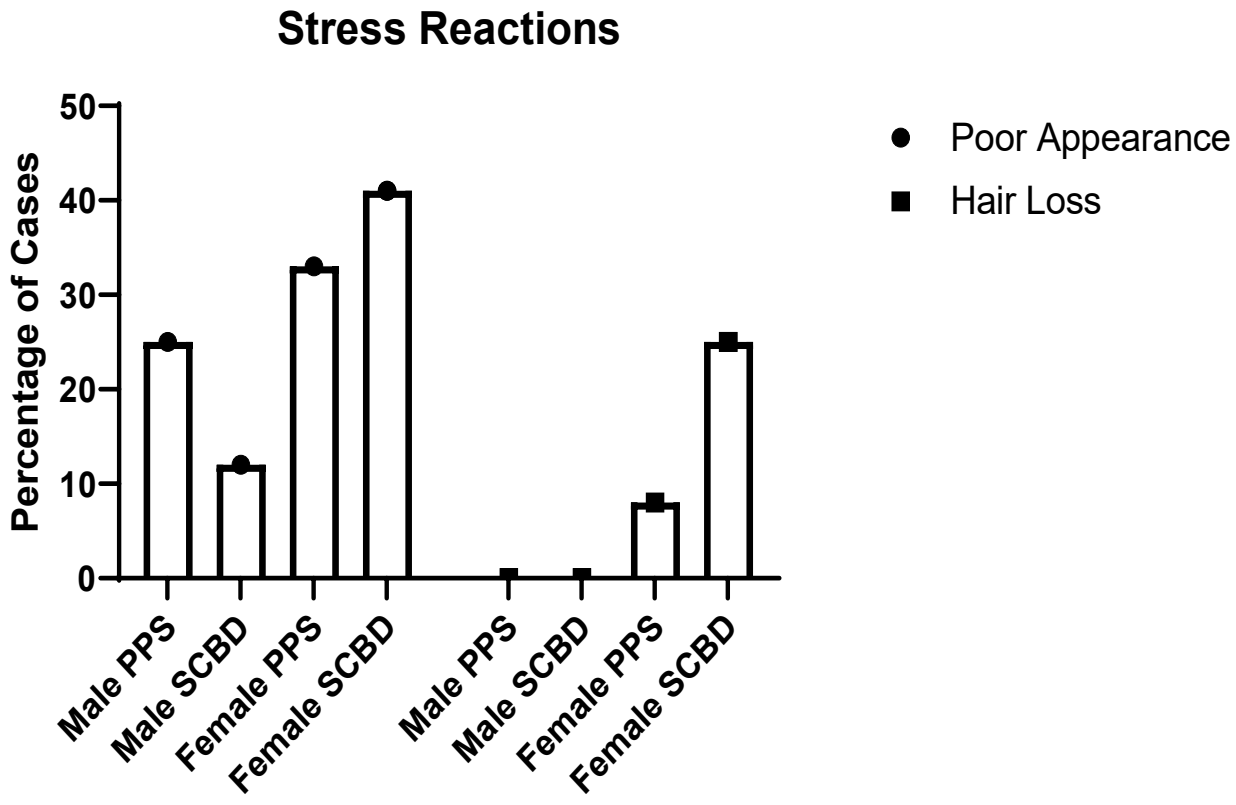


Figure 10) Females had significantly higher percentages of poor appearance such as thinned coat and porphyria stains on the neck than males. Female rats exposed to the stress experienced alopecia not resulting from overgrooming, whereas males did not. Female SCBD rats fared worse than female PPS rats. The trend was reversed in males, with SCBD males displaying fewer distress signs than PPS males. This is an indication that there are many factors that can impact Cannabis pharmacology across sexes.

2.4. Discussion

This model builds off the work of previous authors by extending the duration of the traumatic experience to be 30 days from 21 days (Cohen et al., 2020; Zoladz et al., 2012). This is important given that in humans, PTSD can only be diagnosed after 1 month (Hasin et al., 2013). Exposure to a traumatic experience, as well as social instability, has been demonstrated to produce a reliable model of PTSD-like symptoms in male rats (Cohen et al., 2018; Zoladz et al., 2012). Our model removed the restraint component to eliminate the "learned helplessness" aspect to produce a model more analogous to an inescapable and threatening experience. Although rats age differently compared to humans, the models previously mentioned indicate that adolescent rats developing into adults can be compared with humans (Cohen et al., 2020; Zoladz et al., 2012). Similar to our rat model, in many countries the age of conscription is 18 years of age. In the US, over 5000 individuals under 20 years old across all branches were deployed to Iraq or Afghanistan in support of combat operations (Committee on the Assessment of the Readjustment Needs of Military Personnel et al., 2013). Based on the previous literature, and the ages of many military members in combat worldwide, we feel that this model is as close to human conditions as possible without putting the rats near a live cat.

