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Characterizing the Cognitive and Emotional Effects of delta-9-Tetrahydrocannabinol in Distinct Hippocampal Sub-Regions

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Abstract

The objective of this study is to determine the potential differential effects of THC in the DH or VH sub-regions, as well as the upstream effects on PFC neuronal activity and oscillations. Rodents used for electrophysiology were infused with THC or vehicle in the DH or VH regions, combined with PFC recordings. Additionally, a battery of behavioural paradigms was performed. Deficits in short-term memory when THC was infused into both regions was observed, however working memory was impaired with VH infusions only. This could be due to THC-induced dysregulation in the PFC, as beta oscillations were significantly decreased selectively in the VH. Additionally, a selective increase in anxiety-related behaviours was observed following VH THC infusions, but not in the DH, which could be related to changes in ERK 1/2. These findings have implications for how marijuana may differentially impact emotional vs. cognitive functions through differential effects on hippocampal sub-regions.

Keywords: novel object recognition, y-maze, spontaneous alternations, social interaction, fear conditioning, p70 S6 Kinase, ERK 1/2, open field, light/dark box, THC, schizophrenia, prefrontal cortex, dorsal hippocampus, ventral hippocampus

Co-Authorship Statement

Justine Renard, PhD.- Aided in the teaching of behavioural paradigms.

Hanna Szkudlarek, PhD.- Aided in the collecting and analyzing of electrophysiological data.

Tony Jung, PhD.c – Helped in slicing and histology to determine correct cannula placements.

Walter J. Rushlow, PhD.- Aided in protein extractions of brain samples as well as training of full western blotting process.

Steven R. Laviolette, PhD.- Supervisor, created the project and contributed in the protein extractions of tissue samples.

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Table of Contents

List of Figures

List of Abbreviations

ANOVA	Analysis of variance
BCA	Bicinchoninic acid assay
BLA	Basolateral Amygdala
BSA	Bovine serum albumin
DA	Dopamine
DH	Dorsal Hippocampus
CBD	Cannabidiol
CB1R	CB1 cannabinoid receptor
pERK	Phosphorylated Extracellular signal related kinase
tERK	Total extracellular signal related kinase
GLUT	Glutamatergic
LFP	Local Field Potential
LTP	Long term potentiation
NaS	Nucleus accumbens shell
PCP	Phencyclidine
mPFC	Medial prefrontal cortex
mTOR	Mammalian target of rapamycin
TBS-T	Tris-buffered solution-tween
THC	Delta-9-tetrahyrocannabinol
SEM	Standard Error of Means
VEH	Vehicle Control
VTA	Ventral tegmental area
VH	Ventral Hippocampus

1 Introduction

Marijuana is the most widely abused illicit substance in the world and is typically consumed through inhalation or ingestion. The United Nations reports around 3.8% of the world's population to be regular marijuana users. Due to its easy accessibility, adolescents and young adults alike, generally perceive the drug to be relatively harmless, which may increase its overall usage rates (Ramo et al., 2012). There are numerous well-documented side effects of chronic marijuana usage including the development of marijuana dependence, lung inflammation, cognitive impairments and an increased likelihood of developing symptoms of schizophrenia (Campolongo et al., 2007, Hasin et al., 2015, Taskin et al., 2013). Thus, as more and more nations worldwide are looking to legalize recreational marijuana use, it is imperative to increase our understanding of the potential neuropsychiatric side-effects of marijuana exposure.

Marijuana is composed of a variety of different phytocannabinoids, the most prevalent being delta-9-tetrahyrocannabidiol (THC) and cannabidiol (CBD). THC is considered to be the primary psychoactive component of marijuana and a large body of evidence has shown that THC is the component that contributes to symptoms of psychosis (Colizzi et al., 2018, D'Souza et al., 2016, Herkenham et al., 1991, Renard et al., 2018). These effects of THC are believed to be due to THC-induced modulation of the brain's CB1 cannabinoid receptor (CB1R) system and concomitant dysregulation of mesolimbic dopamine (DA) transmission (Hudson et al., 2017, Renard et al., 2018).

The chemical structure of THC is similar to naturally occurring endocannabinoids such as anandamide. Endocannabinoids are neurotransmitters that play a crucial role in attention, learning and memory. Found abundantly in the hippocampus, CB1R are primarily located on GABAergic interneurons (Masiulis et al., 2011). Once activated CB1 receptors inhibit interneuron activity in the ventral hippocampus, increasing excitation within the hippocampus. THC is a partial CB1R agonist and chronic excitation resultant from marijuana use, has led to impairments in spatial learning and memory as well as emotional processing.

The hippocampus is implicated in a variety of diverse functions, including spatial and contextual processing and emotional memory and processing. However, considerable evidence suggests that these functions are delegated to different ventral vs. dorsal sub-regions of the

hippocampus. For example, whereas the dorsal hippocampus plays a role in spatial cognitive functioning (McHugh et al., 2011, Moser et al., 1995), the ventral hippocampus is more involved in emotional affective learning (Cox et al., 2013, Henke 1990).

Additionally, the ventral hippocampus is heavily connected to various regions of the limbic cortex. As such, over excitation of neurons within the hippocampus affects neuronal activity in other cortical regions downstream or upstream. Importantly, the hippocampus sends excitatory glutamatergic signals to the ventral tegmental area (VTA). Loureiro et al. (2015) demonstrated that ventral hippocampal hyperactivity induced by direct CB1R activation causes an increase in dopaminergic activity in the VTA. This is correlated with increased reward salience and deficits in sociability and social recognition behaviours. Additionally, the ventral hippocampus sends excitatory glutamatergic projections to the nucleus accumbens. CB1R activation in the hippocampus increases glutamatergic firing in the nucleus accumbens, thereby modulating its neuronal activity (Loureiro et al., 2016). This in turn is correlated with an increase in saliency of aversive emotional associative memories (Loureiro et al., 2016). Interestingly, these behavioural effects can be reversed through the infusion of a glutamate antagonist to the nucleus accumbens (Loureiro et al., 2015). Together, this evidence strongly implicates a role for cannabinoid signaling in the ventral hippocampus, specifically in the modulation of affective/emotional processing and behaviours.

Both sub-regions of the hippocampus also send excitatory axon projections to the prefrontal cortex (PFC). The PFC participates in numerous cognitive functions such as working memory, decision making, and goal-oriented behaviour (Kesner and Churchwell, 2011). It is also involved in affective learning such as anxiety, and the consolidation of fear related memories. Experimental evidence has elucidated the involvement of the hippocampus influencing activation of neurons in the PFC during such cognitive and emotional functions (Adhikari et al., 2010, Tierney et al., 2004). In a study performed by Floresco et al. (1997), they demonstrated that a disconnection between the hippocampus and prefrontal cortex attenuated working memory in rats. This implicates a hippocampal to PFC interaction during executive functioning. Additionally, synchronous theta oscillatory activity in both the ventral hippocampus and PFC is correlated with anxiety-like behaviours (Adhikari et al., 2010), further demonstrating the importance of the hippocampal to PFC pathway in cognitive and emotional functioning. Thus,

the aim of this literature review is to explore the neural mechanisms that underlie marijuana-induced deficits, with a focus on the limbic cortex.

1.1 Prevalence of Marijuana Consumption

As stated previously, marijuana is the most widely used drug product in the world. There are about 183 million marijuana users annually and this number has not changed in the past decade (UNODC, 2017). However, North and South America together show a prominent increase in marijuana users from 37.6 million people in 2005 to 49.2 million in 2015 (UNODC, 2017). This rise appears to be most pronounced in the United States.

In the United States of America, 22.2 million people have reported using it within a month according to a 2015 National Survey on Drug Use and Health. The prevalence of daily or almost daily users went from 1.9% in 2002 to 3.9% in 2015 (UNODC,2017). It is increasingly more prevalent among men than in women (Carliner et al., 2016).

Marijuana is most commonly abused within the adolescent to young adult age bracket. In a 2016 survey, 35.6% of those in their 12^{th} grade of high school reported smoking marijuana within the past year; 22.5% within the past month (Johnston et al., 2016). In fact, the age of initial marijuana consumption has been getting progressively younger, as a total of 2.5 million people aged 12 or older consumed marijuana for the first time in the past 12 months in 2014 (CBHSQ, 2015).

1.2 Effects of Acute and Chronic Marijuana Consumption

Marijuana exposure is linked to a wide variety of psychological effects, both on emotional processing and in terms of cognition. Some acute consequences of marijuana consumption include impaired short-term memory, impaired attention, and an increase in anxiety and paranoia. Marijuana also has been shown to have long lasting effects on learning and memory even after the drug has worn off. As a result, an individual who smokes marijuana daily can be functioning at a reduced intellectual level (Schweinberg et al., 2008). There is evidence to suggest students who smoke marijuana daily have poorer educational outcomes than their non-smoking peers. In a case control study, heavy marijuana users were compared to a control group

who reported smoking marijuana at least once in their life but not more than 50 times. There were significant differences found in their educational attainment; the heavy marijuana users were less likely to complete college and had a yearly income of less than $30,000 (Gruber et al., 2003). Heavy marijuana use is also linked to unemployment, criminal behaviour and a perceived lower quality of life. There is also an increased chance of developing dependence, using other drugs, and attempting suicide (Brook et al., 2013, Fergusson et al., 2008).

As mentioned above, frequent consumption of marijuana can not only affect one's socioeconomic status but can also be a gateway to using other commonly abused drugs. For example, individuals who frequently used marijuana were more likely to develop an opioid use disorder (Olfson et al., 2018). Additionally, adolescent rats pretreated with THC had a significant increase in heroin-seeking behaviour during adulthood (Ellgren et al., 2007). This evidence suggests that THC exposure may sensitize the brain to the reward-producing effects of opioids.

Increased marijuana usage has also been linked to the increased likelihood of developing mental health problems in later life (Renard et al., 2017). In fact, our lab has shown that exposure to THC induced long-term deficits in sociability, reduced exploratory behaviour, increase in anxiety, and deficits in prepulse inhibition, all of which are anecdotal to symptoms of schizophrenia (Renard et al., 2017). These rats were chronically exposed to THC during adolescent time period demonstrated cognitive and emotional functioning deficits during adulthood, increasing the probability of developing schizophrenia (Renard et al., 2016, Renard et al., 2017).

Moreover, THC can increase the severity of symptoms in individuals predisposed with schizophrenia. For example, in a study performed by Rodriguez et al., (2017) mice were given a neonatal administration of phencyclidine (PCP) or saline. Administering PCP during critical developmental periods can mimic symptoms of schizophrenia when the rodents are older. On post-natal day 53 mice were then given sub-cutaneous injections of THC or saline for 45 consecutive days. The mice given PCP and saline displayed behavioural deficits in the Morris water maze and pre-pulse inhibition paradigms, consistent with schizophrenia-type disorders. The mice given PCP then THC later in life also displayed deficits in the locomotor paradigm, novel object recognition, Morris water maze and pre-pulse inhibition. Moreover, these deficits were greater than the ones seen in the mice that were only administered PCP.

