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Cannabinoid Transmission in the Basolateral Amygdala Modulates Prefrontal Cortex and Ventral Hippocampal Activity

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ABSTRACT

The cannabinoid system is important for maintaining neuron-to-neuron communication within the mammalian brain. One of the most commonly used substances to alter the cannabinoid system is cannabis. Individuals who are exposed to cannabis report having dissociable effects; both positive and negative. High amounts of THC have been commonly associated with the negative effects of cannabis, whereas CBD can be used to counter these. Pre-clinical evidence suggests that the combination of the two compounds can produce a therapeutic benefit for individuals who are susceptible to the effects of THC. The present study investigates whether the combination of THC+CBD can prevent electrophysiological changes induced by THC. Using In Vivo electrophysiology, simultaneous recordings of single unit activity both in the ventral hippocampal and prefrontal cortex were compared after infusions of cannabinoids into the basolateral amygdala. THC induced changes in the PFC to increase overall activity whereas the combined dose of THC+CBD returned cortical activity to baseline and introduced a potential benefit in reduced hippocampal activity.

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LIST OF ABBREVIATIONS

2-AG	Arachidonoylglycerol
5HT-1A	Serotonin 1A
AEA	Anandamide
ANOVA	Analysis of Variance
BLA	Basolateral Amygdala
BOLD	Blood Oxygenated Level Dependent
CBD	Cannabidiol
CB1R	Cannabinoid Receptor 1
GABA	Gamma Aminobutyric Acid
IV	Intra-venous
LFP	Local Field Potential
NASh	Nucleus Accumbens Shell
PFC	Prefrontal Cortex
THC	Delta-9-Tetrahydrocannabinol
vHPC	Ventral Hippocampus
VTA	Ventral Tegmental Area

1.1 Introduction

Endocannabinoid signalling in the mammalian brain is important for regulating the coordination of communication between brain regions to produce everyday behaviours. A common drug of abuse linked to alterations in this signalling pathway is Cannabis (i.e., Cannabis Sativa). It is well known that individuals can experience both positive and negative affective experiences following cannabis exposure. To study these effects, researchers have focused on two main compounds located in the plant: Delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD). The mind-altering (i.e. psychotropic) effects of cannabis are associated with THC, whereas CBD can treat psychosis symptoms (i.e. antipsychotic) (D'Souza et al., 2004; Mechoulam et al., 1988; Pertwee, 2004; Russo & Guy, 2006; Zuardi et al., 1982; Zuardi et al. 1991). A growing body of literature is now investigating how the combination of the two can prevent disturbances in behavioural and cognitive functioning through its effects on the endocannabinoid system.

Previous studies have examined the potential therapeutic benefits of combined THC+CBD formulations on mental health-related behaviours using systemic administration routes (Boggs et al., 2017, Jacobs et al., 2016; Wright et al., 2013). As of yet, no clear demonstration of the combined and/or synergistic effects of THC and CBD has been investigated with respect to specific regions in the brain. As the amygdala region is involved in general endocannabinoid transmission and has previously produced different effects between THC and CBD in the human brain, we chose this as the primary region of interest in this study (Bhattacharyya et al., 2010; Bhattacharyya et al., 2017; Tan et al., 2010; Tan et al., 2011;). Furthermore, the amygdala is interconnected with both cortical and hippocampal regions that are impacted by THC in cognition, anxiety, memory (Englund et al., 2013; Lichtman et al., 1995; Jentsch et al., 1997; Rubino et al., 2008). The work presented in this thesis is the first attempt to characterize the effects of THC and CBD in the amygdala of rodents using in vivo electrophysiological recordings. Understanding how these compounds impact

distant circuits provides insights in how they act on the brain to produce a potential therapeutic benefit when combined.

1.1.1 The Effects of Cannabis on Mental Health

Cannabis is currently the most commonly used illicit drug in the world. In 2015 in Canada alone, approximately 3.6 million people report using cannabis and usage is expected to increase by the end of the decade (Rotermann & Macdonald, 2018). The passing of medical marijuana laws in select states in the United States of America (USA) have so far seen additional rises in illicit cannabis use (Hasin et al., 2017). Among teenagers, legalization of medical marijuana has led to a decrease in perceived risk and an increased interest to recreationally use (Miech et al., 2015). As compounds associated with cannabis become more commercially available, with the continued legalization of marijuana in US states, and with upcoming legalization in Canada in 2018, more attention is being paid to the potential positive and negative health effects of cannabis use.

Although symptom duration and intensity vary depending on what age one begins recreational cannabis use, users claim to experience acute positive benefits such as reduced nausea, increased relaxation, and a positive high (Mechoulam & Parker, 2012). In contrast, some negative effects that may occur are impairments in attention and memory, increased anxiety, and negative highs associated with psychotomimetic effects (D'Souza et al.,2004; D'Souza et al., 2005; Rottanburg et al., 1982; Volkow et al., 2016). These symptoms differ in duration from short-term to long-term; cannabis, like any drug, has the potential to alleviate or add negative effects. Considerable evidence now links cannabis use with alterations to the endocannabinoid system in the mammalian brain, causing emotional and cognitive dysregulation associated with various neuropsychiatric disorders (Laviolette & Grace, 2006; Parsons & Hurd, 2015; Volkow et al., 2016; Zehra et al., 2018). For example, exposure to cannabis in teenagers is associated with a greater risk for developing psychosis in adulthood (Arseneault et al., 2002; Arseneault, Cannon, Witton, & Murray, 2004; Stefanis et al., 2004). Evidence for these risks are also

supported in animal research. In preclinical models, adolescent exposure to chronic THC causes deficits in cognitive task performance associated with schizophrenia and psychosis (Renard et al., 2017a; Renard et al., 2017b). These deficits are further supported with changes in cortical and sub-cortical molecular signalling pathways found in animal models of schizophrenia (mTOR, GAD 67, & GSK). In addition, there is a growing concern about the epidemiological impact of increased THC content in cannabis products in the past few decades (Cascini, Aiello, Di Tanna, 2012). Increased ratios of THC coupled with the known vulnerability of adolescent populations can potentially lead to an increase in diagnosis rates of psychosis and schizophrenia. Cannabis use does not in itself cause these disorders, yet a growing body of literature finds that individuals with genetic (polymorphisms of COMT and AKT1) and environmental (childhood abuse and familial relatives with these disorders) risks for these disorders may have exaggerated symptom onset, duration, or intensity when chronically exposed to cannabis (Arendt et al., 2008; Caspi et al., 2005; Forti et al., 2012; Henquet et al., 2009; Houston et al., 2008; Radhakrishnan et al., 2014).

Central to understanding the pharmacological and psychotropic effects of cannabis is identifying the compounds responsible. Of over 100 phytochemicals contained in the plant, THC and CBD have been the most widely studied in terms of effects on mental health. Psychotropic effects associated with the plant have been linked to THC (D'Souza et al., 2004; Pertwee, 2004; Mechoulam & Parker, 2012). Opposite to THC, CBD does not produce any mood alterations or highs on its own, yet it has been associated with antipsychotic and antianxiety-like benefits (Russo & Guy, 2006; Zuardi et al., 1982; Zuardi et al. 1991). Both compounds interact with the endocannabinoid system differently to alter the mammalian brain and produce behavioural effects (Atakan, 2012; Morales, Hurst, & Reggio, 2017; Pertwee, 2008;). The dissociation in the effects of cannabis may be due to the way the endocannabinoid system regulates the brain when the additive effects of these compounds interact when combined.

1.1.2 Endocannabinoid System

The endocannabinoid system is important for proper development and functioning of the mammalian brain (Meyer, Lee, & Gee, 2018). This system contains endogenous ligands and receptors that are synthesized and expressed throughout the entire brain, allowing proper cell-to-cell communication between synapses and regulation of brain area coordination (Laviolette & Grace, 2006; Parsons & Hurd, 2015). Both arachidonoylglycerol (2-AG) and anandamide (AEA) are the most well studied endogenous ligands that act on the endocannabinoid system (Devane et al., 1992; Mechoulam et al., 1996). Both ligands act as retrograde messengers that are synthesized and released from the postsynaptic cell to be released into the synapse. From there, they travel to bind and activate cannabinoid 1 receptors (CB1R) located on presynaptic terminals. CB1Rs are one of the most abundant receptors found throughout the mammalian brain (Atakan, 2012; Morales, Hurst, & Reggio, 2017). These receptors are classified as G-protein-coupled receptors with a seven transmembrane domain. The activation of these receptors by these ligands blocks adenyl cyclase and downstream targets to prevent the release of neurotransmitter vesicles (Howlett et al., 1986). Overall, as cells communicate to generate postsynaptic action potentials, the endocannabinoid system responds by releasing 2-AG and AEA to bind to CB1R to cause an overall inhibition on the presynaptic cell and therefore prevents any dysregulation that could be caused by over excitation in the synapse (Hoffman & Lupica, 2000; Kano et al., 2009; Sullivan 1999; Wilson & Nicoll, 2001). In addition to endogenous ligands, synthesized endocannabinoids like THC can bind to CB1R to alter overall brain function.

1.1.3 Properties and Mechanisms of Delta-9 Tetrahydrocannabinol in the Brain

The primary phytochemical responsible for the psychoactive effects of cannabis has been linked to THC and to changes in CB1R functioning. In particular, THC acts as a partial agonist for the CB1R.Because of this, THC can lead to different agonist-antagonist effects depending on the number of receptors currently expressed, the cell type, and the current presence of other endogenous ligands for the CB1R (Morales et al., 2017). Despite this, THC will tend to interact with the CB1R like the endocannabinoids AEA, where the net result on the synapse is to reduce the overall exogenous release of neurotransmitter vesicles from presynaptic terminals (Laaris, Good, & Lupica, 2010; Shen & Thayer, 1998).

As most CB1Rs are located on glutamatergic and gamma aminobutyric acid (GABA) expressing cells, THC will either reduce inhibition or increase disinhibition depending on where it is expressed (Morales et al., 2017). This will determine how the cannabinoid impacts brain functioning. For instance, rodents exposed to THC will have increased extracellular dopamine and glutamate and decreased GABA neurotransmitter levels measured in the PFC (Pistis et al., 2002). Glutamatergic release in the hippocampus is impaired but can be reversed with the presence of CB1 antagonists (Fan et al., 2010). THC can also increase striatal dopamine release, similar to other drugs of abuse, via CB1R and therefore lead to emotional salience misattributions and distortion (Wijayendran et al., 2016). In addition, THC administered to prenatal fetuses or during adolescence results in abnormal development of glutamatergic and dopaminergic signalling in the rodent brain to impact cognition and attention (Castaldo et al., 2007; Renard et al., 2016, Renard et al., 2017a; Renard et al., 2017b).

The interaction of these various neurotransmitter systems during exposure to THC can alter cognitive and behavioural functioning in the mammalian brain. For example, in rodents, THC can cause greater errors in working memory

performance, which is reversed with a CB1 antagonist (Lichtman & Martin, 1996). In both young and adult rodents, systemic THC produces an anxiogenic effect as measured in the elevated plus maze, light-dark, and open field locomotion tasks (Sapyta et al., 2007). In humans, a single administration of intravenous THC can cause healthy individuals to feel increased anxiety, verbal working memory impairments, positive, negative, cognitive and psychosis-like symptoms, along with feelings of a high (D'Souza et al., 2005). Furthermore, individuals with a history of psychotic symptoms experience similar deficits after acute THC administration (D'Souza et al, 2006). Chronic exposure to THC during critical neurodevelopmental periods is associated with schizophrenia-like deficits in adulthood, coupled with a loss in cortical interneurons and modulation of the mesolimbic system (Renard et al, 2017a; Renard et al., 2017b).

Cortical overexcitation via loss of inhibitory interneuron regulation may result in dysregulated GAMMA oscillatory activity, a neuropathological feature associated with schizophrenia (Lee et al., 2010; Symond et al. 2005). Furthermore, administration of THC can alter GAMMA oscillations in both the prefrontal cortex and the hippocampus, two regions that are involved with schizophrenia (Renard et al., 2017a; Renard et al., 2017b; Robbe et al., 2006;). Studying how THC alters the endocannabinoid system is therefore important to understanding how mal-adaptive effects can arise from use in healthy and clinical populations.

1.1.4 **Properties and Mechanisms of Cannabidiol in the Brain**

In contrast to THC, CBD has very low affinity as a CB1 receptor antagonist (Thomas et al., 2009), leading researchers to search for alternative sites of action to explain its effects. CBD can act as a partial agonist to the 5HT-1A serotonin receptor (Pertwee, 2004; Russo et al., 2005), a weak partial agonist for D2 receptors (Seeman et al., 2016), a weak negative allosteric modulator to Mu opioid receptors (Kathmann et al., 2006) and activates the GPR55 receptor (Ryberg et al., 2007). Recently, researchers have focused on CBD's involvement with the

5HT-1A receptor to regulate cell-to-cell communication. In general, activation of the 5HT-1A autoreceptors located on post synaptic somato-dendritic sites causes inhibition of that cell's firing output (Polter & Li, 2010; Tada et al., 2004;). Following this cell firing inhibition, CBD administration could potentially inhibit post-synaptic transmission and therefore impact the activity of projected areas. For instance, intracranial infusion of CBD in the shell of the nucleus accumbens (NASh) can attenuate ventral tegmental area (VTA) dopamine neuron firing (Norris et al., 2016; Renard et al., 2016).

Similar to THC, the effects of CBD are determined by the location of its target receptors, with activation in different brain regions potentially causing different behavioural effects. Although CBD does not show any psychotropic effects, research has linked its potential in having anxiolytic, anti-inflammatory, and anti-psychotic-like properties (Russo & Guy, 2006; Zuardi et al., 1982; Zuardi et al. 1991). Anxiolytic effects of CBD have been associated with behavioural changes in forced swim tasks (Sartim et al., 2016), elevated plus maze tasks (Campos and Guimares, 2008), facilitating fear extinction (Bittencourt et al., 2008; Do Monte et al., 2013), and impairing fear-associated memories (Gomes et al., 2010, Norris et al., 2016, Stern et al., 2017). CBD has also shown antipsychotic-like benefits by reducing sensory-motor gating deficits (Renard et al., 2016), preventing short-term THC-induced memory and social interaction impairments (Malone et al., 2009; Morgan et al., 2010), and dopamine-related psychomotor sensitization (Renard et al., 2016). Although very few clinical trials exist in the literature, research demonstrates that daily oral doses of CBD administered to schizophrenic populations exhibit improvements in negative and cognitive symptoms comparable to those treated with traditional antipsychotic medication (Leweke et al., 2012). The normal circulation of endocannabinoid levels can also interact with CBD. Acute administration of CBD increases overall AEA levels by blocking reuptake and fatty acid amide hydrolase inhibition (Bisogno et al., 2001; Ligresti et al., 2006). This effect on increased release of AEA in the synapse would reduce the overall release of neurotransmitter vesicles from presynaptic terminals, and therefore inhibit cell-

to-cell communication (Hoffman & Lupica, 2000; Kano et al., 2009; Sullivan 1999; Wilson & Nicoll, 2001). Due to its interactions with the endocannabinoid system and the independent benefits that it might have on behaviour, CBD appears to have therapeutic potential for users.

1.1.5 Therapeutic potential of combined THC and CBD formulations

Although the mechanisms underlying CBD's ability to interact with the endocannabinoid system are still being characterized, growing evidence suggests that it may be used to counter some of the negative side effects of THC use. In non-human primates, THC+CBD ratios of 1:1 and 1:3 can reverse temporary THC-induced cognitive and visuospatial attention impairments (Jacobs et al., 2016; Wright et al., 2013). Pre-treatment at a systemic 1:20 ratio can prevent the social interaction deficits associated with THC (Malone et al., 2009). Furthermore, THC-induced conditioned place aversion can be blocked at a 1:1 combined CBD ratio, despite it having no preference effects by itself (Vann et al., 2008).

In human studies, the dose used to demonstrate CBD's potential restorative effects is much higher. For example, individuals given 10mg of THC display increases in skin conductance responses and subjective anxiety ratings to fear stimuli whereas CBD alone (at 600mg) does not elicit any change compared to placebo (Fusar-Poli et al., 2009). In recreational users, those who smoked cannabis with higher CBD to THC concentrations did not display impairments on memory in contrast with those who had lower concentrations of CBD and did have memory issues (Morgan et al., 2010). In healthy participants, THC IV infusions cause deficits in hippocampus-dependent episodic memory performance but pre-treatment with 600mg CBD improved performance to placebo levels (Englund et al., 2013). In addition, a 1:2 oral dose combination prevented THC-induced impairments in emotional facial recognition (Hinodocha et al., 2015). Although more studies are needed, considerable evidence suggests that CBD may prevent some of the neuropsychiatric effects seen with THC, yet not in all situations. In

some situations, CBD can induce an effect independent of the presence of THC, for instance CBD is able to block fear memory reconsolidation with or without THC (Stern et al., 2012; Stern et al., 2015). This could suggest that CBD can prevent THC deficits in some cases and act on the brain independently of THC in other cases. Furthermore, some ratios of THC:CBD may even allow a user to experience a higher dose of THC while avoiding some of the negative side-effects (Boggs et al., 2017). Overall, CBD's potential to prevent or reverse the effects of THC when combined may depend on the dose ratio and the animal species researched.