Our model demonstrates that the PPS model significantly reduces exploratory behaviour in the OFT with PPS compared to control in males. A similar trend was observed in the EPM, but significance was not met. PPS and *Cannabis* do not have the same effect on male rats, which is consistent with previous literature (Bangasser and Wiersielis, 2018, 2018; Colom-Lapetina et al., 2019). CBD, which is thought to be anxiolytic, worsens the exploratory behaviour of female rats exposed to PPS.

CBD in males showed a trending increase but did not meet significance. Serum corticosterone levels, whether exposed to PPS or not. Opposite to males, CBD significantly decreases serum corticosterone levels in females, whether exposed to PPS or not. Female rats exposed to PPS had significantly lower levels of basal corticosterone.

Female rats exhibited lower levels of cytokines in the IL family compared to males. Female rats exhibited higher levels of RANTES, Fractaline MIP-1α and VEGF. This makes sense, given that the latter cytokines belong to the CCL family, which is responsible for the recruitment and migration of inflammatory cytokines within the IL family (Fitzgerald et al., 2001).

Both male and female rats exposed to PPS gained significantly less weight over the course of 30 days than controls. PPS males given CBD gained significantly more weight than PPS males alone. However, the opposite effect was seen in females. PPS affected the appearance and aggression level of 25% of males and 46.1% of females, including causing 33.3% of the females treated with CBD to experience alopecia unrelated to grooming.

Given these results, we can conclude that the model produces a valid PTSD-like response in males and that CBD may be helpful in males to alleviate metabolic and anxiety symptoms. Although the response is not observed in the females in behavioural testing, the elevated levels of inflammatory cytokines, weight loss, cortisol differences, and poor appearance should be taken as a valid model given that these responses were higher than in males. Contrary to what is widely believed, CBD was detrimental to female rats exposed to PPS.

Due to the differing effects related to differences in male and female hormones, future studies will need to include large numbers of males and females. Our findings show major and significant differences between male and female rats in nearly every behavioural and chemical test

to date. More work will be needed to investigate the variation across the sexes in the brains of the rats using imaging and other pathological techniques.

Chapter 3: General Discussion

Our studies demonstrate a clear sex difference between male and female SD rats when responding to exposure to chronic traumatic stress, as well as a sharp contrast in response to consumption of self-administered *Cannabis* oil. Other animal models of PTSD have only used acute models, with the most extended stress exposure being 21 days (Cohen et al., 2018; Korem and Akirav, 2014; Zoladz et al., 2012). Both animal and human studies in PTSD and *Cannabis* have often focused mainly on male subjects, leaving knowledge gaps in female responses to stress and ***Cannabis*** individually, as well as together (Calakos et al., 2017; Liebschutz et al., 2002; Yang et al., 2019).

Many studies have focused on individual pathological components of PTSD such as CRF fluctuation, orexin dysregulation, or specific areas within the brain; mainly the hippocampus and the amygdala (Bangasser and Wiersielis, 2018; Cohen et al., 2020; Klinger et al., 2019; Krusemark et al., 2013). This study captured many pathological components of PTSD to examine further which connections could be made between systemic abnormalities. Our Results showed that given a minimal number of animals, we are able to elicit significant changes in behavioural outcomes in the EPM and OFT tests. Although replications will be needed for better validity, there was a noticeable effect in male vs female outcomes as a baseline as well as with the response to Cannabis. Male behaviour was significantly improved with Cannabis, whereas it was detrimental to females exposed to stress. More studies examining males and females exposed to Cannabis will be necessary, and more detailed serological and pathological testing to determine precise reasons for these outcomes. We examined HPA axis dysfunction via corticosterone levels and adrenal gland weight, cytokine levels, body weight, and behaviour, all of which are affected by PTSD (Cohen et al., 2018; Du et al., 2017; Heim, 2020; Sindhu et al., 2017; Zoladz et al., 2012). Both males and

females exhibited changes in body weight, corticosterone levels, adrenal gland weights, and serum cytokine levels. Although only corticosterone and cytokines showed significant differences, there were profound differences between males and females, which is in need of further exploration.

Although there have been reported benefits from *Cannabis* use, there have been few studies that have focused beyond smoking the dried flower with varying levels of THC (Bonn-Miller et al., 2014; Loflin et al., 2017). Prior animal studies with *Cannabis* have used synthetic cannabinoid receptor agonists and antagonists by injection (Korem and Akirav, 2014). Although still an obscure topic, it is thought that there may be a greater benefit when WPE's are used to deliver what is known as the entourage effect. This is a benefit of all cannabinoids and terpenes from the plant acting together synergistically to provide maximum benefit (Russo, 2019). This study used a WPE and allowed animals to self-administer the *Cannabis* oil orally at will. It is reasonable to argue that self-administration will be less stressful to the animals than injection. To our knowledge, this has been the first study to compare male and female rat responses to WPE *Cannabi*s oil via self-administration.