1.3 Cognitive Impairments Induced by THC Exposure

The literature with regards to the association between marijuana and cognition is inconsistent. Some studies show marijuana users display relatively poorer cognitive performance (Bolla et al., 2002). Other studies report no significant effects of THC on cognition (Ruhl et al., 2014). These inconsistencies may be because marijuana only affects certain aspects of cognition. For instance, there might be a modest but reversible impact on global memories but a more deleterious effect on specific sub-domains such as verbal and/or visual memory processing. Moreover, marijuana containing higher THC content has been shown to produce more pronounced deficits and longer lasting effects (Pope et al., 2010). Bolla et al., (2002) performed a battery of neurocognitive tests on heavy marijuana users and observed a dose-dependent relationship. Those who smoked more marijuana joints per week displayed a decreased performance in verbal and visual memory tasks, executive functioning and psychomotor speed (Bolla et al., 2002). Additionally, while some studies report these cognitive deficits being reversible after 28 days (Pope et al., 2001), the study performed by Bolla et al., (2002) observed these deficits lasted longer than 28 days; this is further evidence that the higher the marijuana consumption, the greater the severity of cognitive deficits. Moreover, the greater the THC concentration the more severe the ensuing cognitive deficits (Pope et al., 2001).

These reports are consistent with a wide body of evidence demonstrating that THC is the main component of marijuana responsible for inducing deficits in cognition. For example, repeatedly injecting adolescent mice with THC demonstrated deficits in spatial memory (Schreiber et al., 2018). This is demonstrated in non-human primates as well. For example, in Rhesus monkeys, chronic injections of THC caused deficits in response inhibition and a loss of control over impulsive behaviours (Jacobs et al., 2016).

Although THC is the main component of marijuana responsible for cognitive deficits, it is selective with regards to what forms of cognition become impaired. As mentioned above THC impairs spatial memory (Ruhl et al., 2014, Schreiber et al., 2018). The literature is inconsistent as to whether or not it impairs associative memories (Renard et al., 2017, Ruhl et al., 2014). Zebrafish given an acute dose of THC displayed no differences in a colour discrimination (Ruhl et al., 2014), however adult rats chronically given THC as adolescents displayed deficits in a novel object recognition paradigm (Renard et al., 2017). Many explanations can be offered to

explain this variation. Perhaps acute administration of THC shows no effect on associative memories, but chronic administrations displays deficits. Additionally, these deficits seem to be dose-dependent, hence a lower THC concentration may not exhibit deficits. Moreover, the colour discrimination task tested long term memory effects whereas novel object recognition tested for short-term memory.

Additionally, memory processing is composed of various stages (e.g. encoding, recall and extinction) and THC may not exert its effects on all processing stages. In an interesting study done by Ranganathan et al., (2017), participants were given THC intravenously and asked to participate in a verbal learning and memory task. This task was administered either before or during THC administration. The study reported that the participants were impaired in memory encoding but not in recall. In other words, only those given THC before the task demonstrated memory impairments. Likewise, in the study performed by Renard et al., (2017), THC was administered before performing any behavioural paradigms, another suggestion of THC affecting memory encoding and possibly not recollection.

Higher executive functioning may also be impacted by THC administration. For example, THC consistently affected working memory regardless of acute or chronic administration protocols (John et al., 2018, Keeles et al., 2017, Tunbridge et al., 2015). Interestingly, in an early study done by Hart et al., (2001) participants who smoked marijuana showed no noticeable impairments in cognitive flexibility. However, it could be because the task used to measure cognitive flexibility was too simplistic. Indeed, later studies using more complex measurements of cognitive function did observe impairments in cognitive flexibility (Egerton et al., 2005, Kangas et al., 2016, Sokolic et al., 2011). For example, in reversal learning paradigms participants need to display a strong ability to reverse previously related associations between a stimulus and reward. Rats administered THC displayed deficits in reversal learning thereby demonstrating cognitive inelasticity (Egerton et al., 2005, Sokolic et al., 2011).

1.4 Emotional Learning and Memory Impairments Induced by THC Exposure

In addition to cognitive impairments, THC can also alter the processing of emotional information. Specifically, THC is known to induce anxiety. Mice given both acute and chronic injections of THC display increased anxiety, as they tend to spend less time and make less arm

entries in the open arms of an elevated plus maze (Kasten et al., 2017, Murphy et al., 2017). This effect appears to be dose dependent, as lower doses seems to reduce stress whereas higher doses increases stress as well as a negative mood overall (Childs et al., 2017). The amount of stress felt also impacts the amount of anxiety expressed. Fukos et al., (2009) demonstrated that in stressed conditions, rats on high doses of THC were prone to anxiolytic-like behaviours, whereas low doses of THC induced anxiogenic effects. Similarly, with the cognition studies, in the research mentioned here THC was given right before beginning the behavioural paradigms, suggesting THC affects the encoding phase of emotional learning and memory.

Not all emotionally processed information is affected by THC in the same way. For example, in a study by Ballard et al., (2012), human participants were given THC and then asked to perform tasks assessing facial emotion recognition. They found that THC significantly impaired recognition of fear and angry faces but only marginally impaired recognition of sadness and happiness (Ballard et al., 2012). This may suggest that THC affects specific, emotional processing circuits in the brain.

In addition to negative, aversive learning phenomena, THC directly interacts with the endocannabinoid system in the brain, responsible for reward-related learning. As such, THC, which causes maladaptive endocannabinoid signaling, can increase the development of addictive and drug-seeking behaviours (Rameakers et al., 2016). Additionally, marijuana consumption has been associated with a lack of motivation and non-drug rewarding deficits, though these effects have been poorly studied. For example, in a study performed by Lawn et al., (2016), individuals consuming marijuana demonstrated a decreased likelihood to make high effort choices to receive a monetary reward, thereby showcasing a lack of motivation. With the impending legalization of marijuana, it would be interesting to explore these effects deeper by understanding the brain regions that modulate these THC influenced deficits.

1.5 Pinpointing Brain Regions affected by THC

So far, the studies described above have only quantitatively measured the cognitive and behavioural effects of THC. These behavioural deficits have yet to be tied to specific neural mechanisms in the brain. Importantly, since THC is a CB1R partial agonist, it will influence brain regions with high CB1R concentrations, such as the limbic cortex. In fact, THC exposure

causes altered glucose utilization in the nucleus accumbens, basolateral amygdala and the hippocampus, all critical regions of the limbic cortex (Whitlow et al., 2013) involved in mediating stress, reward and certain aspects of memory. Thus, the limbic cortex is an area of great interest in determining the specific neural mechanisms of THC-induced deficits.

THC can directly activate the mesolimbic dopamine system (Tanda et al., 1997), and can potentiate brain reward phenomena (Gardner et al., 1988). Similar to drugs such as cocaine, heroin and nicotine, THC produces its rewarding actions in the ventral tegmental area (VTA) and nucleus accumbens shell (NaS); the VTA being the origin of the mesolimbic dopamine system and the NaS being the region where most dopamine fibers terminate (Zangen et al., 2006). This rewarding effect is induced due to the increased VTA dopamine neuronal activity caused by THC exposure (Renard et al., 2017).

While dopamine neurons are known to play a pivotal part in the brain-reward circuit, another cell type that is understudied but does have a crucial role in the reward circuit are GABAergic neurons. CB1 receptors are commonly located on GABA neurons, thus it is possible THC works directly on GABA neuron activity. This turns out to be true as THC in the VTA can decrease GABAergic neuron activity, thereby increasing dopamine activity downstream which results in an increase in reward sensitivity (Friend et al., 2017).

The effects of THC in the VTA and NaS do little to explain the cognitive and anxiety impairments caused by this drug. The prefrontal cortex (PFC), a part of the limbic cortex, plays a pivotal role in both cognitive processes as well as emotional learning and memory. It is responsible for executive functions such as working memory, attention and cognitive flexibility. Hence, cognitive deficits observed from systemic administration of THC can be traced back to the PFC. In fact, CB1R agonist WIN-55, 212-2 modulates bursting mode firing during affective processing which in turn increases responses to fear conditioned cues (Laviolette and Grace, 2006).

1.6 The Role of Cortical Oscillation States in Cognitive and Emotional Processing

One way of measuring neuronal population activity is by looking at brain wave oscillations. Oscillations are studied through local field potentials (LFPs) which represent the activity of a collection of nearby neurons (Buzaki, 2004). Synchronized LFPs are most common

during cognitively demanding tasks (Buschman and Miller 2007). Beta (15-30 Hz) and low gamma (30-80 Hz) oscillations have been associated with higher cognitive functions such as working memory and cognitive flexibility (Glykos et al., 2015, Stanley et al., 2018). Beta wave synchrony helps to construct category ensembles, a task that requires some cognitive flexibility (Stanley et al., 2018).

Specifically, within the PFC, these oscillations seem to be modulated by GABA neuronal activity (Glykos et al., 2015). Chronic administration of THC attenuates GABAergic function in the PFC, leading to a hyperactive state (Renard et al., 2017). This also leads to a decrease in gamma oscillatory activity; gamma oscillations are often associated with emotional memory information processing (Renard et al., 2017). It is possible that THC may alter GABAergic neuron morphology, thus decreasing their function. An interesting study done by Kolb et al., (2018) saw chronic injections of THC altered dendritic arborisation and spine density in the PFC.

CB1 receptors are also abundantly found in the hippocampus, a pivotal region of the limbic cortex. Similar with the PFC, the CB1 receptors are also found on GABA neurons and once activated, will cause a decrease in excitatory activity. Activation of the CB1 receptors by the CB1R agonist CP55940 reduced power of local field potential oscillations and reduced spike timing coordination (Robbe et al., 2006). This alteration of neuron activity could be associated with marijuana-type deficits. Furthermore, the hippocampus can be divided into two regions, the ventral hippocampus (VH) and dorsal hippocampus (DH). The VH is responsible for more affective type learning and memory while the DH is responsible for cognition. Specifically, when the VH is lesioned, rats tend to freeze significantly longer in a fear conditioning paradigm (Cox et al., 2013). Meanwhile, when the DH is lesioned, rats demonstrate impairments in the Morris water maze (McHugh et al., 2011).

Oscillatory activity in the hippocampus is thought to play a role in memory acquisition as well as consolidation. Gamma oscillations are frequently associated with associative memory encoding (Trimper et al., 2017), as well as anxiety and emotional memory processing (Headley & Pare, 2013). In fact, these oscillations are generated during events that elicit an emotionally aroused behaviour (Headley and Pare, 2013). Beta oscillations are associated with a neuronal network tuned for novelty and this in turn may allow for new learning to occur (Franca et al., 2014).