1.2 **Cannabinoid modulation of the basolateral amygdala, ventral hippocampus, and prefrontal cortex**

The endocannabinoid system is involved with multiple brain regions that are important for regulating cognition, emotion, memory, and anxiety (Laviolette & Grace, 2006; Mechoulam & Parker, 2012). The interference of these processes from cannabis may be a result of how THC and CBD potentially interact within these regions. For example, in chronic cannabis users, CB1Rs are downregulated in areas such as the hippocampus and PFC, which are important for cognition and memory (Hirvonen et al., 2011). Structural neuroimaging and resting state studies have also shown reductions in hippocampal, prefrontal cortex, and amygdala brain volume and activity in chronic cannabis users (Block et al., 2000; Yucel et al., 2008). Furthermore, acute administration of CBD has been shown to decrease blood oxygenated levels in both the amygdala and hippocampus (De Crippa et al., 2004). All three regions are comprised of the mesolimbic system, which has been implicated in reward, learning, anxiety, and cognition (Laviolette, 2017). It is therefore important to understand how these regions are influenced by phytocannabinoid exposure to determine how THC and CBD may produce their differential effects on the brain and neuropsychiatric phenomena.

1.2.1 **Cannabinoid Effects on the Basolateral Amygdala**

Anatomically, the amygdala is clustered into functionally distinct regions. The Basolateral Amygdala (BLA) is one area considered important for emotion and anxiety regulation (Janak & Tye, 2015). The BLA has cortical-like features, as it is compromised of two main cell types: glutamatergic pyramidal projection neurons and GABAergic local interneurons (Ramikie et al., 2012). The CB1R is highly expressed in the BLA, with mRNA studies revealing that approximately 95% of cells co-express GABAergic markers (Marsicano & Lutz, 1999). This suggests its predominant expression is on BLA interneuron terminals. Given the location of these receptors, activation via cannabinoid transmission decreases spontaneous and evoked transmission on the BLA pyramidal neurons (Katona et al., 2001). Therefore, activation of these CB1Rs is predicted to indirectly increase pyramidal neuron firing by decreasing GABA interneuron inhibition, therefore causing an increase in activity to downstream targets of the BLA (Ramikie et al., 2012).

Efferent projections from the BLA go to multiple neural regions, but of most importance to this study are those going to the hippocampus and PFC (Knapska et al., 2007; Sah et al., 2003). For example, cannabinoid-mediated modulation of these specific pathways can alter anxiety and emotional memory processing (Felix-Ortiz et al., 2014; Laviolette & Grace, 2006). In addition, being interconnected with these regions positions the BLA as a central structure involved in coordinating salient environmental stimuli and consolidating that information to form emotional memories.

Considerable evidence demonstrates that cannabinoid transmission within the BLA can affect emotion regulation and memory. Activation of intra-BLA CB1Rs via an agonist will potentiate the acquisition of a fear memory, whereas a CB1R antagonist has been shown to block the formation of emotional associative memories (Tan et al., 2011). Endocannabinoid-mediated activation in the BLA also causes animals to have increased anxiety and prevents the formation of aversive

memories (Munguba et al., 2011). Micro-infusions of THC into the BLA alters memory and causes an anxiogenic effect on elevated plus maze and locomotion tasks (Rubino et al., 2008). Furthermore, activation of CB1R in the BLA can switch morphine's rewarding effects into aversive memory place preferences (Ahmad et al., 2016). In humans, acute oral administration of THC in healthy subjects not only increases anxiety and symptoms associated with psychosis but can cause an increased blood oxygenated level in the amygdala when viewing negative stimuli (Bhattacharyya et al., 2017). This effect was correlated with PET imaging expression of CB1R in the amygdala, demonstrating the connection between the effects of THC on emotional stimuli mediated through the cannabinoid receptor. The same lab previously showed that an oral dose of CBD did not produce any changes in the same behavioural measures relative to placebo, but found a significant decrease in amygdala BOLD signalling compared to THC administration (Bhattacharyya et al., 2010). These findings suggest that both CBD and THC can have independent effects on amygdala activity to alter affective processing and associated behaviours.

1.2.2 Effects of Cannabinoids in the Ventral Hippocampus

The hippocampus' involvement in regulating emotion, stress, and learning and memory processes makes it a potential target for the effects of cannabis use. In particular, the ventral hippocampus is more involved with emotion and anxiety phenomena as opposed to the dorsal region, which is important for spatial and contextual memory processing (Fanselow & Dong, 2010). The ventral hippocampus region in rodents is functionally analogous to the anterior hippocampus in humans (Fanselow & Dong, 2010). Reductions of anterior hippocampal volume occur in chronic cannabis users, with the most severe reductions in individuals exposed to higher ratios of THC to CBD (Demirakca et al., 2011).

Within the ventral hippocampus, cannabinoid transmission is important in regulating salience of environmental cues and in emotional memory formation. Similar to the BLA, CB1R expression is mainly located on GABAergic interneurons (Katona et al., 1999), which can cause an overexcitation towards the system via interneuron inhibition when activated. Considerable evidence now suggests that overexcitation of vHPC is responsible for behavioural deficits associated with schizophrenia (Grace et al., 2010). For example, intracranial microinjections of a CB1R agonist into the hippocampus impairs performance on radial arm maze memory tasks similar to the effects seen with systemic THC (Lichtman et al., 1995). Systemic THC and WIN55 (i.e., a full CB1R agonist) cause impairments in a delayed nonmatch sample task which simultaneously co-occurs with alterations in hippocampal cellular firing (Hampson & Deadwyler, 2000). A high dose of THC (5 ug) injected into the vHPC makes rodents more anxiolytic on the elevated plus maze task (Rubino et al., 2008). Direct activation of the CB1R using WIN55 in the vHPC can potentiate the acquisition of both reward and fear memories at non-rewarding or low foot shock conditions while also impairing social recognition (Loureiro et al., 2015). This potentiation of non-salient cues is associated with changes in the mesolimbic system with vHPC micro infusions of WIN55 increasing activity in both NASh medium spiny and VTA dopamine neurons (Loureiro et al., 2015; Loureiro et al., 2016). Furthermore, THC can also impact single unit and local field potential (LFP) recordings in awake animals. Both systemic and intracranial hippocampus injections of a CB1 full agonist decreases power for LFP across Theta, Gamma, and Ripple events (Robbe et al., 2006). Although single unit recordings were not analyzed, systemic injections of THC also decreased LFP power in the same frequency bands (Robbe et al., 2006). Acute administration of THC can reduce resting state ventral hippocampal activity, further implicating the compound as a potential anti-psychotic to counter overexcitation of the circuit (De Crippa et al., 2004).

Alterations from the vHPC can therefore impact other regions that are interconnected. The vHPC shares unique neuronal connectivity between the PFC

and the amygdala, unlike the dorsal hippocampus. Specifically, ventral CA1/subiculum regions of the ventral hippocampus have re-occurring connections with the posterior BLA and prelimbic regions of the PFC (Pitkanen et al., 2000; Cenquizca & Swanson, 2007). This pathway, along with known properties of BLA cannabinoid transmission, makes the ventral hippocampus an interesting target to examine the local effects of THC and CBD administration in the context of mesocorticolimbic circuitry.

1.2.3 Effects of Cannabinoids on the Prefrontal cortex

The prefrontal cortex is an important region to study the effects of cannabinoid drugs on cognition, attention, and memory. In rodents, the pre-limbic region of the PFC is interconnected with the BLA, potentially implicating this pathway as important for endocannabinoid transmission (McGarry & Carter, 2017; Tan et al., 2010; Tan et al., 2011). Similar to the BLA and vHPC, CB1R is highly expressed within the PFC interneuron populations. Endocannabinoids, when expressed in the pre-limbic regions of the PFC, can inhibit evoked excitatory postsynaptic currents and therefore impact other interconnected regions (Lafourcade et al., 2007). In addition, THC given systemically can also cause an increase in mRNA expression in the PFC for c-fos, a marker for neuronal activation (Egerton et al., 2001). In humans, the PFC and anterior cingulate have increased blood oxygenated levels after smoking cannabis (Kanayama et al., 2004). All these changes in the PFC can have implications for behavioural task performance. Working memory tasks such as the T-maze and radial arm maze require the prefrontal cortex to maintain and make decisions based on cues found in the environment. Systemic injections of THC cause impairments on these working memory tasks (Jentsch et al., 1997; Lichtman et al., 1996). Low doses of THC micro-infused into the PFC surprisingly makes an animal more anxiolytic, in contrast to high doses that produce an anxiogenic effect (Rubino et al., 2008). Activation of CB1R with low doses of a full agonist can also potentiate the acquisition of a subthreshold fear memory (Draycott et al., 2014). Combined these

findings suggest that the PFC is a region susceptible to endocannabinoid modulation and that unique connections shared with both the vHPC and BLA make it a region of special interest in the neural circuitry described below.

1.2.4 Cannabinoid modulation of the vHIPP-PFC-BLA circuit

As discussed above, the cannabinoid signaling system, through either endogenous or exogenous compounds, can substantially alter behaviour and neuronal functioning separately in the BLA, vHPC, and PFC (Draycott et al., 2014; Loureiro et al., 2015; Loureiro et al., 2016; Robbe et al., 2006; Tan et al., 2011). However, each area functions independently and is part of a dynamic system with recurring connections between these regions. The effects of cannabis, specifically THC, may alter how each region coordinates with other regions. For instance, systemic THC decreased functional connectivity between the amygdala and PFC in humans, during poor performance on the re-appraisal of negative stimuli compared to controls (Gorka et al., 2015). Oral administration of CBD can also decrease at rest BOLD signalling in both the amygdala and hippocampus (De Crippa et al., 2014).

In rodents, systemic THC can alter neuronal activity in the PFC and vHPC (Aguilar et al. 2016; Robbe et al., 2006). One study found that average firing rates of single unit neurons in the PFC decreased, whereas no change was found in cells recorded in the ventral hippocampus (Aguilar et al., 2016). Furthermore, THC increased coherence between both regions in the Delta frequency band which is associated with activity at rest. A caveat to this study was that no consideration between cell types were controlled for during single unit recordings. Therefore, the combined activity of principal and interneuron populations could have possibly masked any independent effects under the presence of THC. In the current literature, no study has yet addressed how THC can alter single unit populations in both the vHPC and PFC. Furthermore, the combination of THC+CBD has yet to be explored in the modulation of neural circuit dynamics in the mammalian brain.

1.3 **Research Aims and Hypothesis**

The endocannabinoid system is critical for the proper health and development of the mammalian brain (Meyer, Lee, & Gee, 2018). Mental health disorders such as schizophrenia have been linked to disruption of the cannabinoid system in mesolimbic areas of the brain (Laviolette & Grace, 2006). One pathological manifestation of schizophrenia is persistent thoughts of hallucinations and delusions. Cannabinoid compounds may modulate the mesolimbic system and may be partially responsible for inappropriately reinforcing the distorted emotional significance of everyday events within the schizophrenic population (Laviolette & Grace, 2006).

To investigate this hypothesis, our research group has tested how THC can modulate the cannabinoid system within mesolimbic areas of the brain. In the BLA, PFC, and vHPC, we demonstrate that THC, or the activation of its target receptor, CB1R, can impair cognition, anxiety, sociability, learning and memory, and reward-related behaviours (Ahmad et al., 2016; Draycott et al., 2014; Loureiro et al., 2015; Loureiro et al., 2016; Renard et al., 2017a, Renard et al., 2017b; Tan et al., 2010, Tan et al., 2011). Building on this research, we wish to investigate how to prevent these impairments by countering the effects of THC. As previously discussed, CBD combined with THC can restore impairments in cognition, attention, sociability, anxiety and emotional processing (Englund et al., 2012; Fusar-Poli et al., 2009; Jacobs et al., 2016; Malone et al., 2009; Wright et al., 2013). Therefore, this phytocannabinoid combination has potential therapeutic benefits in select behavioural paradigms and neuropsychiatric tests. However, the precise mechanisms by which CBD may counteract the effects of THC on brain circuits linked to cannabis-related neuropsychiatric disorders has not yet been clarified.

Our research group has previously demonstrated that cannabinoid transmission in the BLA can alter pyramidal cell activity in the PFC (Tan et al.,

2010; Tan et al., 2011). This was done using a full, synthetic CB1R agonist, WIN55,212-2, as a method to modulate the endocannabinoid system. However, we have not yet examined how the effects of plant-derived, pure THC (a partial agonist of the CB1R), may alter neuronal activity states in the PFC. Furthermore, systemic administration of THC can induce changes in the single unit and local field measures between the PFC and vHPC (Aguilar et al., 2016). Given the position of the BLA as a region that projects directly to both areas (Knapska et al., 2007; Sah et al., 2003), we wanted to explore how THC might impact this circuitry. Furthermore, CBD can act as a potential therapeutic when combined with THC and independently it can alter neuronal activity of other regions in the mesolimbic system such as the NASh (Boggs et al., 2017; Norris et al., 2016). The current literature has yet to explore whether the combination of THC+CBD can reverse electrophysiological changes induced by THC.

My overarching hypothesis is that acute exposure to THC in the BLA is sufficient to cause an overexcitation of both ventral hippocampal and prefrontal cortex as reflected by increased neuronal and oscillatory activity states and that co-administration of CBD will prevent this effect.

In this thesis, I have addressed this hypothesis with the following specific experimental aims:

1) **Characterize the effects of intra-BLA THC administration on single unit and LFP activity in the PFC.**

2) **Characterize the effects of intra-BLA THC administration on single unit and LFP activity in the vHPC.**

3) **Determine if CBD co-administration with THC can block the effects of THC alone on the PFC-vHPC circuit.**

2. Methods

2.1   Animals and Housing

Adult male Sprague-Dawley rats were obtained from Charles River (Quebec, Canada) and maintained an average weight of 300-400g on testing day. Rodents were pair housed in a temperature controlled 12-hour light-dark cycle at the animal care facility at the University of Western Ontario. Water and food were given ad libitum and housing conditions consisted of being placed in a plexiglass box with corn bedding and environmentally enriched objects (chewing wood blocks, paper towel, and paper nesting material).

2.2   In Vivo Electrophysiology

All methods for preparation of single unit recordings in the PFC and vHPC follow protocols previously described (Laviolette et al., 2005). All recordings were performed under urethane anesthesia (1.5/kg, i.p, Sigma-Aldrich) and rodents were placed in a stereotaxic frame with a heat pad to maintain a body temperature of 37 degrees Celsius. Scalpel incisions were made on the surface of the head and holes were drilled in the skull overlying on the targeted structures of the study: mPFC (AP: +3mm, L+/- 0.8 to +/ 1mm, DV -2.5 to -4.5 mm from the dural surface), BLA (AP: -3.0mm, L=/- 5.0mm, DV - 7.4mm from dural surface), and vHPC (AP: -5.6, ML: 5, DV:(-6-7.5mm).

Electrodes were pulled from borosilicate glass with an average impedance of 6 and 8 MΩ and were filled with 2% Pontamine Sky Blue solution (Sigma-Aldrich). For recordings, extracellular signals were amplified using a MultiClamp 700B amplifier (Molecular Devices) and recorded using a Digidata 1440A acquisition system (Molecular Devices) with pCLamp 10 software. Two channels were used (PFC channel 1 and vHPC channel 2) and were sampled to obtain both single unit recordings (bandpass 0.5 and 3 kHz) and local field potentials (low pass at 0.3 kHz). Micro electrodes were connected to the channels via a tungsten wire

and electrodes were slowly inserted to both areas of the brain. Once in, electrodes were left to rest for fifteen minutes before searching for isolated recordings. Baseline spontaneous activity was recorded for at least 5 minutes before micro infusions of drugs into the BLA.

Neuroexplorer (Nex Technologies) was used for LFP, crosscorrelation, and coherence analysis. Preprocessing consisted of decimating the signal to 1kHz and low pass filtering (IIR butterworth filter at 100, filter order 3, 0.5 shifts in a 2 s window. Spectrogram analysis settings pre-processed data with a maximum frequency of 100, 2048 frequency values, normalized to raw PSD. Oscillations were segmented based on frequency range: Delta (0.5-4Hz), Theta (4-7Hz), Alpha (7-14Hz), Beta (14-30Hz), Low Gamma (30-58Hz), and High Gamma (62-80Hz).