Cannabis has demonstrated the ability to rapidly and relatively safely complement traditional pharmaceutical treatments with limited side effects and potentials for addiction in comparison to other categories of drugs such as benzodiazepines (Bonn-Miller et al., 2014; Corroon et al., 2017; Hawkins et al., 2012). *Cannabis* has been effective in reducing opioid medications for pain with conditions like Cancer and Epilepsy ("*Cannabis* and cannabinoids for medical purposes - Canadian Cancer Socie," n.d.; Tzadok et al., 2016) and has lowered the death rates from opioids in some US states that have a legal framework. It has also shown the potential to be a harm reduction agent in cases of SUD to alcohol, as well as opioids (Cohen et al., 2020).

Clinical evidence has been limited, and not many RCTs have been completed with a significant number of subjects to bear conclusive results (Babson et al., 2017; Bonn-Miller et al., 2014; Loflin et al., 2017). Treatment for PTSD presently is a highly time-consuming process that involves both psychotherapy and pharmacotherapy (Alexander, 2012; Flanagan et al., 2016; Shalev et al., 2012). Both take a significant amount of time before the patient will begin to see the benefits of either treatment. Preliminary RCTs have shown that PTSD patients can rapidly benefit from the consumption of *Cannabis* (Babson et al., 2017; Bonn-Miller et al., 2014). Information regarding the interactions of *Cannabis* with other pharmaceutical agents are poorly known and should be addressed to determine if patients can use *Cannabis* together with current first-line pharmacotherapy.

Although the goal of the study was to use as few animals as possible in line with the 3R's of animal use, at least one, or more replications of the study will be needed to obtain more significant data. More numbers will allow for more powerful data analyses and to allow to test other factors in the HPA axis that are affected by stress and Cannabis simultaneously.

The baseline difference in behavioural outcomes, as well as body weight, cortisol, and cytokine levels, were not expected to be as dramatically different as observed. This makes conclusions more difficult to draw, especially when we try to factor in the effects of Cannabis. Although we controlled for variations in cortisol levels throughout by only collecting at the time of euthanization, and collected at similar times during the day with respect to each group, perhaps increased frequency of collection can be appropriate if we can be certain that it will not cause added stress to the animals. Due to facility constraints, male and female rats could not be conducted at the same time. In order to mitigate seasonal differences and other fluctuations, we spaced the

groups at exactly 1 year apart to ensure the season would not cause any effects. One major limitation impossible to overcome in our situation is the fact that male researchers inevitably cause more stress to rodents than female researchers (Katsnelson, 2014.). These differences are highly important and should be explored further. If the animal model can be replicated, better conclusions will be possible, and we can improve on the design with closer monitoring of weight, estrous cycle in females, and comparison of sex hormone levels between males and females throughout the 30 days and at intervals during behavioural testing.

We can conclude from our studies that male and female SD rats respond very differently to PTSD, as well as to the administration of *Cannabis*. Female rats did not perform as poorly as males in behavioural tests but suffered similar metabolic issues like lower weight gain and hair loss. *Cannabis* was anxiolytic for male rats but exacerbated female symptoms in behavioural tests, as well as promoted higher rates of alopecia to female rats exposed to PTSD. Although this makes it more difficult to compare the treatment of PTSD with *Cannabis* because of the vast baseline differences, the finding is important because there is limited data from females in both animals and humans. PTSD is known to be a very multifaceted disease in which sociological, psychological, genetic, and various external factors play a role in the development and treatment of the disease (Bangasser and Wiersielis, 2018; Calakos et al., 2017; Yang et al., 2019). Further studies should include both male and female subjects in higher numbers or replications for better statistical validity, as well as important pharmacokinetic and pharmacokinetic data regarding effects, safety, and interactions with other pharmaceutical compounds. Multidisciplinary studies assessing the use of *Cannabis* in tandem with psychotherapy should also be completed to see if sessions may be facilitated with the consumption of *Cannabis.*

With *Cannabis* legal in many countries, we are in a golden window of opportunity to study *Cannabis* and human ECS. Due to recent legalization, there has been a significant shift in public opinion, and research has expanded dramatically, allowing for more discoveries with respect to the plant itself, as well as uses for the expanding plethora of compounds contained within. This golden window should be used to study what could be considered "healthy" human subjects that may have been reluctant to consume *Cannabis* previously but may now be more open due to the legal status. This will yield important information on the pharmacokinetics and pharmacodynamics in healthy subjects, which can be used to assess effects on short-term and long-term use objectively, with as limited stigma and bias as possible.

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