1.7 Molecular Pathways Involved in Cannabinoid Control of Emotional and Cognitive Processing

Numerous molecular signaling pathways have been linked to the ability of THC to modulate cognitive and affective processing. As previously stated, THC is a partial agonist to the CB1R, located on presynaptic neurons. These receptors have a role in the modulation of the extracellular signal-related kinase (ERK) pathway activity (Derkinderen et al., 2003). ERK, a mitogen-activated protein kinase, plays a role in synaptic plasticity and is responsible for the induction of long-term potentiation (LTP). Therefore, blocking CB1R activation reduces activation of ERK1/ERK2 subtypes, resulting in a decrease of synaptic markers (Karanian et al., 2005).

ERK activity also modulates synaptic output, implicated in learning and memory formation. Particularly, ERK is involved in reward reinforcement. Zhang et al., (2016) demonstrated CB1R antagonism attenuated the acquisition of morphine-induced conditioned place preference, along with a downregulation of ERK in the hippocampus and nucleus accumbens. Hence, it can be speculated that ERK activation via CB1 receptors is part of the underlying mechanism behind reward learning and memory, therefore involved in associative learning.

In terms of the functional relationship between ERK activation and THC exposure, prolonged THC exposure has been shown to induce an increase in phosphorylated ERK expression in the hippocampus and prefrontal cortex, thereby increasing synaptic plasticity (Rubino et al., 2005). Additionally, inhibiting ERK activation paralleled a lack of tolerance to THC-induced hypolocomotion suggesting its role for THC-induced tolerance (Rubino et al., 2005).

Another pathway modulated by CB1R is the mammalian target of rapamycin (mTOR) pathway, involved in synaptic plasticity and memory formation (Hoeffer and Klann, 2010). Chronic adolescent THC exposure was reported to dramatically decrease PFC expression levels of mTOR, concomitant with affective and cognitive schizophrenia-like deficits (Renard et al., 2016). Interestingly, blocking mTOR activation using rapamycin- an mTOR inhibitor- negates the effects of THC, suggesting mTOR is involved in marijuana-induced deficits (Ibarra-Lecue et al., 2018). The protein mTOR also regulates protein translation via activation of p70S6 kinase

(Ma and Blenis, 2009). Chronic exposure of THC during adolescence causes a decrease in phosphorylated p70S6 kinase in the PFC during adulthood (Renard et al., 2017). This is resultant of an increased stimulation of dopamine receptors, which downregulates p70S6 kinase in the PFC (Fasano et al., 2008).

P70S6 kinase activation has a positive correlation with fear retrieval in the basolateral amygdala (BLA), suggesting enhanced fear retrieval is associated with an increased activation in the BLA (Zubedat and Akirav, 2017). Even within the dorsal hippocampus, there is an upregulation of p70S6 kinase during context dependent fear retrieval (Gafford et al., 2013). THC has been noted to cause an increase in anxiety and emotional saliency and as such, may have differential effects on p70S6 kinase expression in different limbic regions.

1.8 Hippocampal-Prefrontal Cortex Pathway

During the formation of memories or performance of mental tasks, various brain regions need to communicate with each other to process and extract the necessary environmental information. As a result, abnormal activity in one brain region may affect the downstream or upstream regions it sends axonal projections to. As mentioned previously, THC acts upon the limbic reward circuitry, responsible for reward seeking and impulsive behaviours. First of all, the VTA sends dopaminergic projections to the NAc, which then sends glutamatergic projections to the medial PFC (Figure 1). Ramaekers et al., (2016) demonstrated that THC decreases connectivity of the NAc to the PFC as well as increasing impulsivity, suggesting a role of the NAc to PFC pathway in THC-induced impulsive behaviours. Additionally, the PFC and BLA send excitatory projections to the NAc and this effect was inhibited by THC (Pistis et al., 2002), which may play a role in the rewarding aspects of marijuana.

The PFC will also directly send excitatory glutamatergic projections to the VTA and conversely, dopamine neurons from the VTA will send signals to the PFC (Figure 1). Pistis et al., (2002) reported acute THC administration increased dopamine output to the PFC, demonstrating aberrant cannabinoid activity can modulate neuronal activity in the PFC. This in turn may cause aberrancies in emotional saliency and memory formation.

Another circuit involved in the processing of emotionally salient memories is the BLA to PFC circuit (Figure 1). Both brain regions contain high levels of CB1 receptors, which modulate

the dopamine neurotransmitter system, involved in addictive behaviours and the neuropathology of various psychiatric disorders. Aberrant endocannabinoid signalling in the BLA-PFC pathway may lead to dysregulation of dopamine neurotransmission (Tan et al., 2014). Behaviourally, this may lead to disruptions in emotional processing, learning and memory, all of which are involved in neuropsychiatric disorders.

The hippocampus is widely connected to a variety of brain regions involved in both cognition and emotional processing (Figure 1). Parallel to the sub-region function the DH, involved in cognitive functions, has some of its projections with the cingulate and orbital cortical areas and VH, involved in affective behaviour, is closely connected to the amygdala and nucleus accumbens (Caliskan & Stork, 2018).

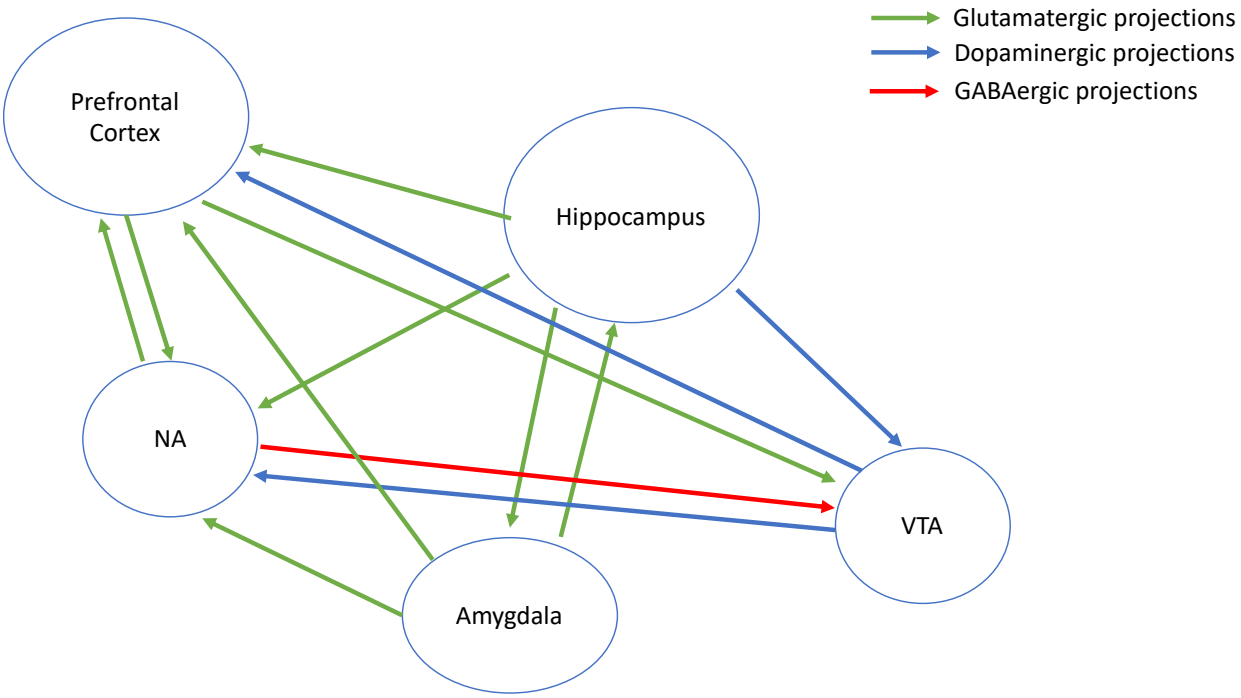


Figure 1 Limbic cortex emotional learning and cognitive functioning pathways.

Hippocampal CB1 receptor activation via WIN-55- a receptor agonist- sends an increased amount of excitatory projections, thereby increasing cell firing in the NaS (Loureiro et al., 2016). This led to an aberrant amplification of emotionally salient memories, specifically in fear and reward related memories (Loureiro et al., 2016. Similarly, VH CB1R activation increases VTA dopamine cell firing and bursting while simultaneously decreasing VTA non-dopamine neurons (Loureiro et al., 2015). With regards to behaviour, this increases reward saliency while causing deficits in sociability and social recognition (Loureiro et al., 2015).

Communication between the hippocampus and PFC is responsible for higher cognitive functioning such as working memory and attention. Both dorsal and ventral hippocampal sub-regions send excitatory glutamatergic signals to the medial prefrontal cortex (mPFC). This induces an excitatory response in the pyramidal cells of the PFC (Degentais et al., 2003). A study by Degentais et al., (2003) also observed an inhibitory response after the excitation, suggesting an engagement of pyramidal and non-pyramidal neurons that may constrain the excitation in response to the hippocampal inputs.

While there is limited research involving pharmacological interventions in the hippocampal-PFC pathway, there have been multiple studies observing oscillatory activities. These oscillations are often linked to various cognitive and emotional processes. Hippocampal theta (4–8 Hz) and gamma (30–80 Hz) oscillations play a fundamental role in coordinating assembly firing across the hippocampo-prefrontal cortical regions and are believed to be critical for working memory and encoding episodic memories (Robbe et al 2006). Theta oscillations also work in combination with alpha oscillations (8-13 Hz). This theta-alpha wave combination binds together hippocampal and PFC activity during memory retrieval (Herwig et al., 2016).

1.9 Research Hypothesis

The primary research objective of the thesis project was to pinpoint a specific neural basis for THC-induced deficits in cognition and emotional learning, focusing in on the hippocampus-prefrontal cortex pathway. As discussed above, the hippocampal formation is a critical region of interest associated with marijuana-induced neuropathology as well as its anatomical sub-regions, and their distinct roles in cognitive and emotionally-motived learning and memory.

The overarching goal of the thesis is to determine the potential differential behavioural, neuronal and molecular effects of THC in the DH vs. VH, and how these effects may modulate hippocampal regulation of the PFC in terms of PFC cell activity and oscillatory activity states. My General Hypothesis is that ***THC directly infused into the VH or DH will result in differential effects on cognitive and affective processing through dissociable modulation of the PFC.*** The hypothesis has been addressed with the following Specific AIMS:

Aim 1: Characterize the effects of THC exposure in the DH or VH on select measures of spatial and non-spatial cognitive functioning, and emotional/affective memory processing.

Aim 2: Characterize the effects of DH and VH THC exposure on upstream PFC neuronal activity patterns and oscillatory states associated with emotional and cognitive processing.

Aim 3: Characterize the effects of THC exposure on protein levels in the DH and VH associated with learning and memory processing.