Both crosscorrelation and coherence analysis are statistical tests used to assess the degree of connectivity between multiple regions. In this case, both tests were used to determine whether LFP signals in the vHPC co-occurred with LFP signals in the PFC across recording sessions. For the auto-correlation, the vHPC signal was correlated in reference to the PFC signal and averaged across a five-minute baseline to produce a single data point. This was repeated to obtain a data point for a five-minute post-drug infusion period. Both values are represented as a correlation value, with the highest possible crosscorrelation 1.0 representing both LFP signals completely overlapping at the same time point. Using a repeated measures ANOVA, both correlation values are compared between groups to determine changes in PFC-vHPC LFP signal overlap. The coherence analysis takes a similar approach but differs in two respects. First, it compares vHPC to PFC signal overlap as a function of frequency (Delta, Theta, Alpha, Beta, & Gamma). Thus, data points obtained are restricted to each oscillation range. Second, more than one data point is obtained with respect to each oscillation range because this analysis is not restricted to using data at the same time-point. Instead it looks at overlap across the entire five-minute baseline compared to the entire five-minute post-infusion period. Therefore, crosscorrelation compares overlap

between PFC-vHPC at the same time point whereas coherence is only interested in total signal overlap within each oscillation range.

PFC pyramidal cells were identified based established criteria of firing frequency (<10 Hz), waveform shape, duration of action potential (> 2.5 ms), and burst firing. Bursting patterns were identified when a cell fires 3 consecutive spikes with an inter-spike interval of <45ms. Percentage of burst spike count was determined by dividing the total bursts by the total amount of spikes in the same five-minute period. Selection for vHPC principal neurons in the current dataset were for cells that had baseline firing rates of 0.5-5hz (Goonawardena, Riedel, & Hampson, 2011). The total sample of animals used in each group are: Vehicle N = 8, THC N = 9, CBD N = 8, THC+CBD 100ng:100ng N = 10, THC+CBD 100ng:500ng N = 4, CBD 500ng N = 3.

2.3 Drug Preparation

Both THC (Cayman Chemical) and CBD (Tocris) were used in the study. THC stored in ethanol was dissolved in cremophor and saline (1:1:18). Ethanol was evaporated from the working solution through evaporation nitrogen gas. CBD in powder form was dissolved in cremophor and then into saline. Both THC and CBD mixtures independently and combined consisted of a final concentration of 5% cremophor in the working solution. Drugs were micro infused into the BLA with a microinjector (Hamilton Syringe 10 ul) at a volume of 0.5 ul slowly over one minute.

2.4 Histology

Upon completing electrophysiological recordings, rodent brains were extracted and placed in a 10% formalin solution for at least 24 hours. Then brains were moved to a formalin-sucrose solution for at least 5 days. Brains were sliced at 60 µm and mounted on slides. Slides were then stained with neutral red (Sigma Aldrich) and placements for BLA, vHPC, and PFC were verified using light

microscopy. Any rodents showing placements outside the boundaries defined by Paxinos and Watson (2005) were excluded from data analysis.

2.5 Statistical Analyses

Electrophysiological data were analyzed with either a one or two-way ANOVA where appropriate. Post hoc analyses were performed using Fisher's LSD tests.

3. Results

3.1 Histological Analyses

Analysis of histology revealed the Hamilton syringe placements to be localized in the anatomical boundaries of the BLA according to the Rat Brain Atlas (Paxinos & Watson, 1996). Pontamine blue dot and electrode tracks were used to confirm placements of the last recorded cell in the anatomical boundaries of both the prelimbic regions of the PFC and vHPC. Figure 1A presents a microphotograph displaying a representative placement within the PFC. Figure 1B displays a schematic illustration showing representative PFC placements along the rostral-caudal axis. Figure 1C presents a microphotograph of injector placements in the BLA. Figure 1D displays a schematic illustration of rostral-caudal placements within the BLA. Figure 1E is a representative microphotograph of a placement within the vHPC. Figure 1F displays a schematic illustration of rostral-caudal placements within the vHPC.

1A

1B

Bregma 3.72 mm

Bregma 3.24 mm
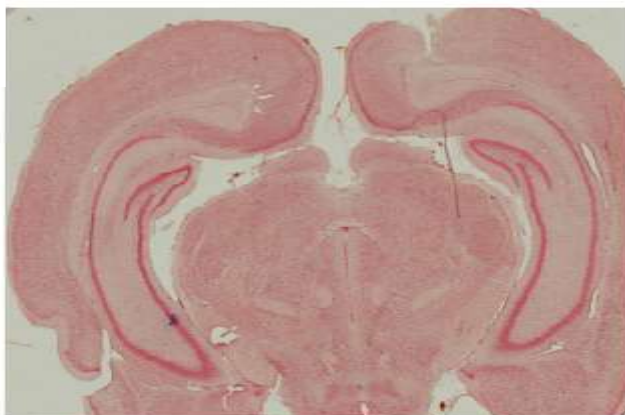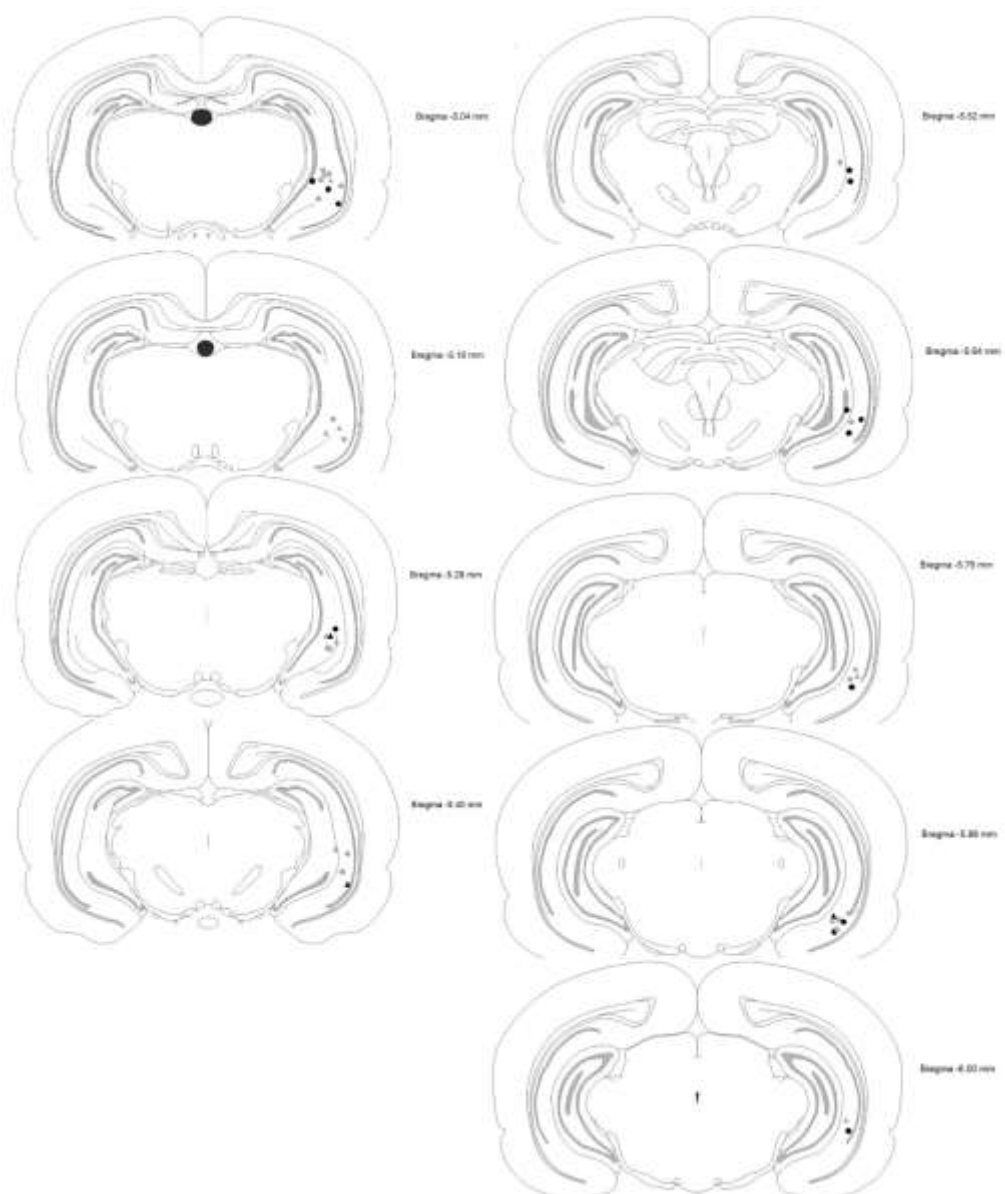
Bregma 3.00 mm

Bregma 2.76 mm
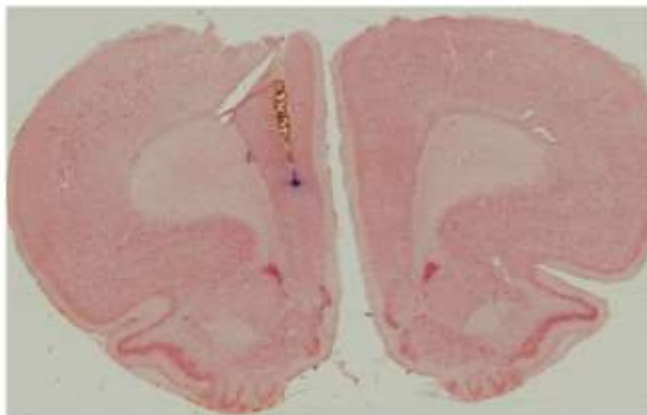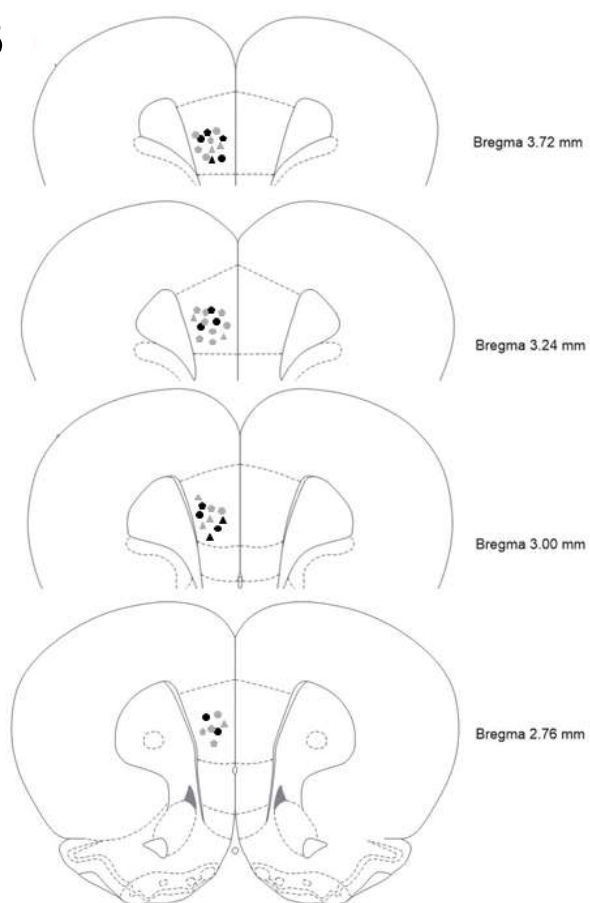
1C
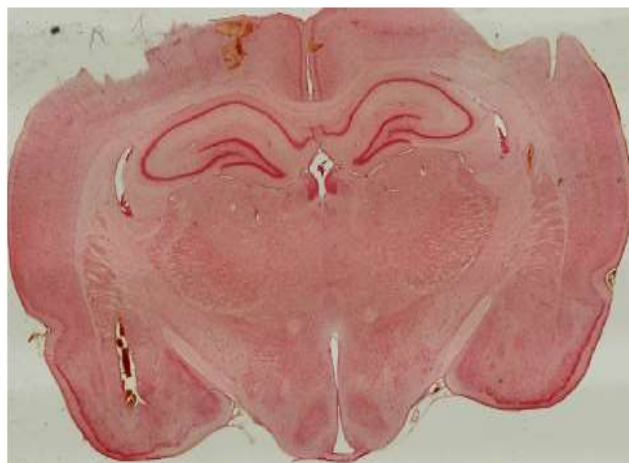
1D
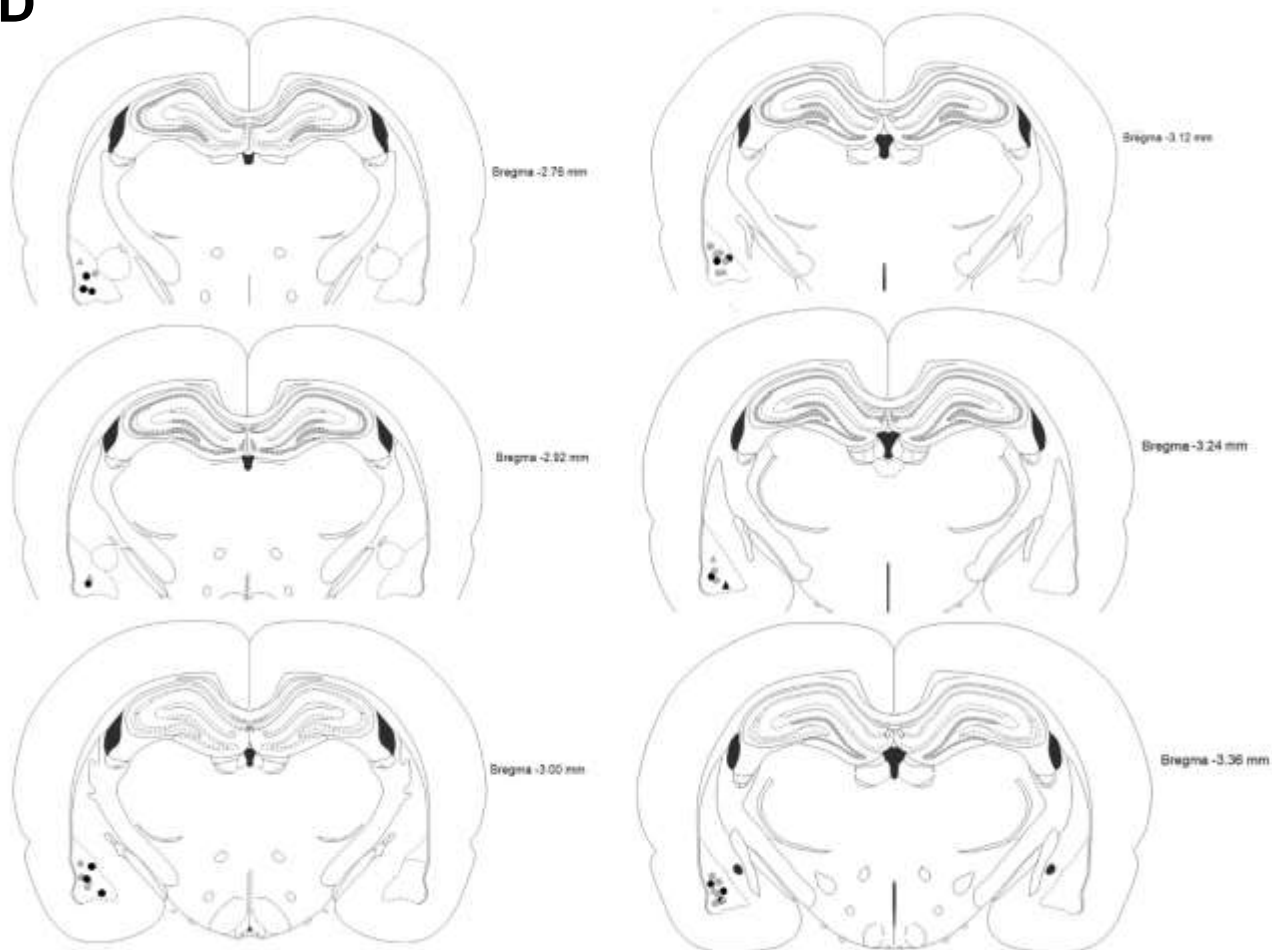
1E

1F

Figure 1. Histological analysis of BLA injection and PFC & vHPC recording sites. A) Microphotograph of a presentative PFC recording site. B) Schematic representation of PFC recording locations. C) Microphotograph of representative BLA injection site. D) Schematic representation of BLA injector locations. E) Microphotograph of vHPC recording sites. F) Schematic representation of vHPC recording locations. Symbols: ●Vehicle,●THC 100ng,▲ CBD 100ng, ▲ CBD 500ng, ⬠ THC+CBD 100ng:100ng, ⬟ THC+CBD 100ng:500ng.