2 Methods

2.1 Animals and Housing

Male adult Sprague Dawley rats (PND 75) were obtained from Charles River Laboratories (Quebec, Canada). At arrival rats were pair housed under controlled conditions (12-hour light/dark cycle, constant temperature and humidity). Food and water was provided *ad libitum*. All experimental protocols and procedures were approved by the Canadian Council on Animal Care and Animal Care Committee at University of Western Ontario.

2.2 Neuronal Activity Recording and Analysis

Extracellular single unit electrophysiological recordings were performed *in vivo* in the adult rodents. In total 36 rats were used for this experiment and were separated in the following groups: VH-VEH- 8, VH-THC- 10, DH-VEH- 7, DH-THC-11.

The recordings were taken from glutamatergic (GLUT) mPFC neurons and THC/VEH infusions were made at either VH or DH. Rats were anesthetized with urethane (1.4g/kg, i.p., Sigma-Aldrich) and placed onto a stereotaxic frame with body temperature maintained at 37°C. An incision at the scalp was made and a hole was drilled in the skull overlaying the targeted structure at the following coordinates: mPFC: AP +2.5 to +3.4 mm from bregma, LM: ±0.8 mm,

DV: -2.5 to 4.5 mm from the dural surface. Recordings were taken with glass electrodes (average impedance of 6–10 MΩ) filled with 2M sodium acetate solution containing 2% pontamine sky blue (Sigma-Aldrich). Extracellular signals were amplified (×5000) using a MultiClamp 700B amplifier (Molecular Devices), digitized at 25 kHz and recorded on the computer using a Digidata 1440A and pClamp software (Molecular Devices). The wideband signal of PFC recordings was fed to two channels of the digitizer and filtered to obtain single unit recordings (band pass between 0.3 and 3 kHz) and local field potentials (LFPs; low pass at 0.3 kHz).

GLUT mPFC pyramidal neurons were identified based on established criteria: firing frequency <10 Hz, waveform shape, action potential duration >2.5 ms. Cells exhibiting 2 or more consecutive spikes with an interspike interval of < 45 ms were classified as burst firing cells. The electrophysiological properties of spontaneously active pyramidal neurons were sampled in the mPFC by making vertical passes of the electrode through the pyramidal cell body region. Once an individual GLUT neuron was located its baseline activity was recorded for 5 minutes. For intra-VH or DH micro infusions of VEH or THC (100 ng/0.5 µl), a 10 µl Hamilton syringe was slowly lowered into the VH or DH using the following coordinates: DH: AP – 4.3 mm from bregma, LM: ±2.8 mm, DV: -2.5 mm from the dural surface; VH: AP: -5.6 mm from bregma, LM: ± 5 mm, DV: -6.8 mm from the dural surface. Cellular activity was recorded for 30 minutes post infusion. The basal firing rate and bursting rate were sampled.

LFP signals were analyzed using Neuroexplorer (Nex Technologies). First of all, the signals were decimated to 1000 Hz and lowpass filtered (IIR Butterworth filter at 100 Hz; filter order set to 3). A spectrogram function was used to calculate the power of oscillations at frequencies between 0-100 Hz (window length 1 s; shift 0.5 s). One minute long recording epochs were utilized for estimating the average power spectrum distribution. Power values for a given frequency were averaged over time of the recording epoch and normalized such that the sum of all power spectrum values equated to one. The total power was calculated by adding all the power values at the frequencies between 0-59 and 61-100 Hz. Power values surrounding 60 Hz were excluded from all calculations. Delta waves were defined as 1-4 Hz, theta waves were 4-8 Hz, alpha waves were 8-13 Hz, beta waves 13-30 Hz and finally gamma waves 30-100 Hz.

For histological analyses, the recording electrodes positions were marked with an iontophoretic deposit of pontamine sky blue dye (-20 mA, continuous current for 15-20 minutes). Afterwards the rat was decapitated, brain harvested and placed in a formalin solution. Coronal

sections (60 μm) containing either the mPFC, DH, and VH were cut on a cryostat and slide mounted. They were later stained with a neutral red staining dye (Figure 2).

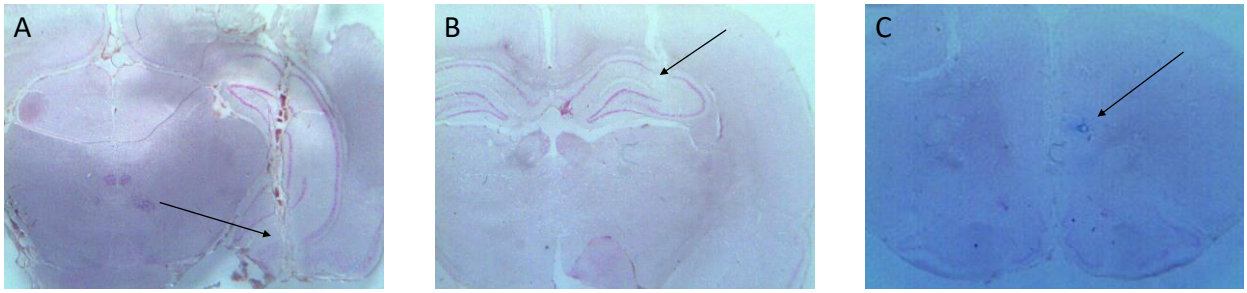


Figure 2 Visual representation of THC micro-infusions in the A) Ventral hippocampus, B) Dorsal hippocampus and electrode placement in the C) Prefrontal cortex.

2.3 Surgical Procedures

Surgical procedures began at least 3 days after arrival once the rats had weighed at least 300 g. Rats for behavioural testing were anesthetized with an intraperitoneal injection of ketamine (80 mg/mL, Vetoquinol)- xylazine (6 kg/kg, Bayer) mixture. To reduce pain and inflammation, meloxicam (1 mg/kg; s.c., Boehringer Ingelheim) was administered before and after surgeries. The rats were positioned in a Kopf stereotaxic device designed for cannula implantation. Five-millimeter stainless steel guide cannula were implanted bilaterally into the DH for the following coordinates: AP: -4.3 mm L: ± 2.8 mm DV: -2.5 mm from the dural surface, or the VH in the following coordinates: AP: -5.6 mm from bregma, LM: ± 5 mm, DV: -6.8 mm from the dural surface (Figure 3). The guide cannula was secured firmed by jeweler's screws and dental acrylic cement. Post-surgery rats were single housed and behavioural tests began after one week of recovery.

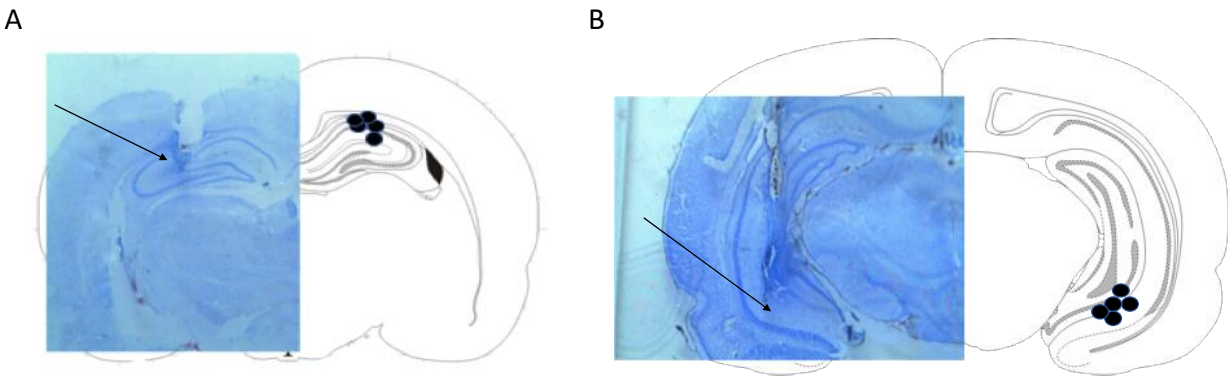


Figure 3 Visual representation of surgical guide cannulas for A) Dorsal hippocampus and B) Ventral hippocampus.

2.4 Intra-VH/DH micro infusions

Intra-DH or -VH micro infusions of either vehicle (VEH, chremophore, NaCl 0.9% mixture) or THC (100 ng/0.5µl) were performed prior to the start of behavioural experiments. A total volume of 0.5 µl per hemisphere was delivered via an injector over a period of 1 minute. Microinjectors were left in place for an additional 1-minute following infusion to ensure adequate diffusion from the tip.

2.5 Behavioural Testing

2.5.1 ***Novel Object Recognition*.** This task measures the ability of the rat to discriminate between a familiar object previously encountered and a novel object. Normal rats will spend more time exploring the novel as opposed to the familiar object. Rats were tested in an 80 cm x 80 cm open roofed arena. They were habituated to the test arena for 20 minutes 1 day before testing. There are two trials in this test, each three minutes long. The first trial is the acquisition trial, where each rat was placed in an arena containing two identical objects in the far corners, 15 cm from the side wall. The rats were freely able to explore the objects after which there was a one-hour delay period before the next trial. During this delay period rats were placed back in their home cage. During the second trial one object was replaced by a novel object in the same location as the previous object. Between all rats both the role (novel or familiar) and relative position of the object was counterbalanced. Object exploration was considered when the head of the rat was facing the object and/or the rat was sniffing the object. Times spent in exploration were videotaped with a video-tracking software (ANY-maze; Stoelting). N: VH-VEH:9, VH-THC:10, DH-VEH:11, DH-THC:9

2.5.2 ***Spatial Object Recognition*.** This paradigm measures the ability of rodents to discriminate between on object in a familiar spatial position versus an object in a novel spatial position. Similarly, with novel objection recognition, normal rats will explore the object located in the novel position. Rats were tested in an 80 cm x 80 cm open roofed arena. This paradigm follows the same protocol as novel objection recognition with one notable difference. During the second trial instead of replacing one object with a novel object, one of the objects from the first trial will be moved to a different location in the arena. Time spent in exploration were

videotaped with a video-tracking software (ANY-maze; Stoelting). N: VH-VEH:8, VH-THC:8, DH-VEH:9, DH-VEH:8

2.5.3 ***Object Recognition in Temporal Order*.** This task measure temporal order memory otherwise known as the ability to distinguish past experiences in the order in which they occurred. Rats are habituated to the same arena used for novel object recognition 24 h before testing day. This test consists of two acquisition trials, followed by a test trial. The first trial is the same as the trial described in novel object recognition, where the rats can freely explore two identical objects. Following the one-hour delay period, during the second trial rats are placed back into the arena but the objects from the previous trial have been switched with a different set of identical objects. After another one-hour delay period rats are placed back into the arena. This time, one of the two objects will be the object from the first trial and the other will be the object from the second trial. Between all rats both the role (first or second) and relative position of the object was counterbalanced. Object exploration was considered when the head of the rat was facing the object and/or the rat was sniffing the object. Times spent in exploration were videotaped with a video-tracking software (ANY-maze; Stoelting). N: VH-VEH:10, VH-THC:9, DH-VEH:8, DH-THC:8