3.2 The effects of BLA cannabinoid administration on PFC Single Unit activity

A one-way ANOVA comparing the mean firing rates of single unit activity revealed THC significantly increased pyramidal cell firing in the PFC. Overall, there was a significant difference between groups ($F(5,95) = 2.453$, $p = 0.039$). Following this, a Fisher's LSD post-hoc test comparing groups means revealed that infusion of THC into the BLA significantly increased pyramidal cell firing compared to vehicle ($p = .008$), CBD 100 ng ($p = .003$), THC+CBD 100ng:100ng ($p = .039$), THC+CBD 100ng:500ng ($p = .011$), and CBD 500 ng ($p = .013$) infusions. When CBD was combined with THC at both ratios, this blocked the THC mediated increase. All other groups that contained CBD showed no increase compared to vehicle. In addition, a one-way ANOVA comparison on PFC single unit bursting rate found a significant difference, $F(5,95) = 3.458$, $p = .006$, where THC infusions increased bursting firing compared to all other groups (i.e., Vehicle $p = .001$, CBD 100 ng $p < .001$, THC+CBD 100ng:100ng $p < .003$, THC+CBD 100ng:500ng $p = .004$, and CBD 500ng $p = .003$). Once again, all other groups were no different compared to vehicle.
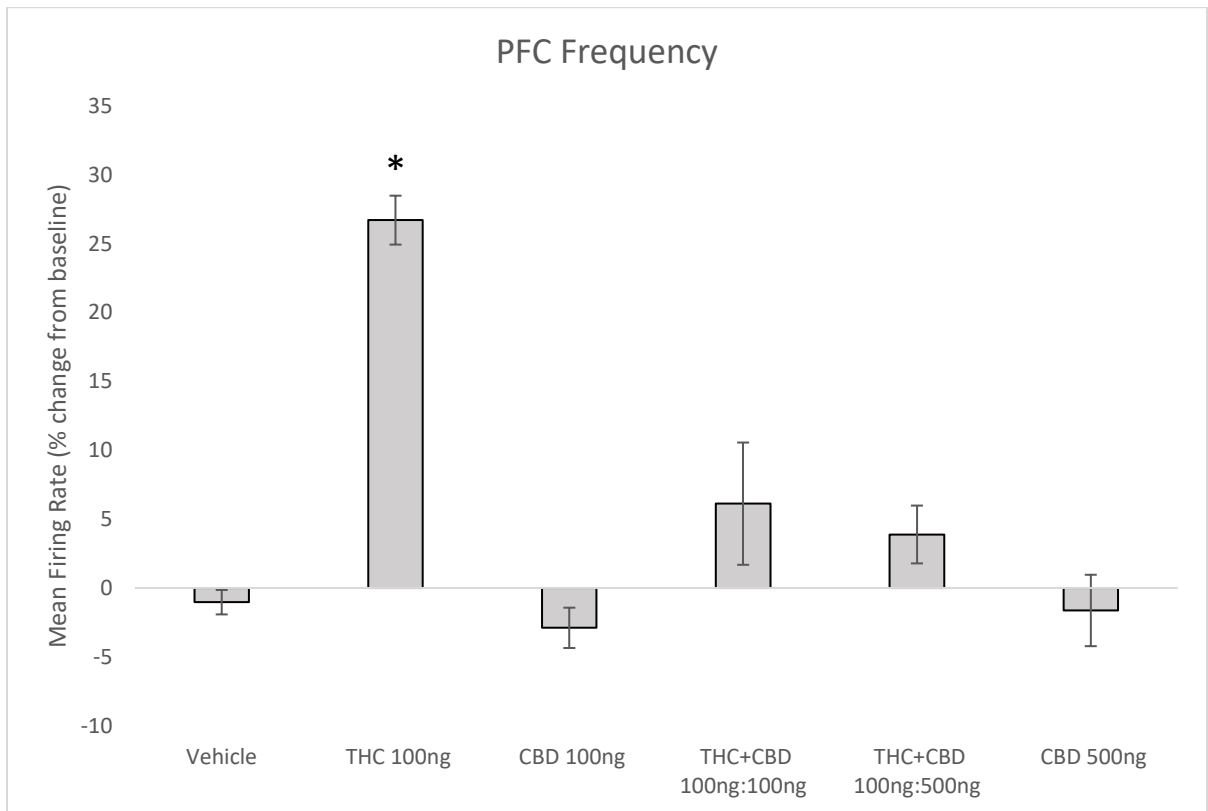
2A

2B

2C
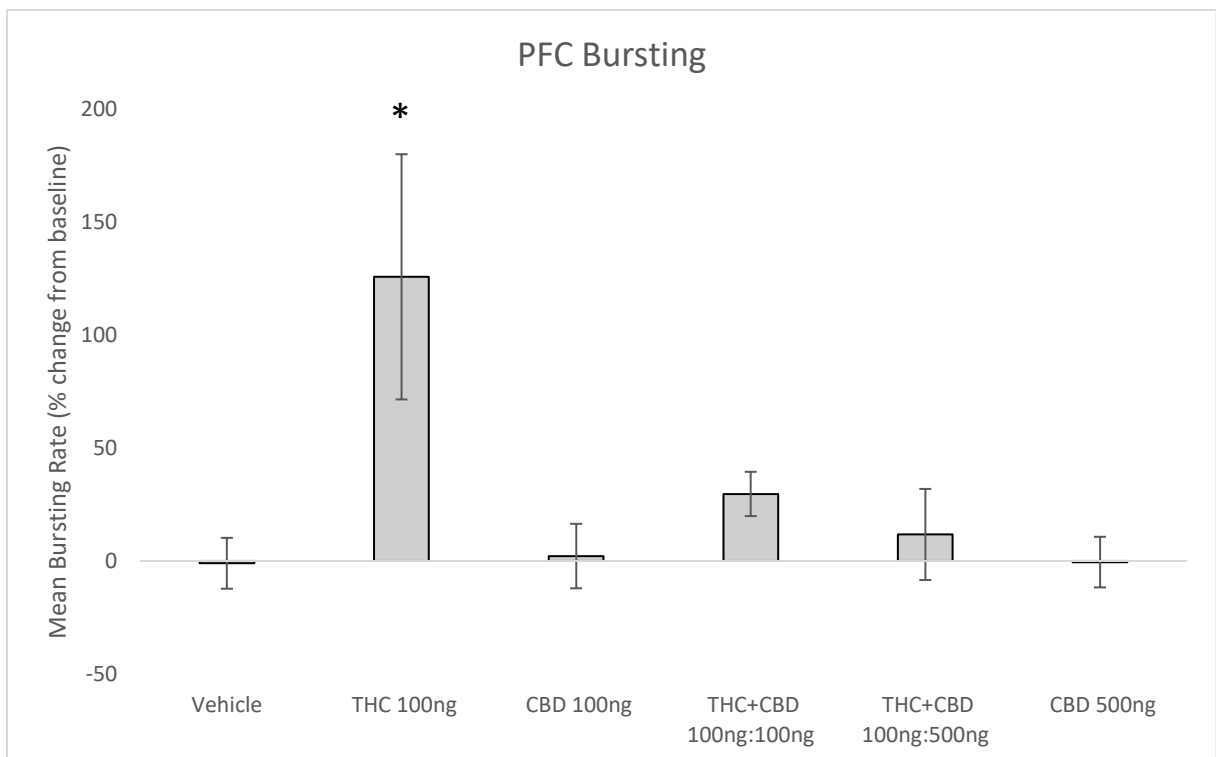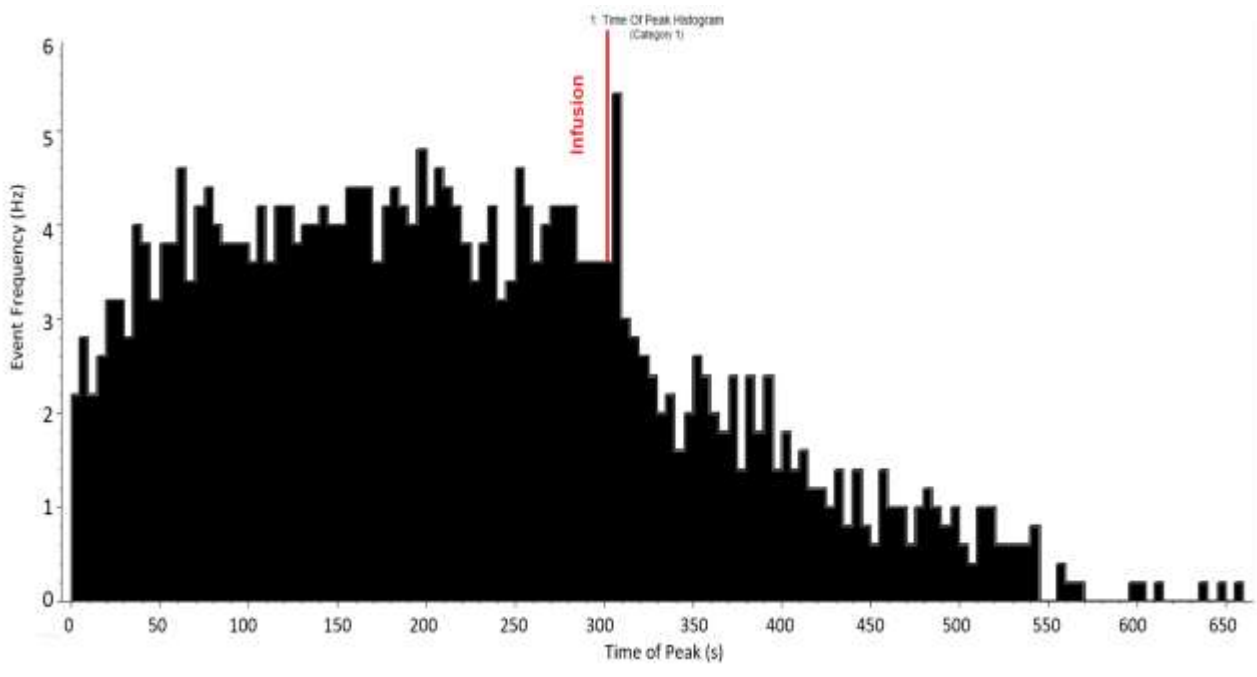
PFC Frequency

2D

PFC Bursting
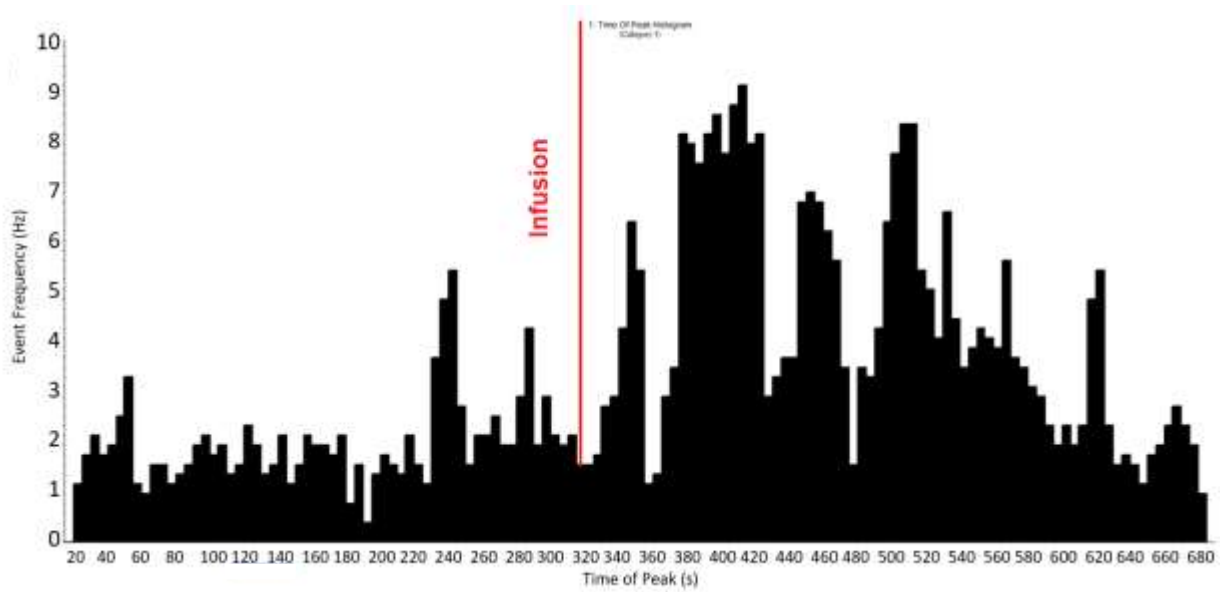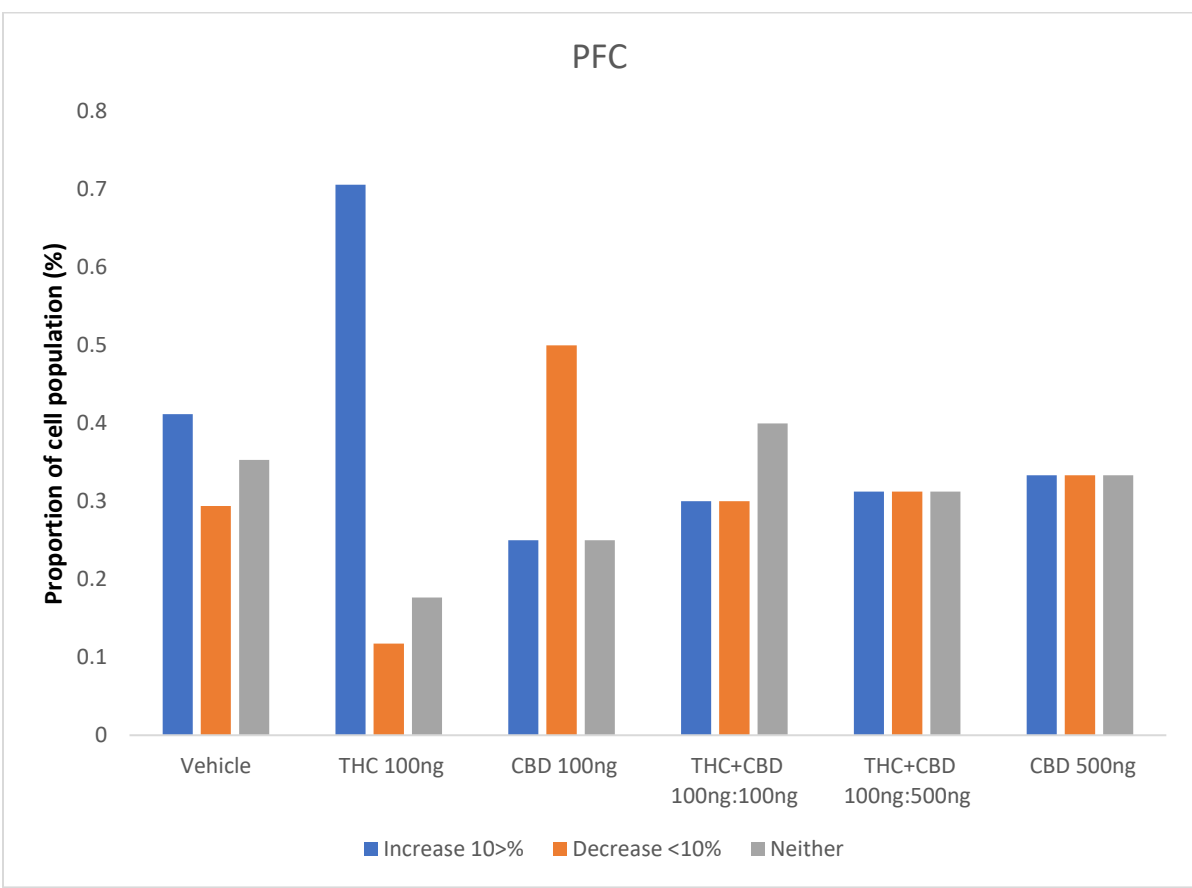
Figure 2. In vivo single unit recording activity in the PFC. A) Representative rastergram showing firing frequency of PFC pyramidal cell neurons during intra-BLA THC microinfusion. B) Percentage of cells within each group classified as cells that increase more than 10%, decrease more than 10%, and cells that do not change beyond +/-10%. C) Percentage difference in mean firing rates. * indicates significantly different from all groups. D) Percentage change in mean bursting rates. * indicates significantly different from all groups. Sample size: Vehicle (N=17), THC 100ng (N=17), CBD 100ng (N=20), THC+CBD 100ng:100ng (N=20), THC+CBD 100ng:500ng (N=15), CBD 500ng (N=12).

3.3 The effects of BLA cannabinoid administration on vHPC Single Unit activity

Overall, all groups containing CBD decreased ventral hippocampus principal cell firing. A one-way ANOVA comparing mean firing rates of ventral hippocampal single unit activity found a significant difference between groups $(F_{(5,103)} = 4.575, p = 0.001)$. A follow up Fisher's LSD post-hoc test compared all groups revealing Vehicle and THC infusions and they were not significantly different from one another, $p = .699$. In contrast, Vehicle infusions were significantly different from all groups that contained CBD: CBD 100ng, $p = .015$, THC+CBD 100ng:100ng, $p = .007$, the THC+CBD 100ng:500ng, $p < .001$, and CBD 500ng, $p = .002$. BLA infusions of THC were also significantly different from all groups that contained CBD: CBD 100ng, $p = .37$, THC+CBD 100ng:100ng, $p = .007$, THC+CBD 100ng:500ng combination, $p = .001$, CBD 500ng, $p = .007$. All groups containing CBD were not significantly different from one another.

3A

3B
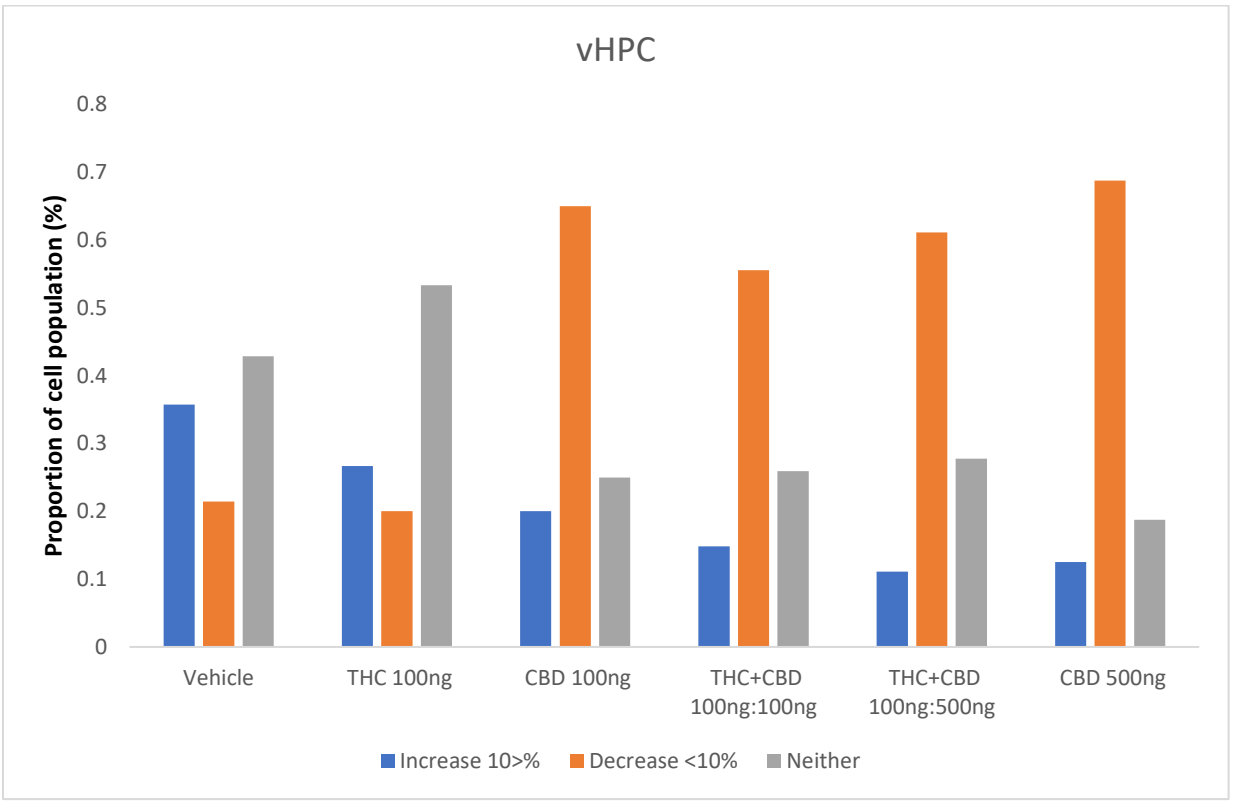
3C
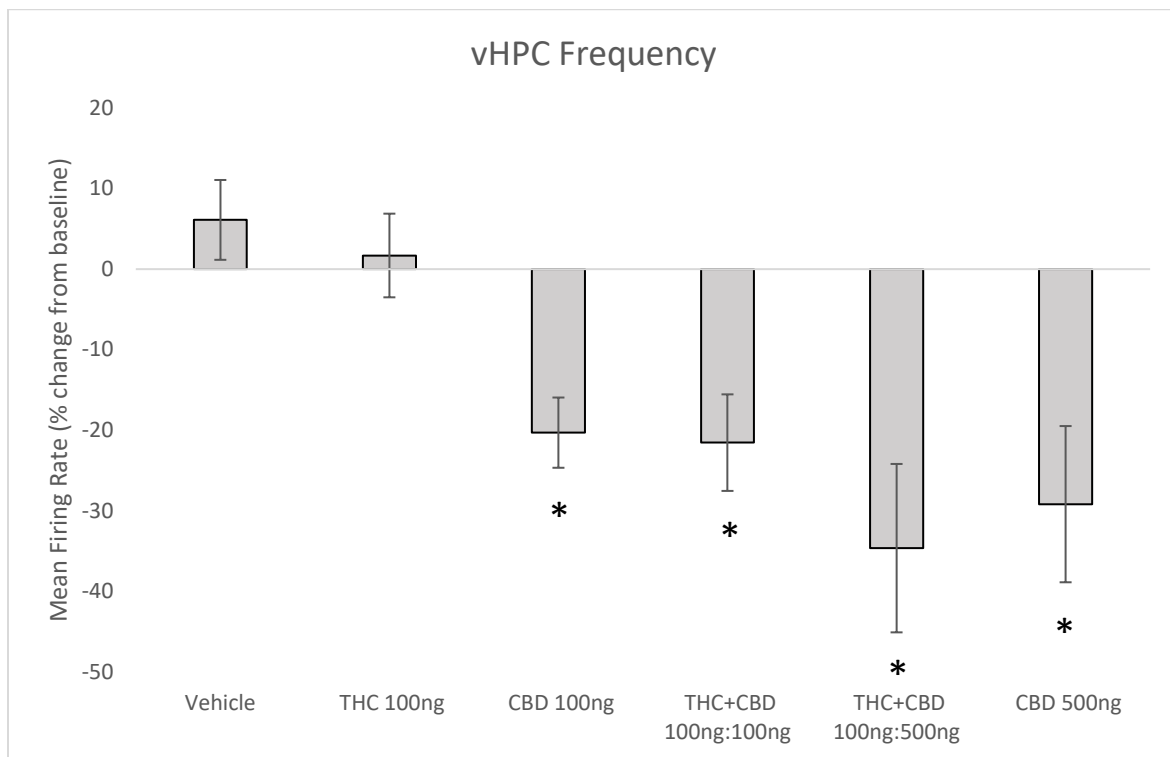
Figure 3. In vivo single unit recording activity in the vHPC. A) Representative histogram showing firing frequency of vHPC principal cell neurons during intra-BLA THC+CBD microinfusion. B) Percentage of cells within each group classified as cells that increase more than 10%, decrease more than 10%, and those that have neither directional change within 10%. C) Percentage difference in mean firing rates. * indicates significantly different from vehicle and THC. Sample size: Vehicle (N=14), THC 100ng (N=15), CBD 100ng (N=20), THC+CBD 100ng:100ng (N=27), THC+CBD 100ng:500ng (N=18), CBD 500ng (N=15).

## 3.4 The effects of BLA cannabinoid administration on PFC LFP activity

Given significant differences at the single unit level, we then analysed data at the local field potential level. This analysis involved comparing all six groups across six oscillation ranges (Delta, Theta, Alpha, Beta, Low Gamma, and High Gamma). Overall, THC caused an increase in power for low and high Gamma. For low Gamma (i.e., 62HZ-80HZ), a one-way ANOVA found a significant difference, $F(5, 85) = 2.442$, $p = .041$ across groups where both THC 100ng and THC+CBD 100ng:100ng were significantly different from Vehicle (p= .037; p = .038) and CBD 100ng (p= .011; p = .010). For High Gamma, a one-way ANOVA found a significant difference, $F(5, 85) = 3.524$, $p =.006$ across groups where only THC significantly

increased power post-infusion compared to all other groups (i.e., Vehicle p = .002; CBD 100ng p= .001, THC+CBD 100ng:100ng p = .001, THC+CBD 100ng:500ng p= .002, CBD 500ng p= .003). One-way ANOVA analysis revealed no significant differences between groups across Delta ($F_{(5, 85)}$ = 1.697, p = .144), Theta ($F_{(5, 85)}$ = .652, p = .661), Alpha ($F_{(5, 85)}$ = .855, p = .515), and Beta ($F_{(5, 85)}$ = 1.201, p = .316).

4A
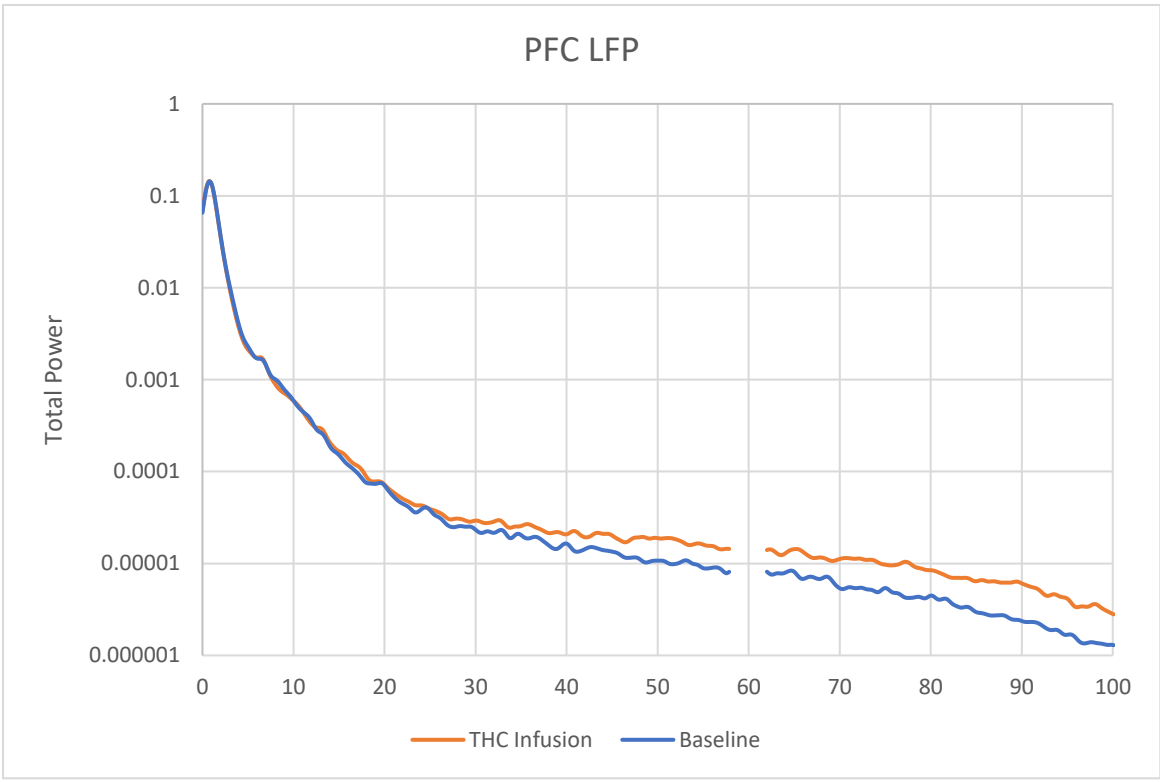
4B
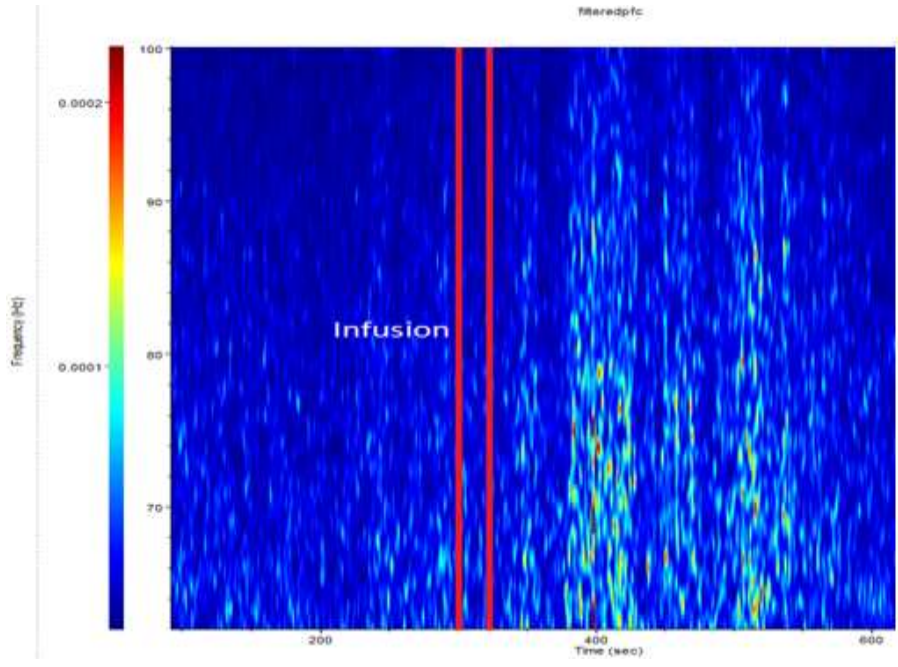
4C
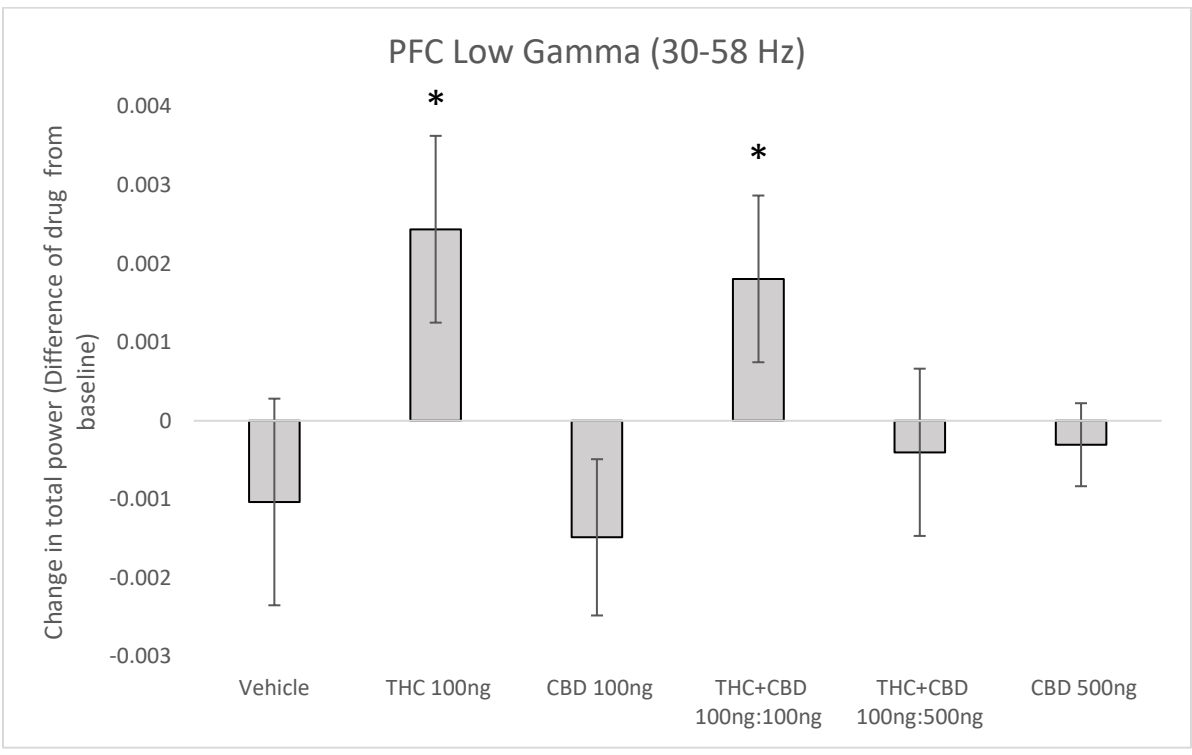
4D

Figure 4. Local Field Potential recordings in the PFC. A) Diagram of a representative cell's LFP total power across 0-100 HZ oscillation range. Orange is total power across five minutes after infusion of THC. B) Spectrogram depicting change in power in the high gamma oscillation (62-80 Hz). C) Change in LFP averaged across groups in low gamma range. * indicates significantly different from vehicle and CBD 100ng. D) Change in LFP average across groups in high gamma range. * indicates significantly different compared to all groups. Sample Size: Vehicle (N=16), THC 100ng (N=16), CBD 100ng (N=20), THC+CBD 100ng:100ng (N=20), THC+CBD 100ng:500ng (N=15), CBD 500ng (N=12).

### 3.5 The effects of BLA cannabinoid administration on vHPC LFP activity

This next section compared LFP difference in the vHPC. A one-way ANOVA analysis revealed no significant difference between groups across Delta ($F_{(5, 88)}$ = .486, p = .786)), Theta ($F_{(5, 88)}$ = 1.946, p = .095)), Alpha ($F_{(5, 88)}$ = 1.167, p = .332), Beta ($F_{(5, 88)}$ = .424, p = .831)), Low Gamma ($F_{(5, 88)}$ = .929, p = .466)), and High Gamma ($F_{(5, 88)}$ = .174, p = .972)) oscillations. Therefore, THC had no single unit effect and no change in LFP in the ventral hippocampus. CBD, in comparison to single unit recordings, did not alter LFPs in the ventral hippocampus.