2.5.4 ***Spontaneous Alternation Task*.** This task measures for spatial working memory. A Y-maze was used for this test. During the beginning of this task each rat is placed at the end of one arm facing the wall. All rats started in the same arm. For 15 minutes each rat is freely able to explore and alternate between arms. Arm entry was considered when roughly 80% of a rat's body was in one arm. A set of alternations was defined as entry into all three arms consecutively. Each trial was video recorded. N: VH-VEH: 10, VH-THC:10, DH-VEH: 9, DH-THC:8

2.5.5 ***Social motivation and social cognition***. Rats were tested using a social interaction procedure as described previously (Loureiro et al., 2015). This task assesses socially motivated behaviour as well as social recognition memory. Rats are habituated to the 3-chamber apparatus for 13 minutes a day before testing. Testing consisted of two successive 8-minute phases. The initial phase tests for social motivation. In this phase, rats are placed in the centre chamber of the apparatus. The other two chambers contain either a stranger male rat enclosed in a small wire cage, or an empty wire cage. Time spent with the stranger rat and empty cage was measured. In the next phase, the empty cage now contained a new stranger rat and as such, the rat from the first phase becomes the familiar rat. Time spent with the stranger rat and familiar rat were

measured. The locations of the stranger rat versus the familiar rat was counterbalanced between the left and right chambers. Time exploration was analyzed using a video-tracking system (ANY-maze; Stoelting). After each test, chamber and cages were cleaned with a 50% ethanol solution to avoid odour cue biases. N: VH- VEH: 8, VH-THC: 10, DH- VEH: 7, DH-THC: 10

2.5.6 ***Light dark box***. This task measures for anxiolytic or anxiogenic- like behaviours. It is based upon a rat's natural aversion to brightly lit environments. The test apparatus consisted of two compartments measured at 50 x 25 x 37 cm. Between the compartments was a 10 cm x 10 cm door. One compartment was dark, covered by a black lid (the dark box), while the other side was light, uncovered and brightly illuminated with a lamp located 120 cm above apparatus floor (the light box). At the start of the experiment, a rat was placed in the light box facing the wall, across from the entry to the dark box. The rat was freely allowed to explore both compartments for a total of 8 minutes. Total time spent in the light versus dark box, first latency from light box to enter dark box and latency time to enter the light box from dark box (latency to second transition) were measured. A zone entry was considered with 80% of the rat's body was in the zone. Experiments were recorded using a video-tracking software (ANY-maze; Stoelting). N: VH-VEH: 14, VH-THC:12. DH-VEH:9, DH-THC:11

2.5.7 ***Context-independent fear conditioning***. This paradigm measures a rat's ability to distinguish between a fear conditioned or unconditioned stimuli. Rats were first habituated to the fear boxes 24 h before conditioning. During conditioning one of two odours, almond or peppermint, were presented. The odour was paired with a subthreshold level (1 sec, 0.4 mA) foot shock to become the conditioned stimulus (CS+). Testing day occurred 24 h after conditioning. During the test phase, rats were re-exposed CS+. Time freezing was recorded for 5 minutes using a video-tracking software (ANY-maze; Stoelting). N: DH-VEH: 8, DH-THC: 9, VH-VEH: 8, VH-THC: 7

2.5.8 ***Open field test***. Rats were placed in an automatic open field activity chamber (San Diego Instruments, San Diego, CA, USA) for 30 minutes. Distance travelled and time spent in the centre versus residual area was recorded and analyzed. N: VH-VEH:8, VH-THC:8, DH-VEH:8, DH-THC:9

2.6 Protein Isolation, Quantification and Western Blots

Prior to sacrificing rats were given a final dose of THC of vehicle. Rats were sacrificed by giving an overdose of sodium pentobarbital (240 mg/kg, i.p., Euthanyl^{TM}). They were then decapitated, brains harvested and flash frozen. Coronal sections (98 μm thick) of either the VH or DH were cut on a cryostat and slide mounted. Bilateral micro-punches of either hippocampal region was obtained for protein isolation. Tissue was collected adjacent to but excluding the site of injection. Protein was isolated using a lysis buffer and quantification was performed using a bicinchoninic acid assay (BCA assay).

For the western blot procedure total protein samples underwent SDS polyacrylamide gel electrophoresis at 120 V, followed by a transfer to a nitrocellulose membrane. The membranes were blocked using a 2.5% non-fat dry milk in TBST solution. Afterwards, the membranes were placed in a 2.5% BSA in TBST solution and probed with the following antibodies with their corresponding dilutions: p-ERK1/2 (1:1000; Cell Signalling Technology), total ERK 1/2 (1:2000; Cell Signalling Technology), p-p70S6 Kinase (1:1000; Cell Signalling Technology), total p70S6 Kinase (1:1000), Alpha-tubulin (1:10,000; Sigma-Aldrich) (loading control). The membranes were left overnight at 4 °C. Species appropriate secondary antibodies were used at a dilution of 1:10,000 in a 2.5% non-fat dry milk TBST solution, and membranes scanned using a Licor scanner.

2.7 Histology

Rats were sacrificed, and brains were harvested and sliced using the method mentioned previously. They were later stained with a cresyl violet dye as described in Loureiro et al., (2015).

2.8 Statistical Analysis

All analysis was done using SPSS (IBM SPSS Statistical Package Version 23), beginning with checking for normal distribution using the Kolmogorov-Smirnov test. The behavioural and electrophysiology data was analyzed using t-tests, or ANOVA where appropriate. Densitometry values for western blots were obtained with the Licor Image Studio Lite software, normalized to alpha-tubulin and analyzed with t-tests. Confidence Interval was set to 95%, $p < 0.05$.

3 Results

3.1 Behavioural Results

Novel Object Recognition

First of all, short-term memory was measured of adult rats who received micro-infusions of THC or VEH in the DH or VH with the novel object recognition paradigm. Two-way repeated measures ANOVA revealed a significant main effect of treatment in the DH on recognition index ($F_{(224)} = 11.845$, $p<0.05$). Similarly, there was also a significant main effect of treatment for the VH groups ($F_{(252)} = 7.443$, $p<0.05$). Intra-VH and intra-DH THC treatment induced deficits in short-term memory deficits compared to the VEH groups (Figure 4).

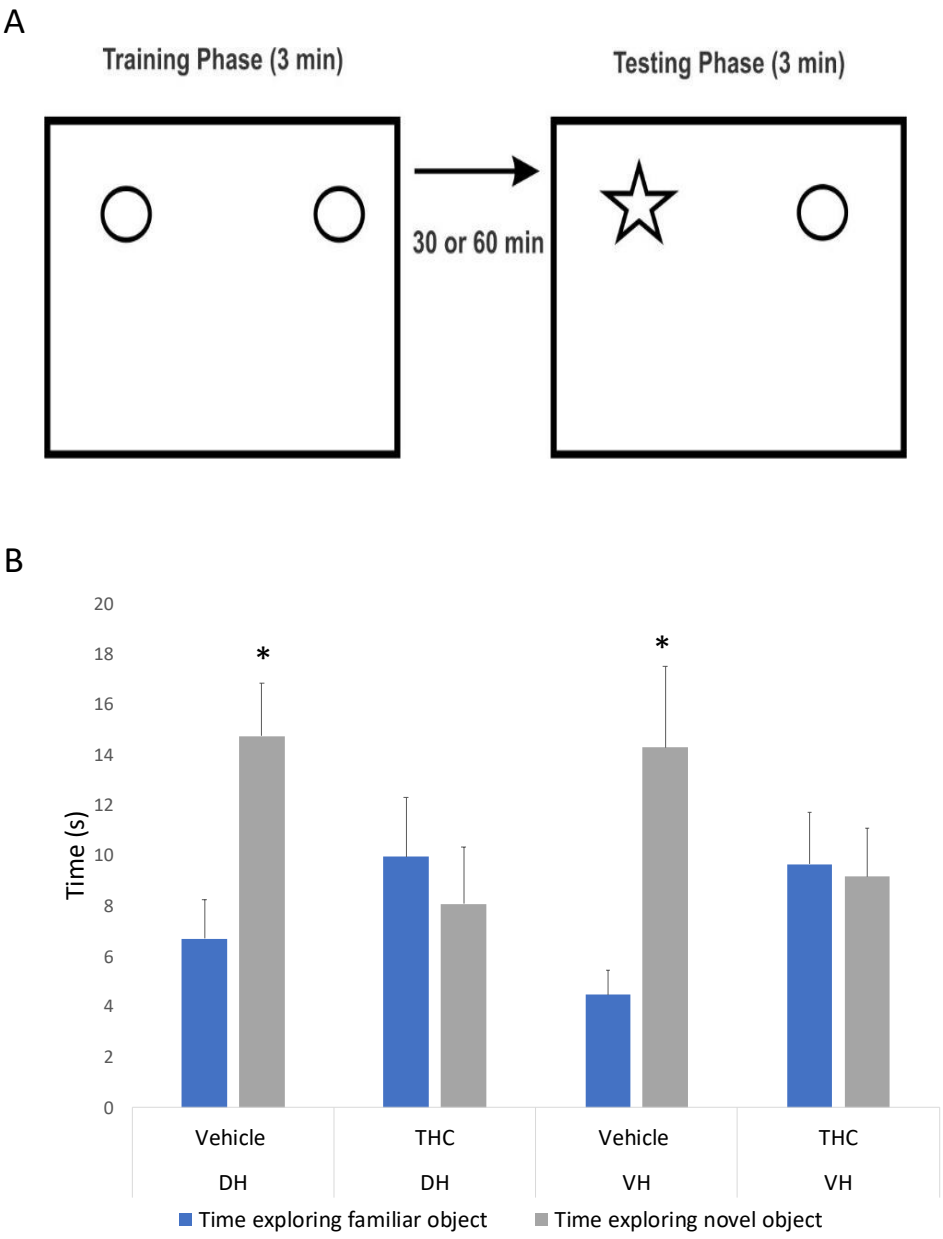


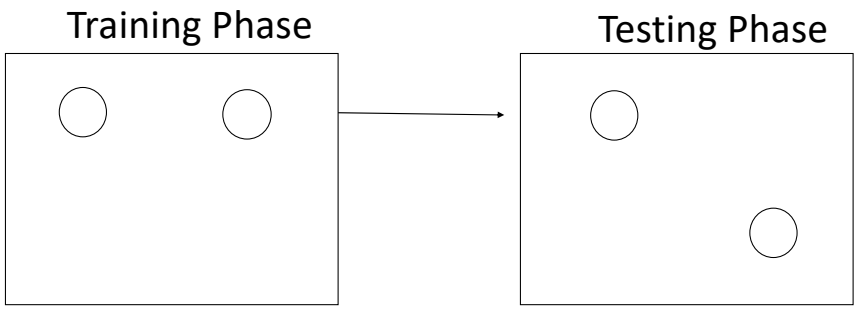
Figure 4 Novel Object Recognition. A) Schematic for novel object recognition paradigm. B) THC DH and VH infused animals displayed no preference for novel or familiar objects. N: VH-VEH:9, VH-THC:10, DH-VEH:11, DH-THC:9, repeated measures ANOVA; *Indicated $p<0.05$, Error bars represent standard error of the means (SEMs).

Spatial Object Recognition

Next spatial short-term memory was measured of adult rats who received micro-infusions of THC or VEH in the DH or VH with the spatial novel object recognition paradigm. Two-way ANOVA revealed no significant main effect of treatment between the VH groups ($F_{(156)} = 3.16$,

p>0.05), however there was a significant effect of treatment between the DH groups ($F_{(557)}$ = 37.295, p<0.05). Intra-DH THC treatment only induced deficits in spatial short-term memory compared to the DH VEH treated group (Figure 5).

A



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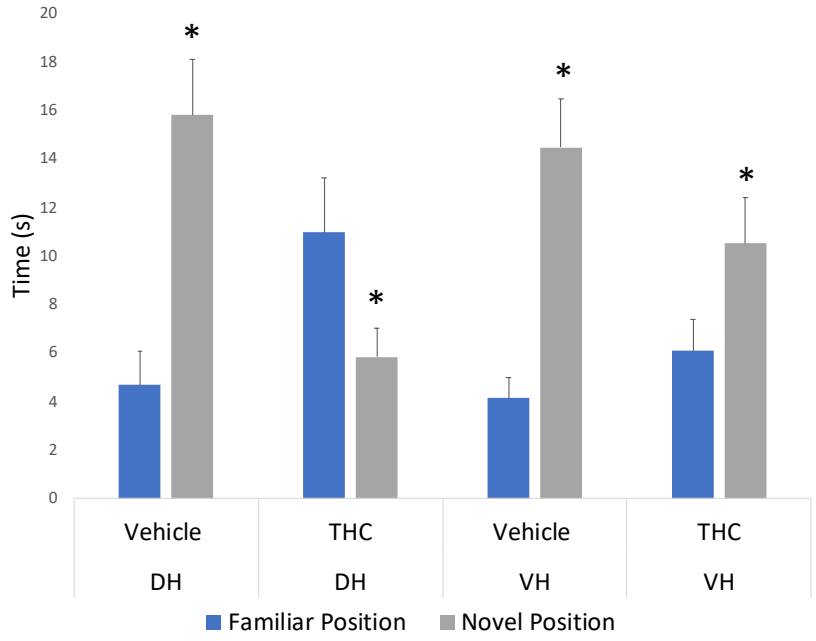


Figure 5 Spatial Object Recognition. A) Schematic for spatial object recognition paradigm. B) THC infused in the DH caused rodents to spend more time exploring the object in the familiar position as opposed to the object in the novel position. N: VH-VEH:8, VH-THC:8, DH-VEH:9, DH-VEH:8, repeated measures ANOVA; *Indicated p<0.05. Error bars represent standard error of the means (SEMs).

Objects in Temporal Order

Next potential differences in temporal order memory was measured between VEH and THC in VH or DH using the objects in temporal order paradigm. Two-way ANOVA revealed a significant effect of treatment between the DH groups ($F_{(277)} = 26.236$, $p<0.05$) and similarly there was also a significant effect between the VH groups ($F_{(451)} = 13.674$, $p<0.05$). Intra-DH and VH THC treatment induced deficits in temporal order memory comparing to the VEH VH and DH treated groups (Figure 6).

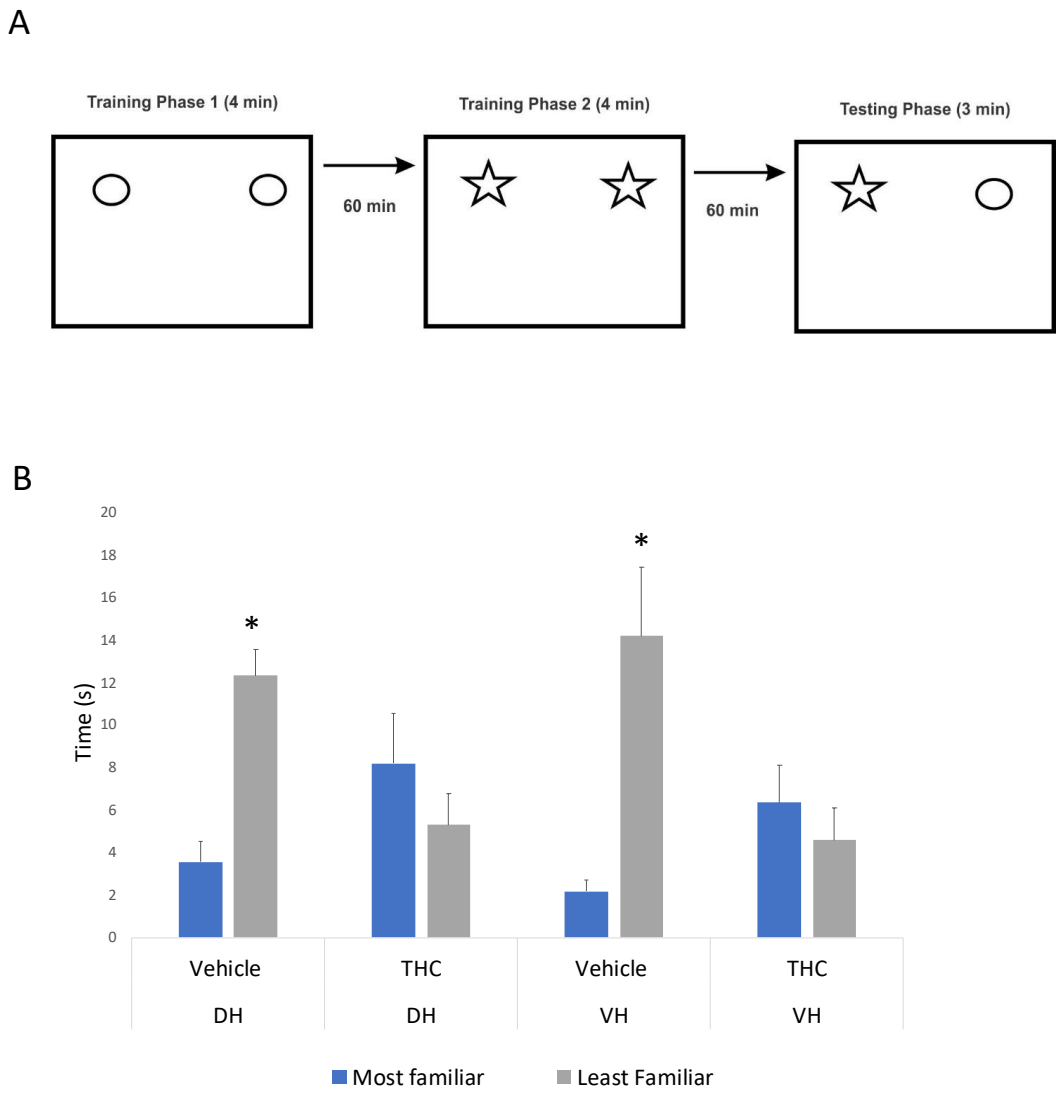


Figure 6 Objects in Temporal Order. A) Schematic of the paradigm. B) Rodents with THC infused in either DH or VH displayed no significant preference for either object. N: VH-VEH:10, VH-THC:9, DH-VEH:8, DH-THC:8, repeated measures ANOVA; *Indicated $p<0.05$. Error bars represent standard error of means (SEMs).

Spontaneous Alternations

Working memory differences between intra-VH and DH, THC and VEH groups was measured using the spontaneous alternations paradigm. Thus far, all analysis between VH and DH have been performed separately and to keep this consistent, t-tests were utilized to measure for significant differences. Two-tailed t-test revealed a significant difference between the intra-VH THC and VEH treated animals ($t_{(18)} = -4.936$, $p<0.05$) but no significant different between the intra-DH THC and VEH treated animals ($t_{(15)} = -1.496$, $p>0.05$). Intra-VH THC treatment but not DH THC treatment induced working memory deficits when compared to the VEH treatment (Figure 7).

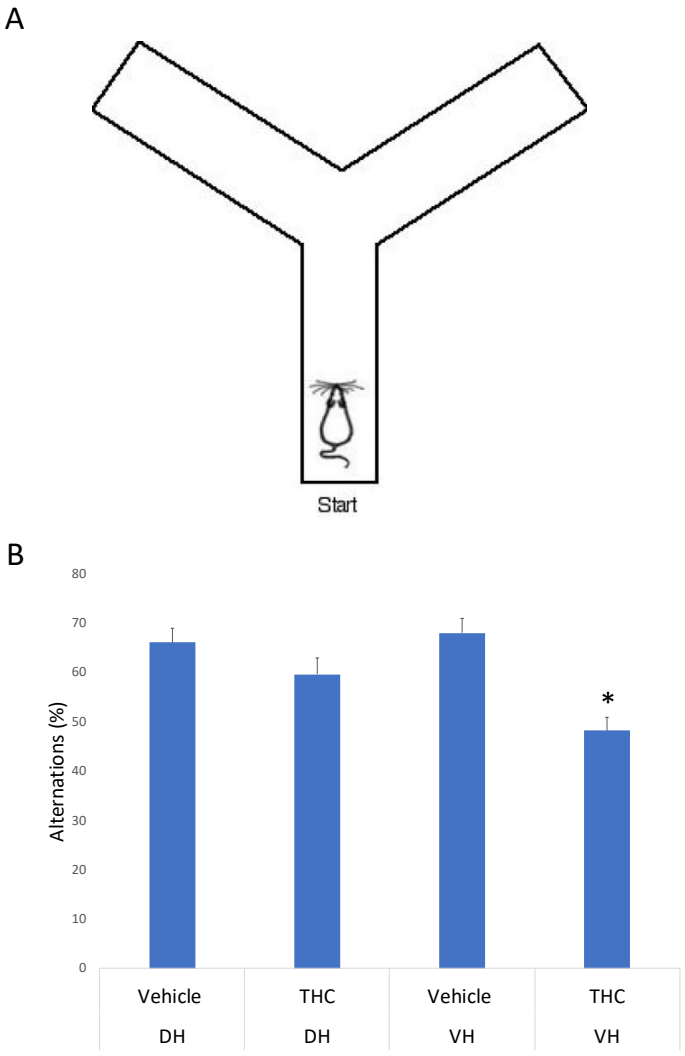


Figure 7 Spontaneous Alternations. A) Schematic of Y-maze. B) THC infused in VH caused a significant reduction in alternations. N: VH-VEH: 10, VH-THC:10, DH-VEH: 9, DH-THC:8, t-tests; *Indicated $p<0.05$. Error bars represent standard error of means (SEMs).