5A

vHPC Low Gamma (30-58 Hz)

5B

vHPC high Gamma (62 - 80Hz)
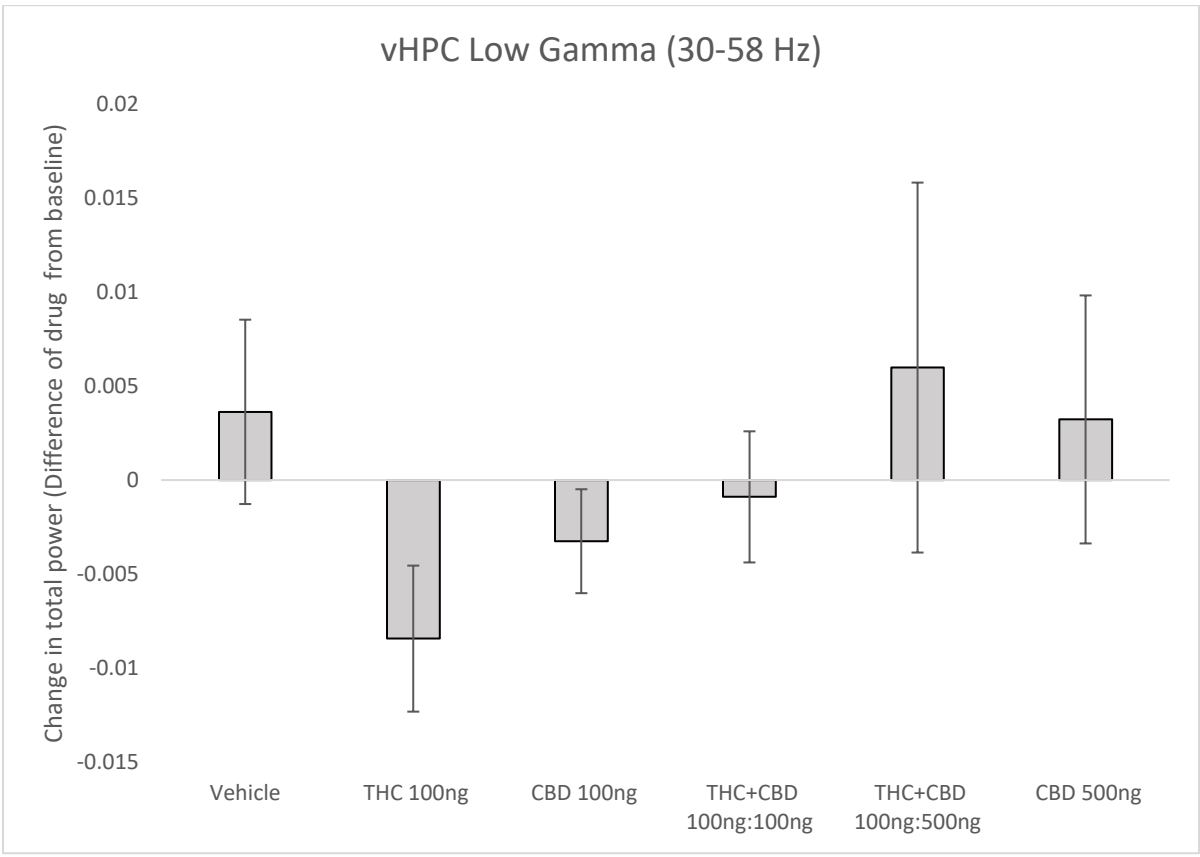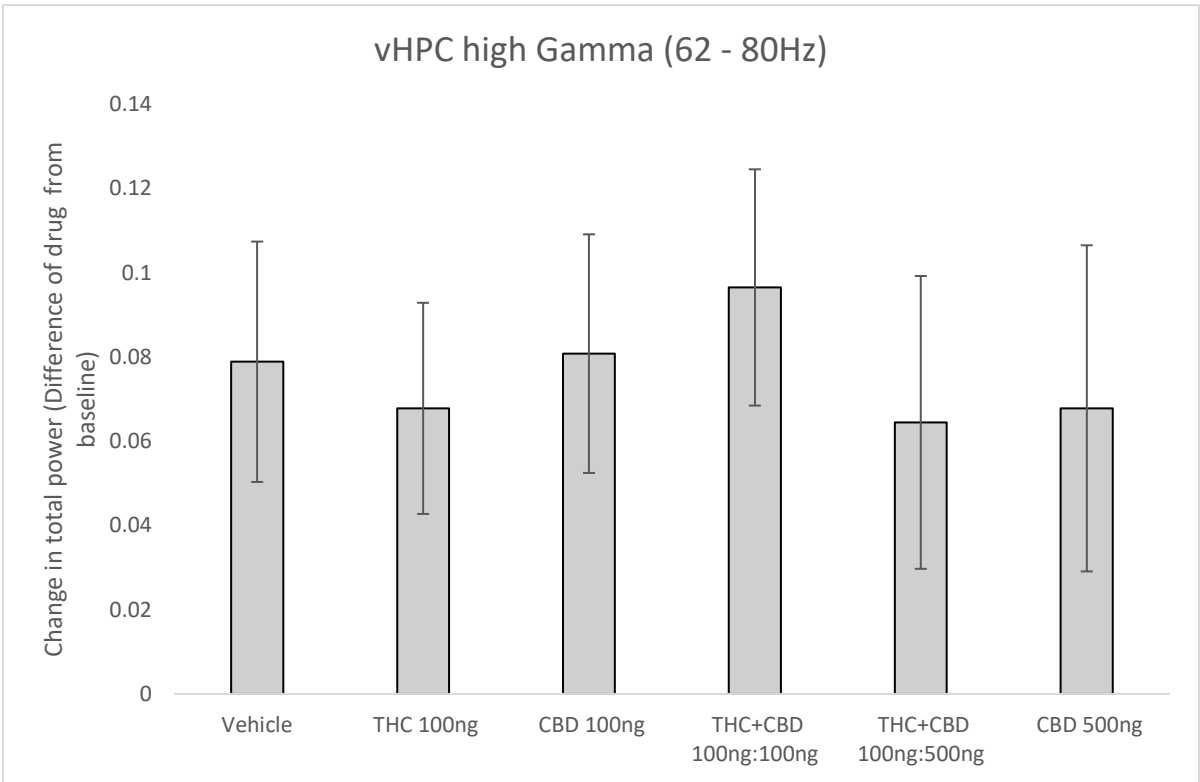
Figure 5. Local Field Potential recordings in the vHPC. A) Change in LFP averaged across groups in low gamma range. B) Change in LFP averaged across groups in high gamma range. Sample size: Vehicle (N=16), THC 100ng (N=16), CBD 100ng (N=20), THC+CBD 100ng:100ng (N=20), THC+CBD 100ng:500ng (N=15), CBD 500ng (N=12).

3.6 Crosscorrelation Analysis between PFC and vHPC

After analyzing the local field potential within a region, we decided to compare changes between regions. This analysis looks at whether THC or CBD alters crosscorrelations or coherence levels between the PFC and vHPC. A two-way repeated measures ANOVA revealed no overall main effect between pre-infusion and post infusion times, $F(1, 129) = .550$, $p = .460$. In addition, no within-subjects interaction between groups and infusion time were revealed in the data set, $F(5,129) = .099$, $p = .992$.
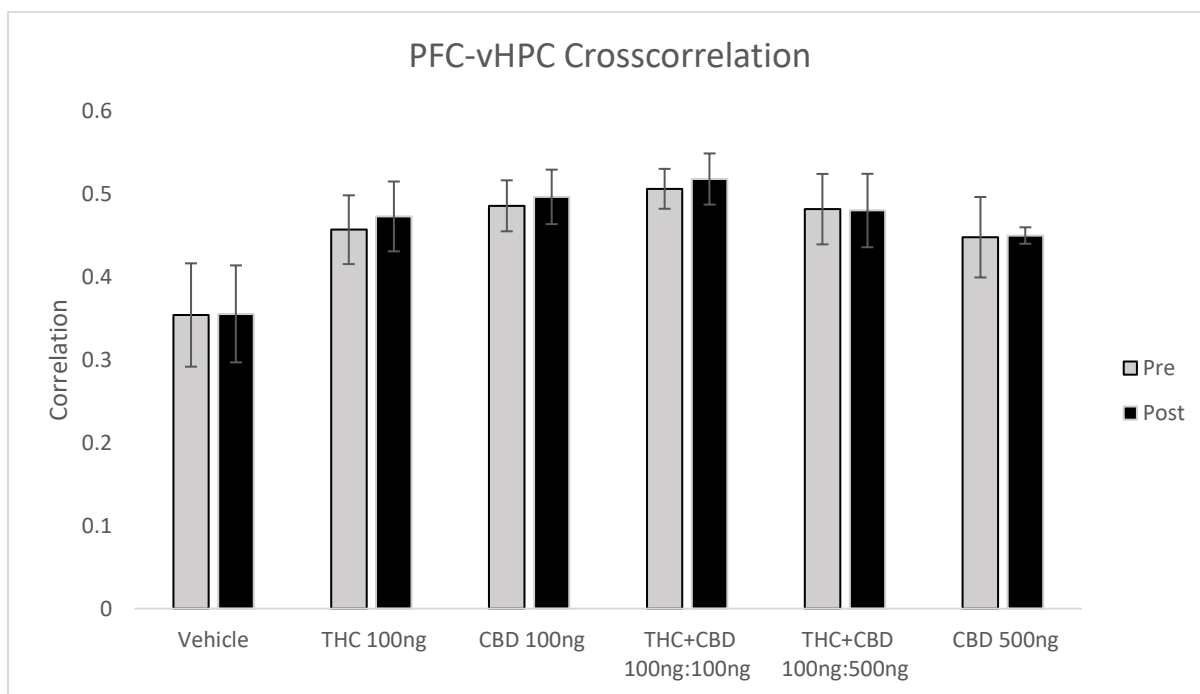
Figure 6. PFC-vHPC Crosscorrelation. A) Correlogram of Crosscorrelation scores between PFC and vHPC connectivity. Sample size: Vehicle (N=20), THC 100ng (N=21), CBD 100ng (N=33), THC+CBD 100ng:100ng (N=23), THC+CBD 100ng:500ng (N=21), CBD 500ng (N=17).

3.7 Coherence Analysis between PFC and vHPC

For our final analysis, we divided the data within each oscillation range and compared between PFC-vHPC activity using a coherence analysis. Overall, there was no significant difference in a two-way ANOVA across all oscillation ranges. Time is listed as the variable between pre- and post-infusion. Overall, the analysis revealed no significant main effect for Delta time ($F_{(1,127)}$ = 1.649, p = .201)) or interaction TimexGroup ($F_{(5,127)}$ = .760, p = .580)); Theta Time ($F_{(1,127)}$ = .232, p = .631)) or interaction TimexGroup ($F_{(5,127)}$ = 1.030, p = .403)); Alpha Time ($F_{(1,127)}$ = .591, p = .002)) or interaction Alpha TimexGroup ($F_{(5,127)}$ = .061, p = .079)); Beta Time ($F_{(1,127)}$ = .010, p = .921)) or interaction TimexGroup ($F_{(5,127)}$ = .492, p = .781)); Low Gamma Time ($F_{(1,127)}$ = .025, p = .875)) or interaction TimexGroup ($F_{(5,127)}$ = .272, p = .927)); High Gamma Time ($F_{(1,127)}$ = .097, p = .756)) or interaction TimexGroup ($F_{(5,127)}$ = .299, p = .913)).

7A

PFC-vHPC Coherence Delta (0.5-4 Hz)

7B

PFC-vHPC Coherence Theta (4-7 Hz)

7C

PFC-vHPC Coherence Alpha (7-14 Hz)

7D

PFC-vHPC Coherence Beta (14-30 Hz)

7E

PFC-vHPC Coherence Low Gamma (30-58 Hz)
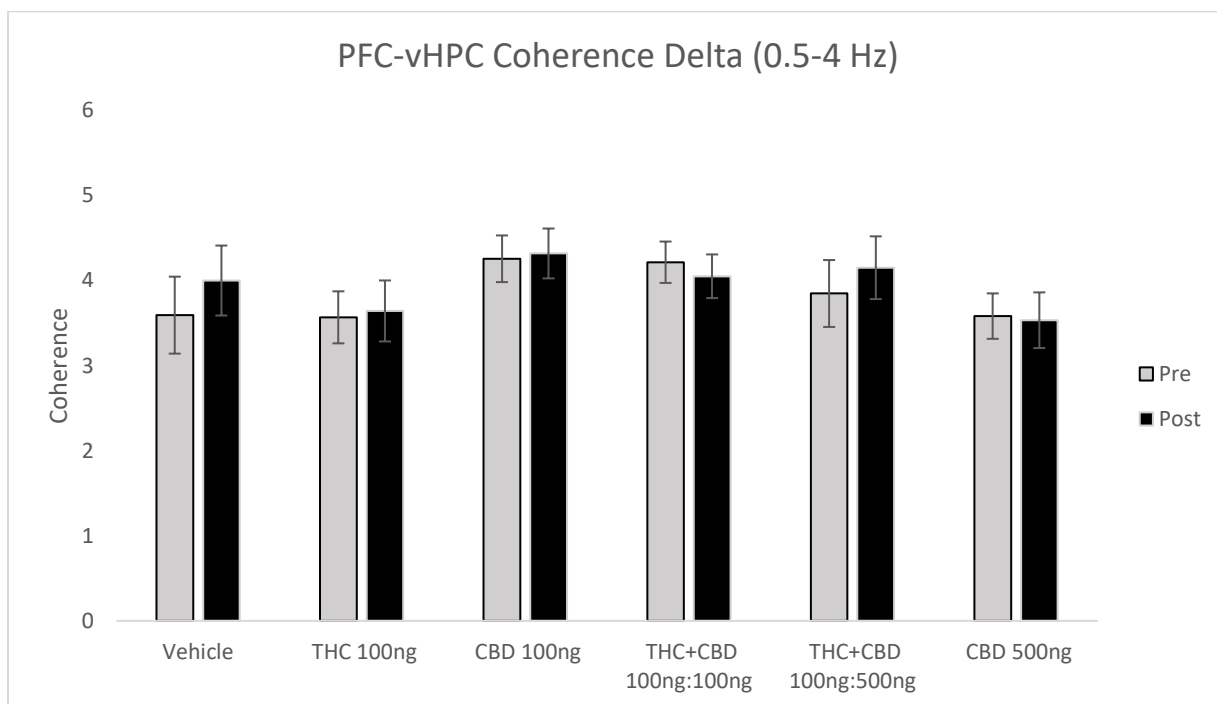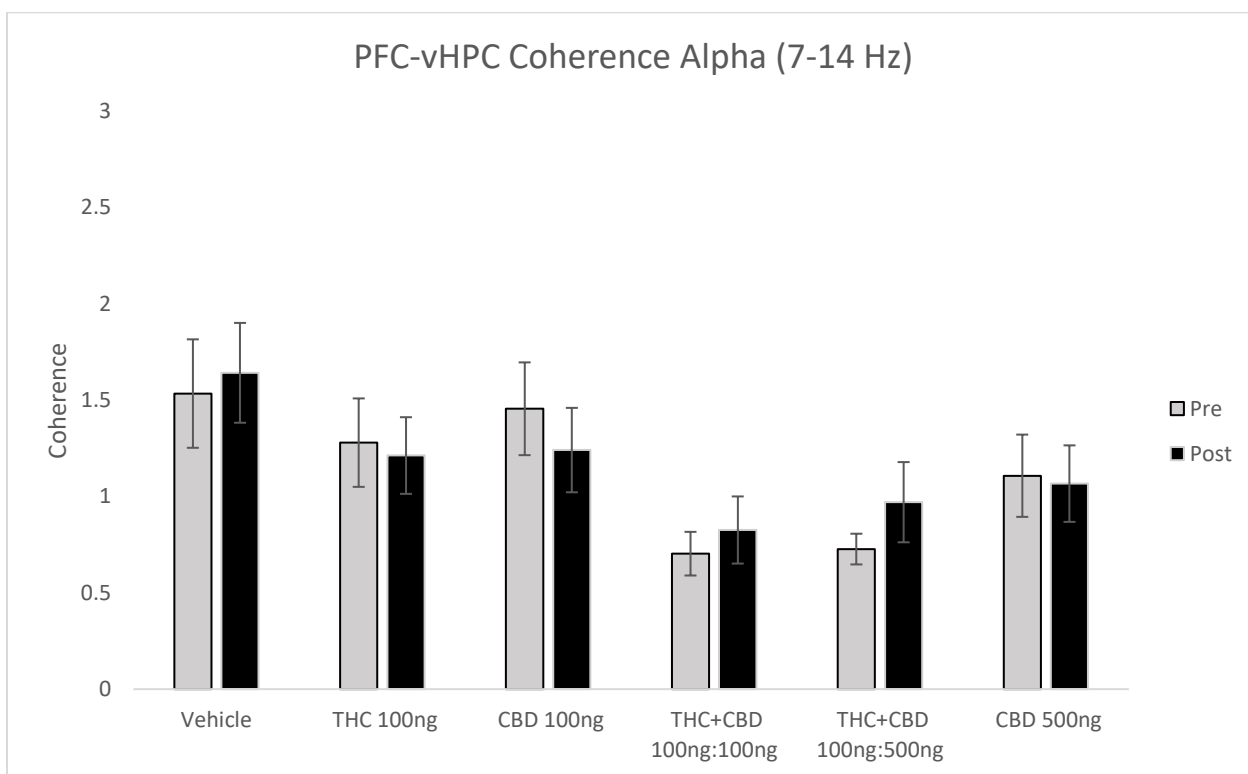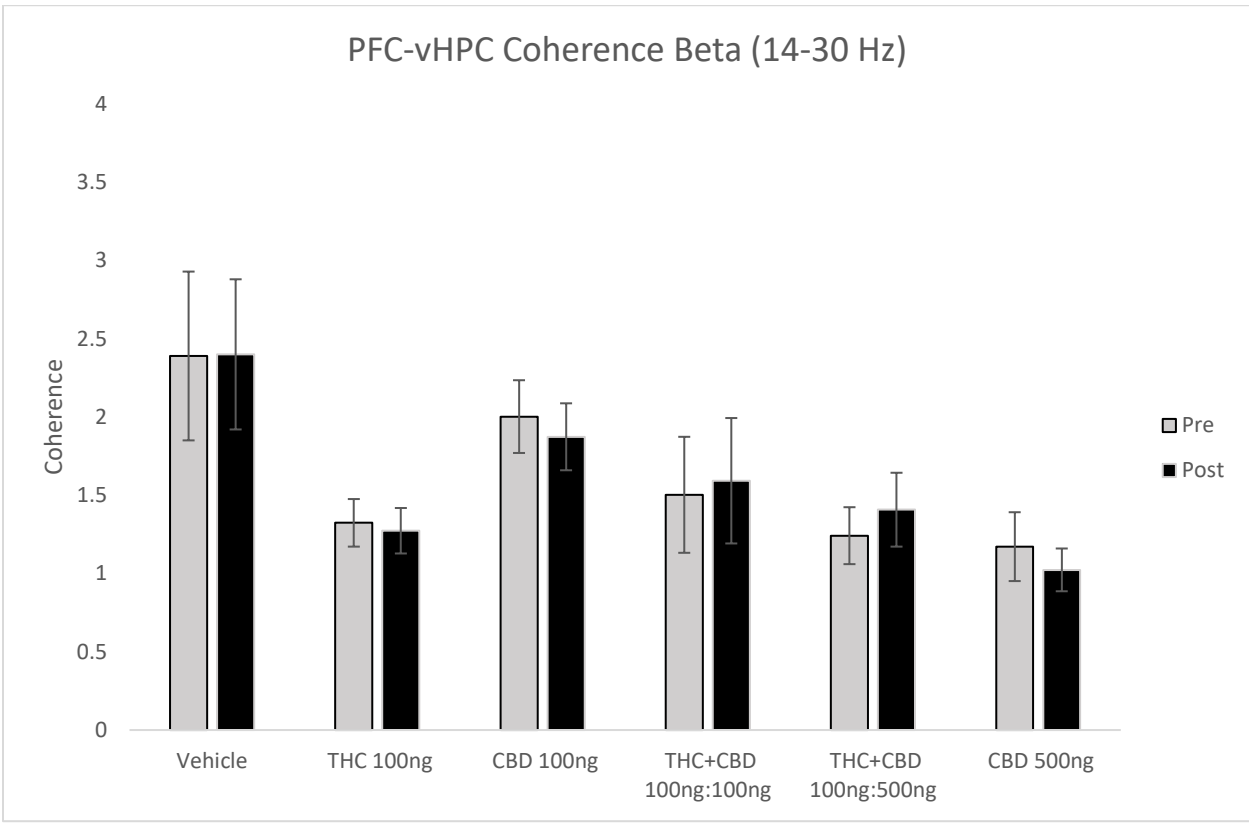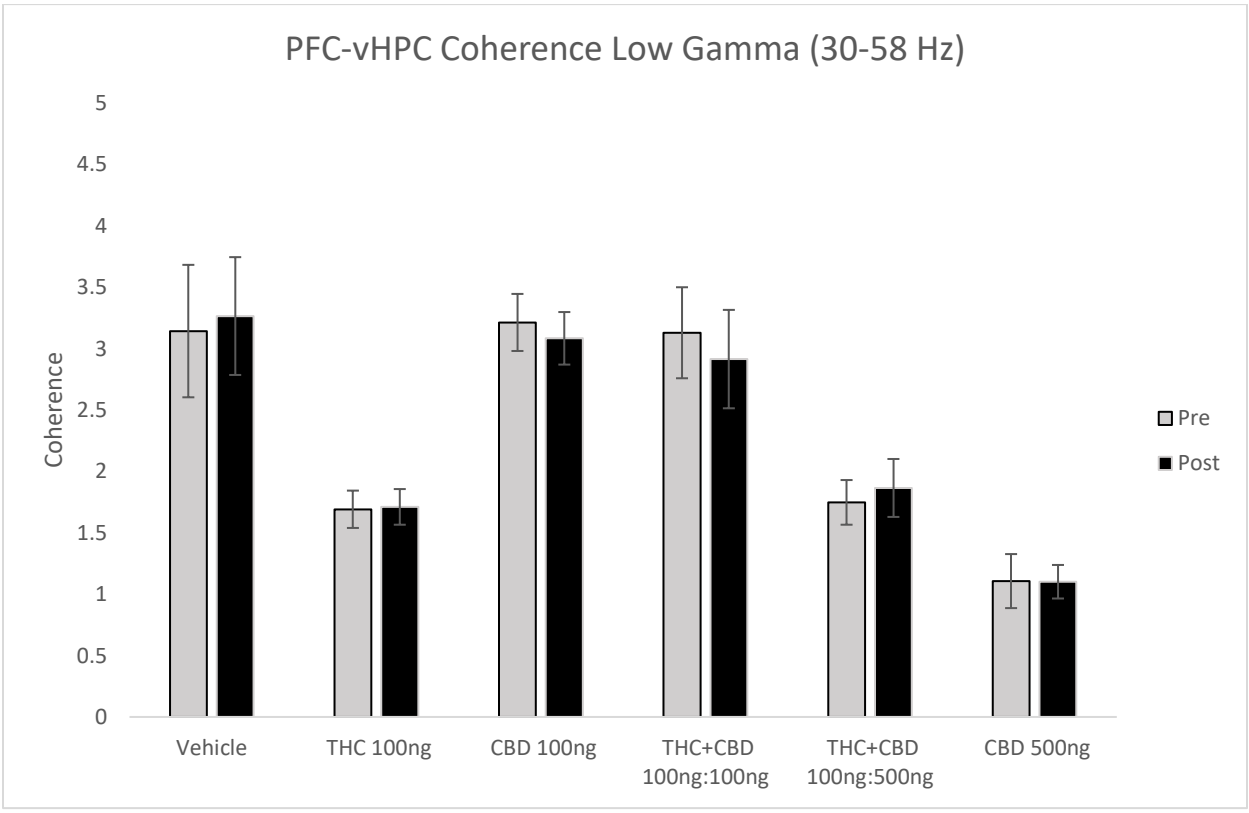
7F
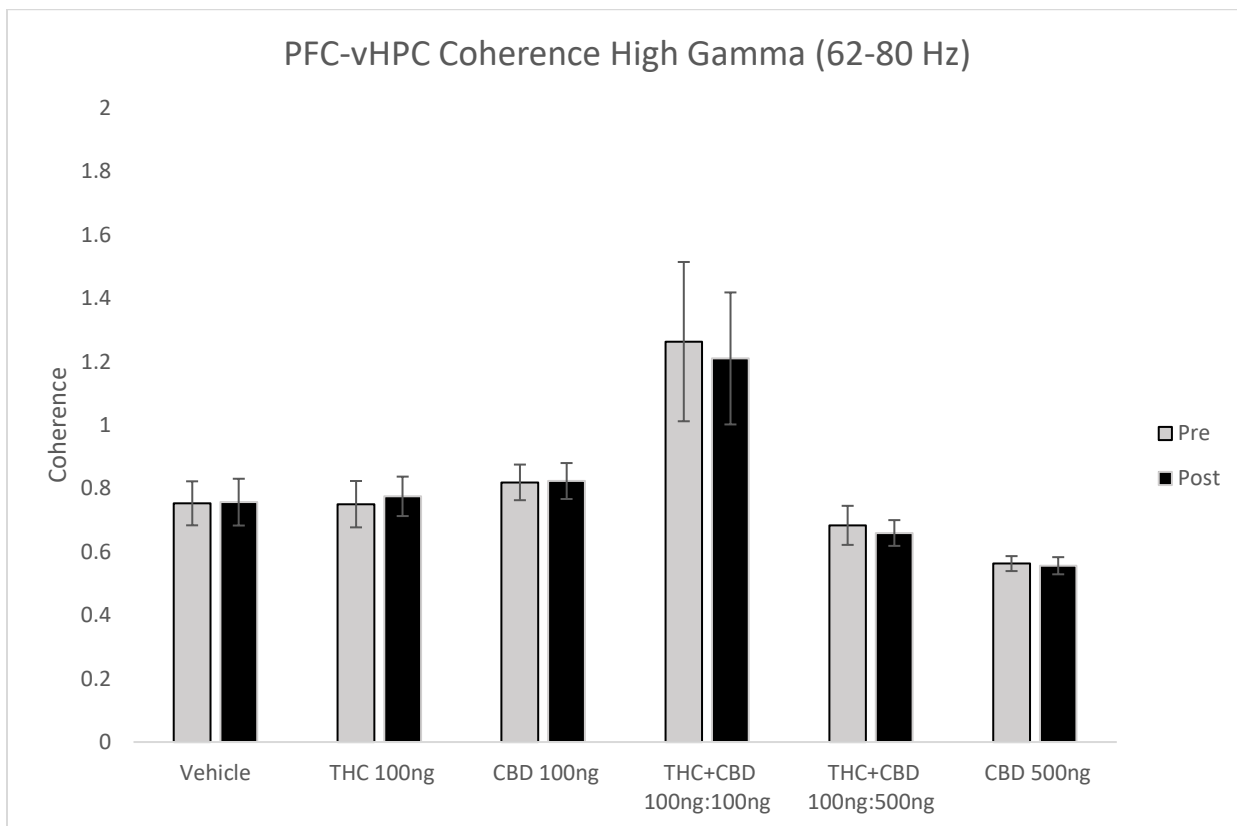
Figure 7. Coherence analysis between PFC-vHPC activity A) Delta coherence. B) Theta coherence. C) Alpha coherence. D) Beta coherence. E) Low gamma coherence. F) High gamma coherence. Sample size: Vehicle (N=20), THC 100ng (N=21), CBD 100ng (N=33), THC+CBD 100ng:100ng (N=21), THC+CBD 100ng:500ng (N=21), CBD 500ng (N=17).

4. Discussion

4.1 PFC single unit activity is altered by THC but prevented by co-administration of CBD

Previous findings from this research group have already demonstrated that a full agonist for the CB1R in the BLA can alter the single unit activity of neurons in the PFC (Tan et al. 2010;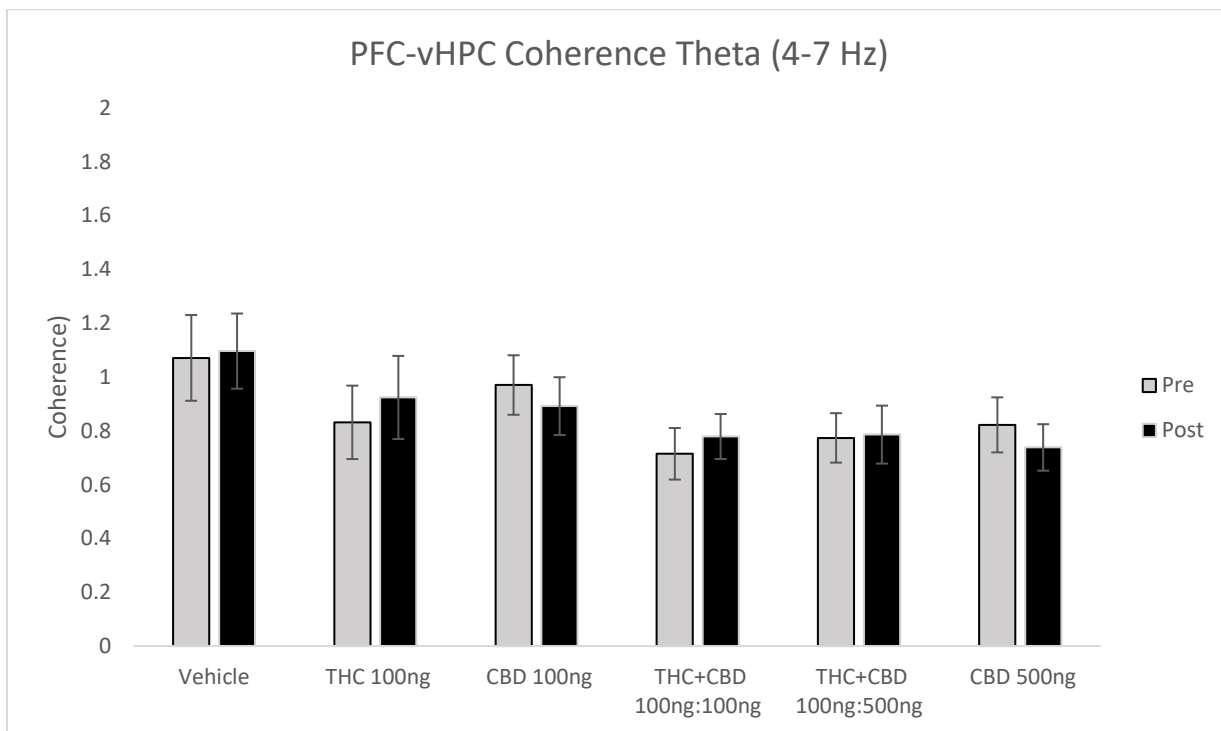 Tan et al., 2011). Given these findings, we performed a series of in vivo single unit recordings in both the PFC and vHPC and investigated the effects of the partial CB1R agonist THC in the BLA. Our analyses revealed that micro-infusions of THC caused an increase in pyramidal cell firing and bursting rates in the PFC.

The majority of CB1R expression in the BLA is located on interneuron populations (Marsicano & Lutz, 1999). Furthermore, application of endocannabinoid agonists on BLA interneurons reduces spontaneous evoked activity, which can potentially reduce overall inhibition on BLA pyramidal cell firing (Howlett et al., 1986; Sullivan 1999; Hoffman & Lupica, 2000; Wilson & Nicoll, 2001; Kano et al., 2009). Given these findings, along with the present results, our proposed model suggests that THC increases activation of BLA pyramidal cell projections to increase spontaneous single unit activity in the PFC. Our lab has already shown that activation of BLA CB1R can potentiate non-salient stimuli during the acquisition of an emotional memory and create a conditioned place aversion of a morphine reward memory (Tan et al., 2010; Tan et al., 2011). As THC is a partial agonist of the CB1R, it is possible that micro-infusions into the BLA could also potentiate non-salient stimuli during emotional memory acquisition. Although we have not tested this in the BLA, THC micro-infusions into the NASh can potentiate the acquisition of a fear memory at sub-threshold levels of foot shock (Fitoussi et al., 2018). Given the well-known psychotropic effects associated with THC, it is possible that increasing activity of the BLA to alter PFC activity leads

to misattributing the salience of normal objects in the environment, a behaviour that is commonly found in psychosis and schizophrenia (Laviolette & Grace, 2006).