Social Interaction

To test for sociability and social cognition between intra-VH/DH THC and VEH groups, the social motivation and recognition tests were utilized. For the intra-DH groups, all groups, regardless of treatment, spent a significantly longer amount of time exploring the stranger rat as opposed to the empty box. A main effect for treatment was not observed ($F_{(399)} = 0.385$, $p>0.05$). Similarly, using a two-way ANOVA to compare the VH treatment groups, a significant main effect for treatment was not observed ($F_{(980)} = 1.264$, $p>0.05$). THC did not induce deficits in social motivation (Figure 8). Two-way ANOVA between the DH treatment groups, for the social recognition test, demonstrated no main effect for treatment ($F_{(43)} = 0.071$, $p>0.05$). However, between the VH treatment groups a significant main effect for treatment was noted ($F_{(7877)} = 8.225$, $p<0.05$). Intra-VH THC treatment only induced deficits in social recognition memory (Figure 8).

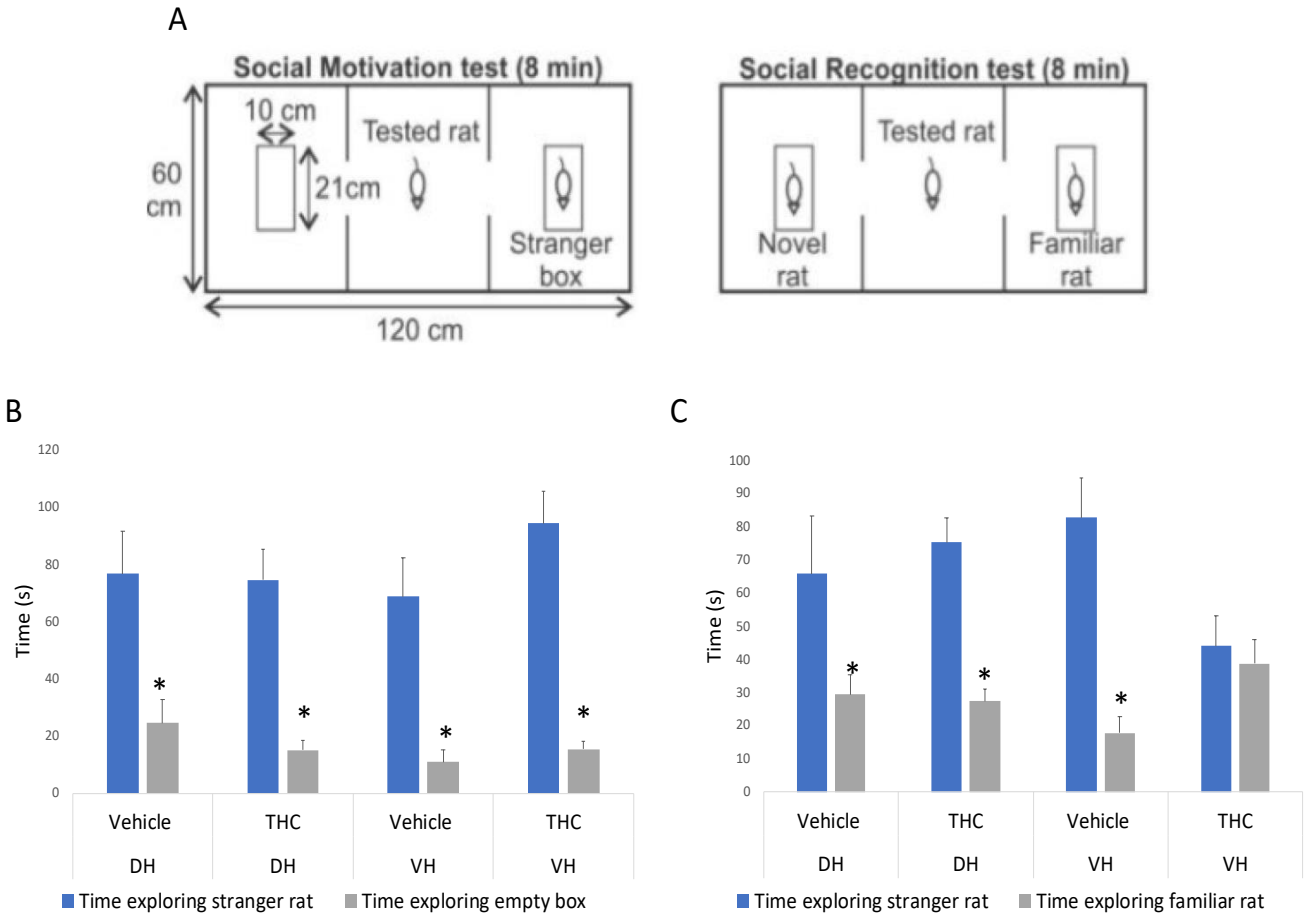


Figure 8 Social Interaction. A) Schematic of social interaction arena. B) Social Motivation test: All rodents spent significantly longer time exploring the stranger rat as opposed to the empty box. C) Social Recognition test: Rodents

that received THC infusions in the VH displayed no preference for stranger or familiar rat. N: VH- VEH: 8, VH-THC: 10, DH- VEH: 7, DH-THC: 10, repeated measures ANOVA; *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Light/Dark Box

To measure levels of anxiety between THC and VEH groups, the light/dark test was used (Figure 9). Two-tailed t-tests found no significant differences in transition to first latency between THC and VEH in VH ($t_{(28)} = -0.382$, $p>0.05$) or DH ($t_{(20)} = -0.642$, $p>0.05$). A significant increase in the transition to second latency was found between intra-VH THC and VEH treated animals ($t_{(14)} = 3.467$, $p<0.05$) but not in intra-DH treated animals ($t_{(21)} = 0.158$, $p>0.05$). Finally, repeated measures ANOVA found no main effect for treatment in time spent between light side and dark side for VH ($F_{(406)} = 1.035$, $p>0.05$) and DH ($F_{(589)} = 2.324$, $p>0.05$). All groups spent more time in the dark side as opposed to the light side (Figure 9).

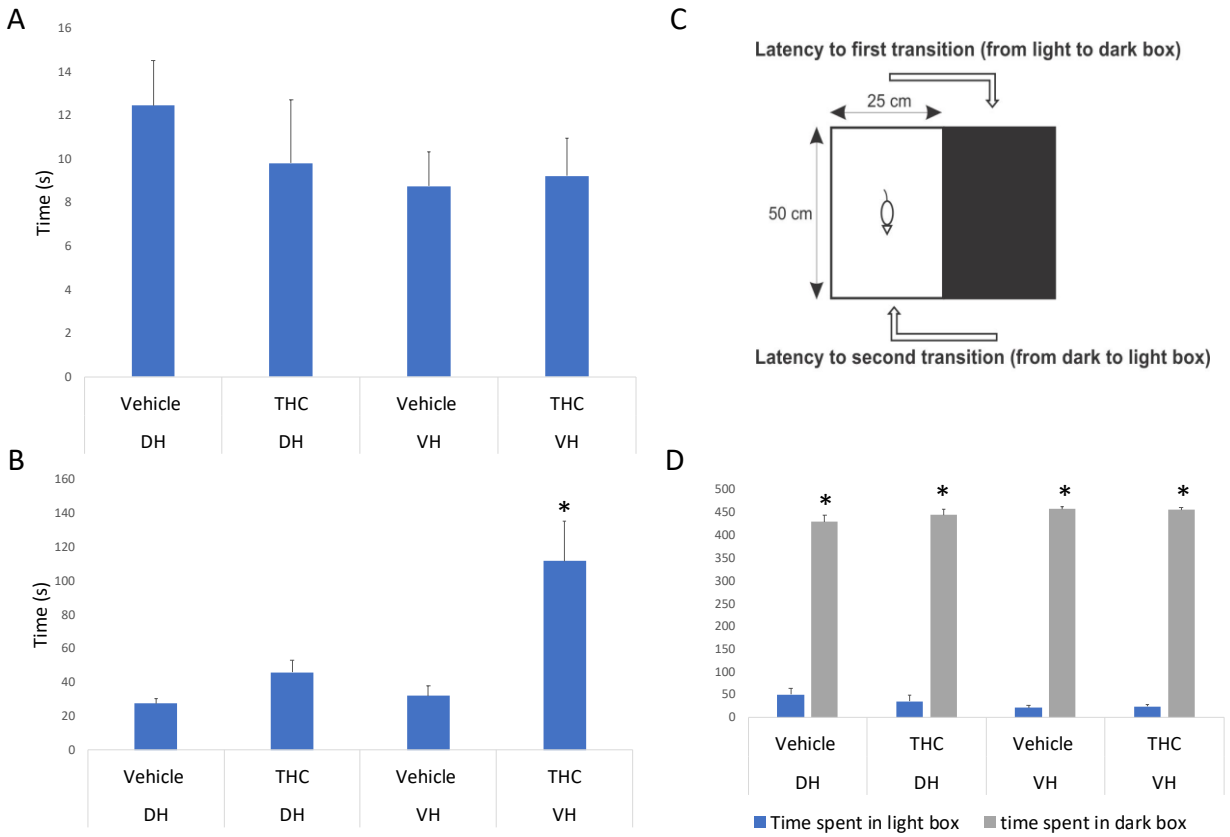


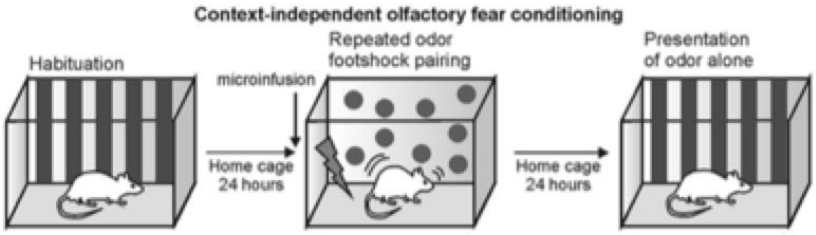
Figure 9 Light dark box. A) No significant difference from transitioning from light to dark side (first transition) B) Rodents with THC infusion in the VH spent a significantly longer time transitioning from dark side to light side

(second transition). C) Schematic for light/dark box paradigm. D) All rodents spent a significantly longer time in dark box as opposed to light box. N: VH-VEH: 14, VH-THC:12. DH-VEH:9, DH-THC:11, t-tests, repeated measures ANOVA; *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Fear Conditioning

To measure for affective learning and memory between THC and VEH groups, context-independent fear conditioning paradigm was utilized. Similar to the spontaneous alternations paradigm, t-tests were utilized to measure for significant differences in fear expression. Two-tailed t-test found a significant increase in percent freezing between intra-VH THC and VEH treated animals ($t_{(13)} = 4.248$, $p<0.05$). No significant differences were observed between intra-DH THC and VEH treated animals ($t_{(15)} = 0.259$, $p>0.05$). Intra-VH THC animals only displayed an increase in emotional saliency (Figure 10).