Next, we investigated whether the combination of THC with CBD at a ratio of 1:1 and 1:5 would block the effects of THC. In both cases, micro-infusions of the combination prevented PFC pyramidal cell increases as seen with THC. In addition, CBD at 100 and 500ng did not alter PFC activity compared to vehicle. While there yet exists a clear mechanism to explain how CBD can reverse the effects of THC, a number of studies have demonstrated the potential therapeutic advantage of the formulated dose. In rodents, systemic combinations of 1:1 or 1:3 can prevent THC induced conditioned place aversion or deficits associated with social interaction (Vann et al., 2008; Malone et al., 2009). In humans, a 1:2 oral dose can prevent impairments in affect recognition induced by THC (Hindocha et al., 2015). Current research has yet to investigate how the combined dose can interact in specific regions of the brain. The current findings are the first to demonstrate that a combined THC+CBD formulation blocks electrophysiological changes induced by THC in the brain.
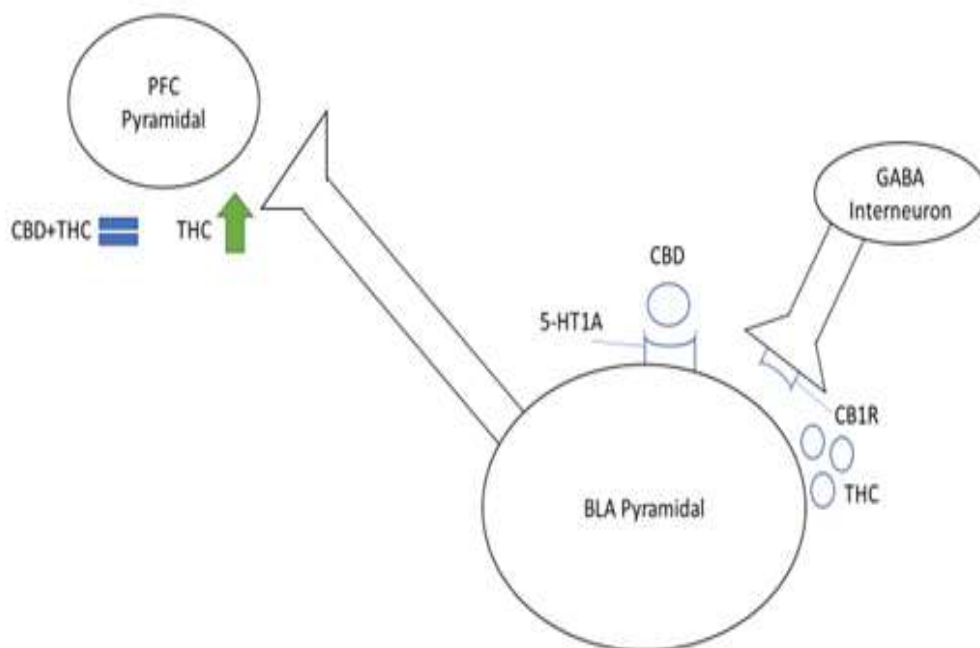
Figure 8A. Proposed model of BLA cannabinoid transmission on PFC activity. THC into the BLA binds to CB1Rs that are primarily located on GABA interneuron populations. Activating these receptors inhibits neurotransmitter release on terminals, therefore causing an overall dis-inhibition of BLA pyramidal cell activity. Increases in BLA activity are associated with changes to PFC pyramidal cell targets, which are associate with increases in single unit firing, bursting, and LFPs for high frequency gamma. The THC+CBD formulation is sufficient to prevent these effects. The mechanism for how these two compounds interacts is still not clear.

4.2 Cannabidiol decreases single unit activity in the vHPC

In the vHPC, THC micro-infusions did not alter single unit pyramidal cell activity compared to vehicle. Previous findings have demonstrated systemic THC infusions show no change in pyramidal cell firing (Aguilar et al., 2016). It is possible that the dose used in the current study was not high enough to induce an electrophysiological change in the vHPC, as a higher dose of BLA THC infusions (1ug) have been associated with anxiolytic effects (Rubino et al., 2008). In contrast to results in the PFC, CBD micro-infused in the BLA caused a decrease in vHPC single unit activity. Surprisingly, this effect was still present when CBD was combined with THC.

Interestingly, intracranial infusions of CBD into the NASh, at this same dose, have previously been shown to attenuate single unit activity of dopamine neurons in the VTA (Norris et al., 2016; Renard et al., 2016). The application of a 5HT-1A antagonist while co-administered with CBD was sufficient to prevent this effect, supporting the 5HT-1A serotonin receptor as one of its main sites of action (Norris et al., 2016; Russo et al., 2005). Activation of the 5HT-1A receptor can cause the inhibition of postsynaptic action potentials, therefore decreasing the cell's firing output (Polter & Li, 2010; Tada et al., 2004). With the current project's findings, our proposed model suggests that CBD, via activation of the 5HT-1A receptors on BLA pyramidal cells, inhibits principal cell firing rates in the vHPC. Using patch-clamp recordings, researchers have demonstrated that inactivating BLA projections to vHPC pyramidal cells causes decreased cell firing because of a decrease in glutamate release (Felix-Ortiz et al., 2013). It is possible that CBD inhibits output of BLA pyramidal cell activity to the vHPC by decreasing glutamate release. Inactivating these pathways is related to social and anxiety-related behaviour changes (Felix-Ortiz et al., 2013; Felix-Ortiz & Tye, 2014). Further research should investigate whether CBD in the BLA is sufficient to alter similar behaviours through inactivating projections to the vHPC.

9A
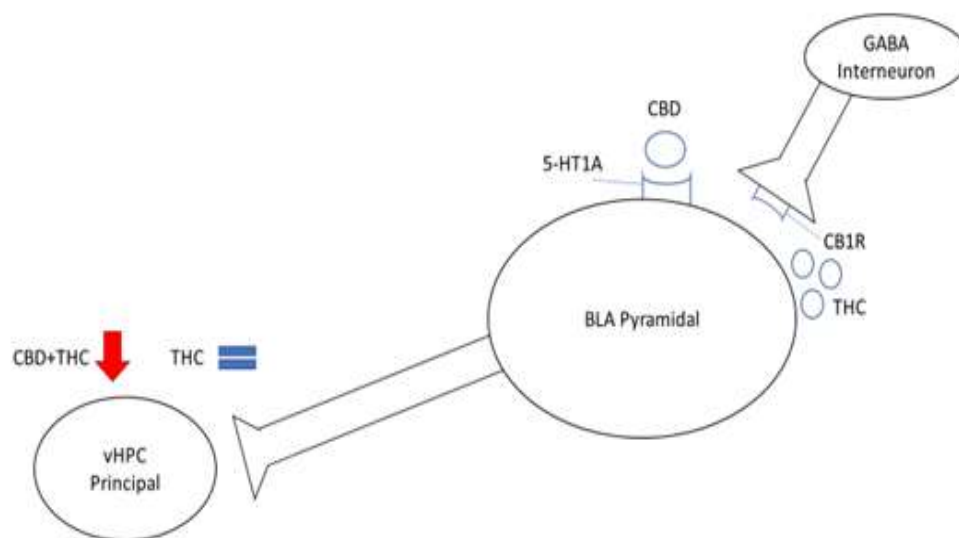
Figure 9A. Proposed model of BLA cannabinoid transmission on vHPC activity. CBD infused into the BLA binds to 5-HT1A receptors located on the soma and dendrites of pyramidal cells. Activating these receptors is associated with reduced EPSP generation to reduce overall action potential firing. This causes a decrease in pyramidal output to vHPC principal neurons, therefore reducing the overall activity of these cells. Surprisingly, the combination with THC is insufficient to prevent this effect, indicating this pathway's potential preference for CBD. Independent of CBD, THC within the BLA does not increase vHPC single unit or LFP activity.

4.3 Cannabinoid transmission alters cortical LFP activity

In contrast to cell-specific firing rates, we next investigated measures of indirect changes in cell input with LFPs. Analysis across oscillation ranges revealed that BLA THC caused an increase in power for gamma oscillations, with the strongest effect seen at the higher frequency (62-80Hz). Furthermore, the combination with CBD prevented this effect and returned post-infusion power changes similar to vehicle. The application of BLA CBD did not alter LFP signals compared to vehicle. In the vHPC neither THC, CBD, nor the combination led to changes in any oscillation patterns.

Coordination of cortical gamma oscillations integrate brain functions associated with cognitive and sensory processing (Basar et al., 2001). Disturbances in this network can potentially make the brain vulnerable to behavioural abnormalities. Network gamma oscillations in rodents are altered by activation of the CB1R, leading to alterations in impairments of cognitive and sensory processes (Hajos, Hoffmann, & Kocsis, 2008). As well, systemic application of THC in both adolescent and adult rodents have previously been shown to alter power for high frequency oscillations (Renard, 2017a; Robbe et al, 2006).

One neuropathological feature of schizophrenia is the disturbance of PFC gamma oscillations, leading to increased hallucinations and deficits in cognitive processes (Lee et al., 2010; Symond et al., 2005). In healthy subjects, acute THC administration has been associated with increase gamma activity and is correlated with increases in symptoms associated with psychosis (Nottage et al., 2015).

Therefore, THC's negative effects can potentially act through alterations in the gamma oscillation range. We demonstrate that a combination of CBD may act to prevent these disturbances, as the literature suggests a therapeutic benefit of higher CBD to THC ratios (Boggs et al., 2017).

4.4 BLA cannabinoid transmission does not alter PFC-vHPC connectivity.

For the final set of data, we performed an exploratory analysis investigating PFC-vHPC connectivity across both general activity (Crosscorrelation) and within oscillations (Coherence). The crosscorrelation takes the average shape of the LFP signal within a five-minute baseline period. The average shape within the vHPC is applied on top of the average shape of the PFC LFP signal. The degree of overlap on both signals is then assigned a correlation value, with the highest crosscorrelation 1.0 meaning the signals completely overlap. Coherence is a similar method, but it looks at comparing the degree of vHPC to PFC signal overlap as a function of frequency (Delta, Theta, Alpha, Beta, & Gamma). In both crosscorrelation and coherence analysis, post-infusion values are compared against baseline values to determine if any significant differences exist between treatment groups.

In both data sets, micro-infusions of THC, CBD, or the combination in the BLA did not cause any significant differences. Our findings are consistent with previous research that showed systemic THC does not alter Theta or Gamma oscillations, although researchers show increased Delta coherence in awake animals (Aguilar et al., 2016). Our findings suggest that while cannabinoid transmission in the BLA is sufficient to alter single-unit and LFP activity, it does not alter the coordination between brain regions. Future studies should explore whether a higher dose of THC or the application of a full CB1R agonist can change the coordinated activity between regions.

4.5 Implications

The current thesis adds to the literature on cannabinoid transmission within the mesolimbic system. Specifically, this is the first study to demonstrate that a combination of THC+CBD is enough to negate the effects of THC on electrophysiological activity of mesolimbic brain areas. Within regions of the BLA, PFC, and vHPC, our research group has demonstrated that THC or the activation of its target receptor, CB1R, can impair cognition, anxiety, sociability, learning and memory, and reward-related behaviours (Ahmad et al., 2016; Draycott et al., 2014; Loureiro et al., 2015; Loureiro et al., 2016; Renard et al., 2017a; Renard et al., 2017b; Tan et al., 2010, Tan et al., 2011). Current literature suggests the combination of THC and CBD can reverse some of these impairments, although the exact mechanism is still not understood (Boggs et al., 2018).

The model presented in Figures 8 and 9 can potentially explain these effects. Activation of CB1R in the BLA increases the activity of PFC pyramidal cells, which can potentiate the processing of emotionally neutral stimuli (Tan et al., 2010; Tan et al., 2011). Furthermore, increased activity of cortical gamma oscillations can impair cognition and produce acute deficits, as seen in schizophrenia (Lee et al., 2010, Nottage et al., 2015; Renard et al., 2017a; Symond et al., 2005). In our current study, the combination of THC+CBD in the BLA blocked this overexcitation of PFC activity. Overexcitation of the vHPC has also been implicated in modulating the mesolimbic system to produce schizophrenic-like deficits (Grace et al., 2010). Although we were surprised to find THC did not increased activity in this region, CBD with or without THC decreased overall principal neuron activity in the vHPC. These findings are supported in healthy human populations, in which oral administration of CBD decreases resting state activity within the amygdala and ventral hippocampus (De Crippa et al., 2004). Given how CBD interacts with the 5HT-1A receptor, decreased BLA pyramidal cell firing is responsible for inducing a decrease in vHPC activity. Our findings provide a novel avenue for investigating the anti-psychotic-like benefits of CBD because

of its ability to alter the vHPC. Modulating the mesolimbic system through cannabinoid transmission may partially be responsible for inappropriately reinforcing the distorted emotional significance of everyday events within the schizophrenic population (Laviolette & Grace, 2006). The combined use of THC+CBD can potentially mitigate this impact on the mesolimbic system by simultaneously preventing overexcitation of cortical and hippocampal activity.

5. Limitations

Although our findings demonstrate a clear relationship between BLA cannabinoid transmission and its effects on the PFC and vHPC, more experiments are needed to better characterize this circuitry. In particular, we could not demonstrate whether cells recorded in either region were directly or indirectly affected by afferent projections from the BLA. For instance, BLA projections not only synapse on pyramidal cell populations but also on GABAergic interneuron populations. Associated changes in either region in this study could be mediated by altering activity in interneuron populations. Increase of cortical GAMMA oscillations are mediated in part by dysregulation of local interneuron populations (Buzsaki & Wang, 2012). A follow-up project should investigate whether the effects seen with infusions made into the BLA directly interfere with the PFC or vHPC or are mediated from a different cell. One method to determine this is orthodromic validation. This would require recording in the BLA to PFC or vHPC innervation before drug infusion. In this paradigm, experimenters would stimulate neurons in PFC or vHPC target regions, which would conduct back up the axon of a BLA pyramidal cell. When co-stimulation of BLA and target structures negate the other area's conduction, the recorded cell is interpreted as a direct projection. Furthermore, recordings of interneuron populations within the vHPC or PFC would indirectly provide detail as to whether THC or CBD may have an indirect effect. In the case of VTA dopamine neurons, previous research has demonstrated changes via nucleus accumbens CBD infusions to be mediated by changes in VTA interneuron activity (Norris et al., 2016).

In the present study, THC did not cause a change in vHPC recorded neurons. One limitation to this interpretation was that we only used one dose of THC. In the case of CBD, two doses were used to confirm its effects. It is possible that a higher dose of THC can still cause a change in vHPC activity, given how previous studies have used BLA infusions of 1ug and above (Rubino et al., 2008). Despite this, our dose was still sufficient to elicit a change in PFC activity. Follow-up research should include a low THC dose (10 ng) that has not elicited any behavioural or electrophysiological changes in rodents (Norris et al., 2016). By adding a lower dose, future research can determine the lower bound of BLA THC infusions that cause electrophysiological changes.

Future Directions

We demonstrated the reversal effects of a combined THC+CBD formulation, however we have yet to demonstrate the underlying mechanism behind this. Although CBD has opposite effects on the CB1R compared to THC, CBD's overall low affinity for this receptor suggests that its main site of action is dependent on a different receptor (Pertwee, 2004). In this case, we could not determine which receptor type is responsible for the decreases found in the ventral hippocampus. Given the pharmacological binding profile of CBD, potential target receptors are 5-HT1A, D2, Mu opioid, or GPR55 (Kathmann et al., 2016; Russo et al., 2005; Ryberg et al., 2007; Seeman, 2016). Previous research has already indicated that blockade of the 5-HT1A receptor prevents the effects of systemic and local CBD on emotional memory acquisition and changes in mesolimbic neuron firing (Katsidoni et al., 2009; Norris et al., 2016). Future research should combine CBD with a 5-HT1A antagonist to determine if CBD's main site of action in the BLA is mediated by the serotonin receptor.

Beyond electrophysiology, future studies should explore the independent and combined behavioural effects of THC and CBD. Activating BLA CB1R and

increasing PFC activity can potentiate non-emotionally salient stimuli to be encoded in a fear memory (Tan et al., 2010). In fear memory formation, CBD infused into the shell of the nucleus accumbens alone is sufficient to block acquisition (Norris et al., 2016). It is possible that the combination of THC+CBD can prevent potentiation of fear memory and can negate changes in cortical activity.

Conclusions

Within the mammalian brain, the proper encoding of environmental stimuli is regulated by the cannabinoid system. Disruption of this system through cannabis can lead to either positive or negative effects, depending on the relative ratio of THC to CBD within the plant. Impaired cognition, anxiety, learning, and memory are associated with activation of the CB1R via THC in the BLA, vHPC, and PFC. Combining CBD with THC appears to prevent these acute impairments and could allow a user to avoid some of the negative effects associated with high amounts of THC. To test this question, we used in vivo electrophysiology to determine if THC+CBD can negate THC-induced overexcitation of PFC pyramidal cells. Not only was the combination effective, it simultaneously decreased ventral hippocampal activity, possibly another benefit to counter the effects of high THC. We demonstrate for the first time that THC+CBD has therapeutic benefits that are demonstrated through neuronal activity in mesolimbic areas of the brain.

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Curriculum Vitae

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Honours and Awards
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Publications
        Adolescent Nicotine Exposure Induces Dysregulation of Mesocorticolimbic
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Conference Presentations

Yeomans, J.S., & **Pereira, B.J**. (2016). Maternal circuits that respond to mouse
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Yeomans, J.S., Wasserman,D.I., Curry,T., **Pereira, B.J**., Tselichtchev, P., Lam, M. (2015). Maternal influences on pup ultrasonic vocalizations in D2 and M5 deficient mice. *Society for Neuroscience*, Chicago, IL, USA.

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Ouslis, N. E., **Pereira, B.J.**, Jeong, J. Y., & Spence, I. (2014, July). Attention and Visuo-spatial Working Memory in Mental Rotation. Poster Presentation at the Canadian Society for Brain, Behaviour, and Cognitive Sciences, Toronto, ON.