A



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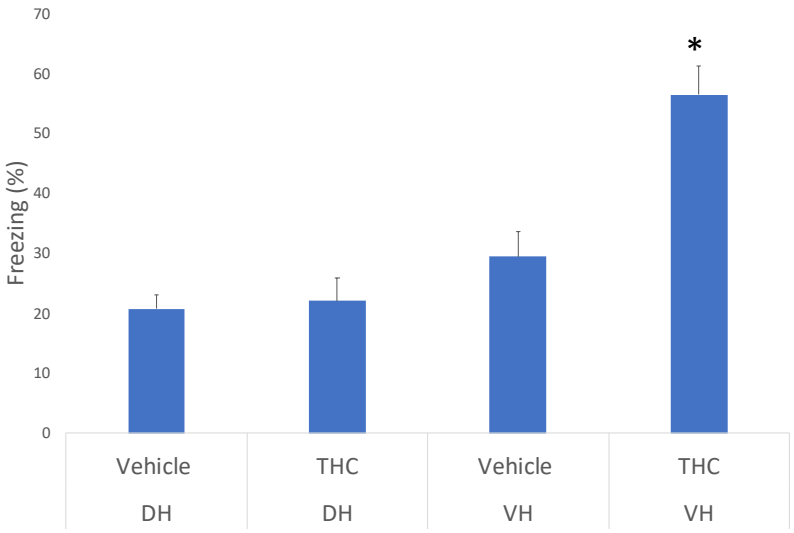

Figure 10 Context independent fear conditioning. A) Schematic for fear conditioning paradigm. B) Significant increase in freezing observed in intra-VH THC treated animals only. N: DH-VEH: 8, DH-THC: 9, VH-VEH: 8, VH-THC: 7; t-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Open Field

The open field test was utilized to determine deficits in locomotion and anxiety levels (Figure 11). Two-tailed t-tests determined no significant difference in total distance travelled for

intra-VH groups ($t_{(14)} = -0.781$, $p>0.05$) and intra-DH groups ($t_{(15)} = 1.510$, $p>0.05$). Furthermore, repeated measures ANOVA determined no significant differences in treatment between distance travelled in the periphery vs. center in intra-VH groups ($F_{(549998)} = 1.610$, $p>0.05$) and intra-DH groups ($F_{(349156)} = 1.029$, $p>0.05$). All groups spent a longer amount of time travelling in the periphery than in the center.

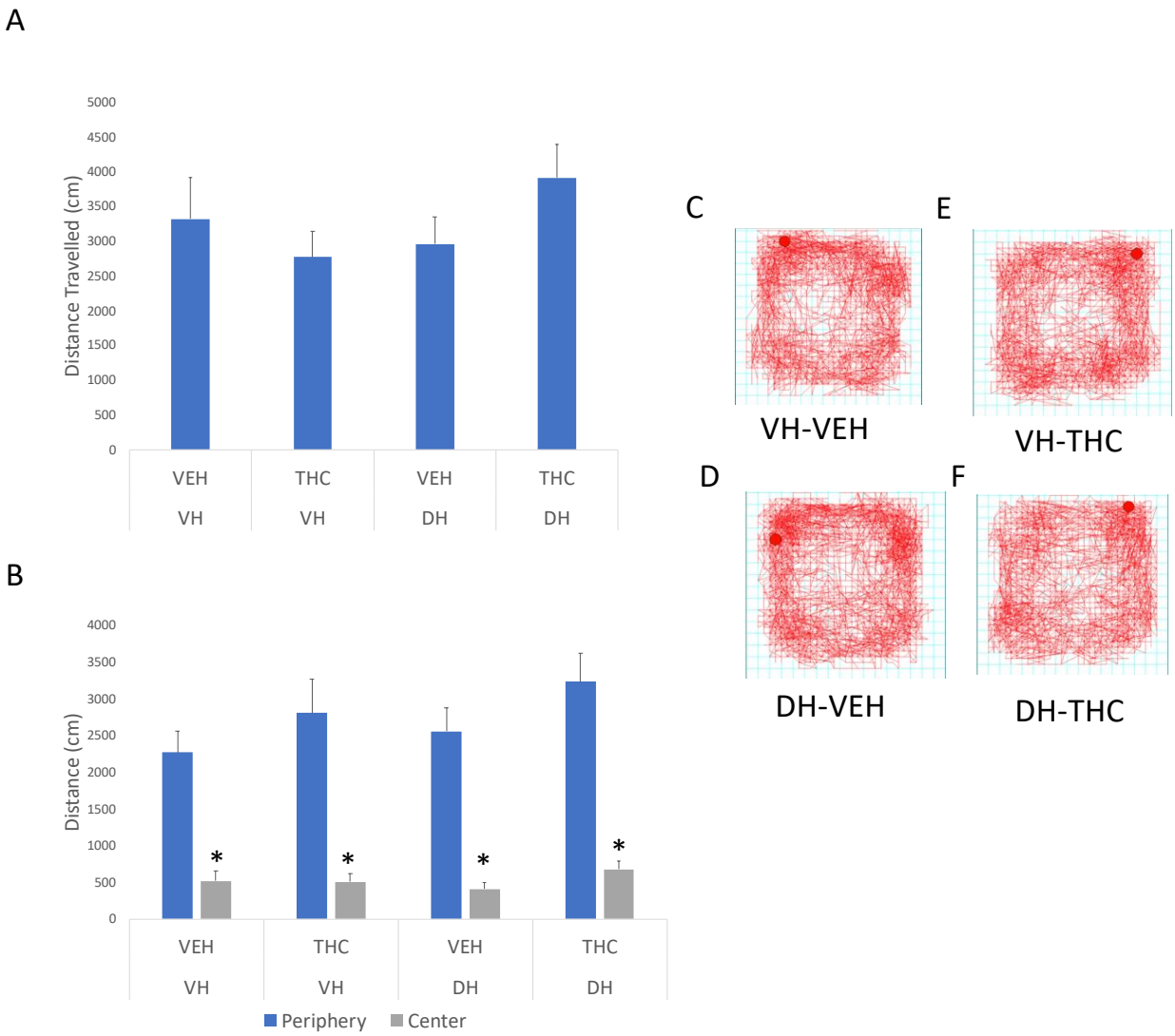

Figure 11 Open field maze. A) No significant differences in total distance travelled between all groups. B) All groups spent significantly longer in the periphery than the center. C) Representative pictogram of intra-VH VEH treated rat's movement pattern over 30 minutes. D) Representative pictogram of intra-DH VEH treated rat's movement pattern over 30 minutes. E) Representative pictogram of intra-VH THC treated rat's movement pattern over 30 minutes. F) Representative pictogram of intra-DH THC treated rat's movement pattern over 30 minutes. N:

VH-VEH:8, VH-THC:8, DH-VEH:8, DH-THC:9; t-tests, repeated measures ANOVA, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

3.2 *In vivo* Electrophysiology Results

Single Unit Firing and Bursting data

To determine whether THC infusions in the DH or VH affected neuronal activity in the PFC, electrophysiology was utilized. After intra-VH VEH treatment, 60% of mPFC cells increased, 30% of cells decreased and 10% displayed no change (Figure 12 top left). Intra-VH THC treatment caused 36% of cells to increase firing, 44% to decrease and 20% displayed no change (Figure 12 top right). Intra-DH VEH treatment caused 46% of cells to increase, 37% to decrease and 16% displayed no change in firing (Figure 12 bottom left). Finally, intra-DH THC treatment caused 44% of cells to increase firing, 41% to decrease in firing and 15% displayed no chance (Figure 12 bottom right).

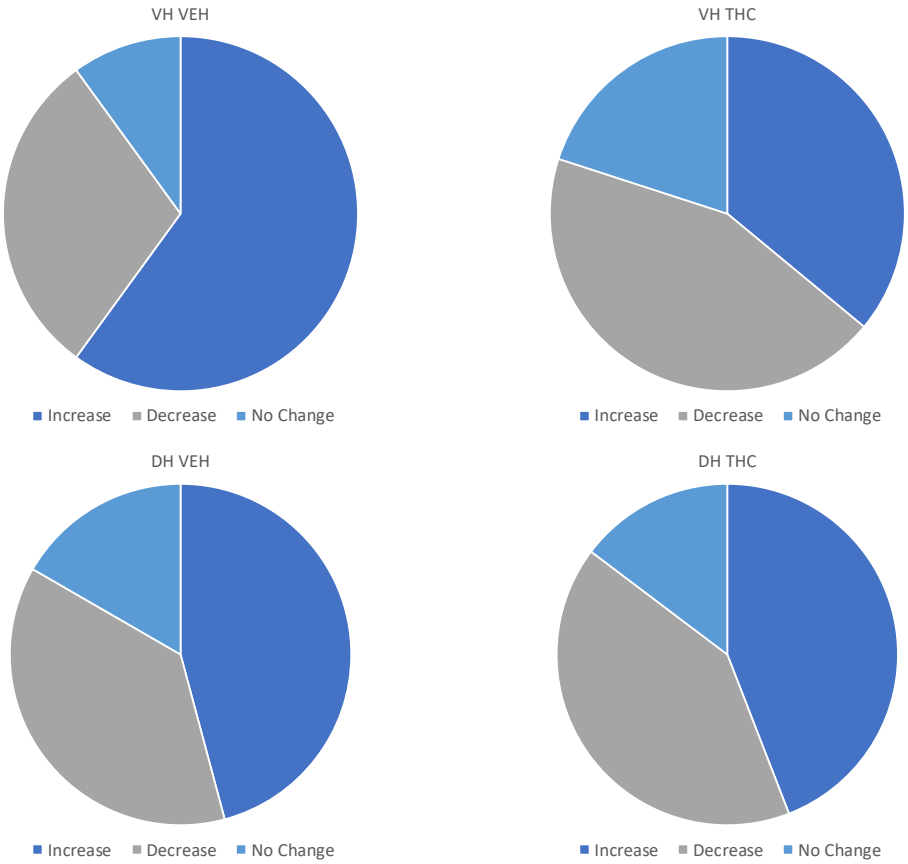

Figure 12 Single Unit cell firing changes after THC or VEH treatment.

Cell firing and bursting activity was measured in the mPFC using single unit *in vivo* electrophysiology (Figure 13). Two-tailed t-test found cells decreasing firing after intra-VH THC treatment was significant compared to the VEH group ($t_{(21)} = -4.126$, $p<0.05$) and cells increasing firing after intra-DH THC treatment was significant compared to the VEH treated group ($t_{(32)} = 6.051$, $p<0.05$) (Figure 13). Cells with a decrease in bursting activity after intra-VH THC treatment was significant compared to the VEH group ($t_{(23)} = -2.255$, $p<0.05$) (Figure 13). Additionally, cells increasing in bursting activity after intra-DH THC treatment was significant compared to the VEH group ($t_{(28)} = 4.104$, $p<0.05$).

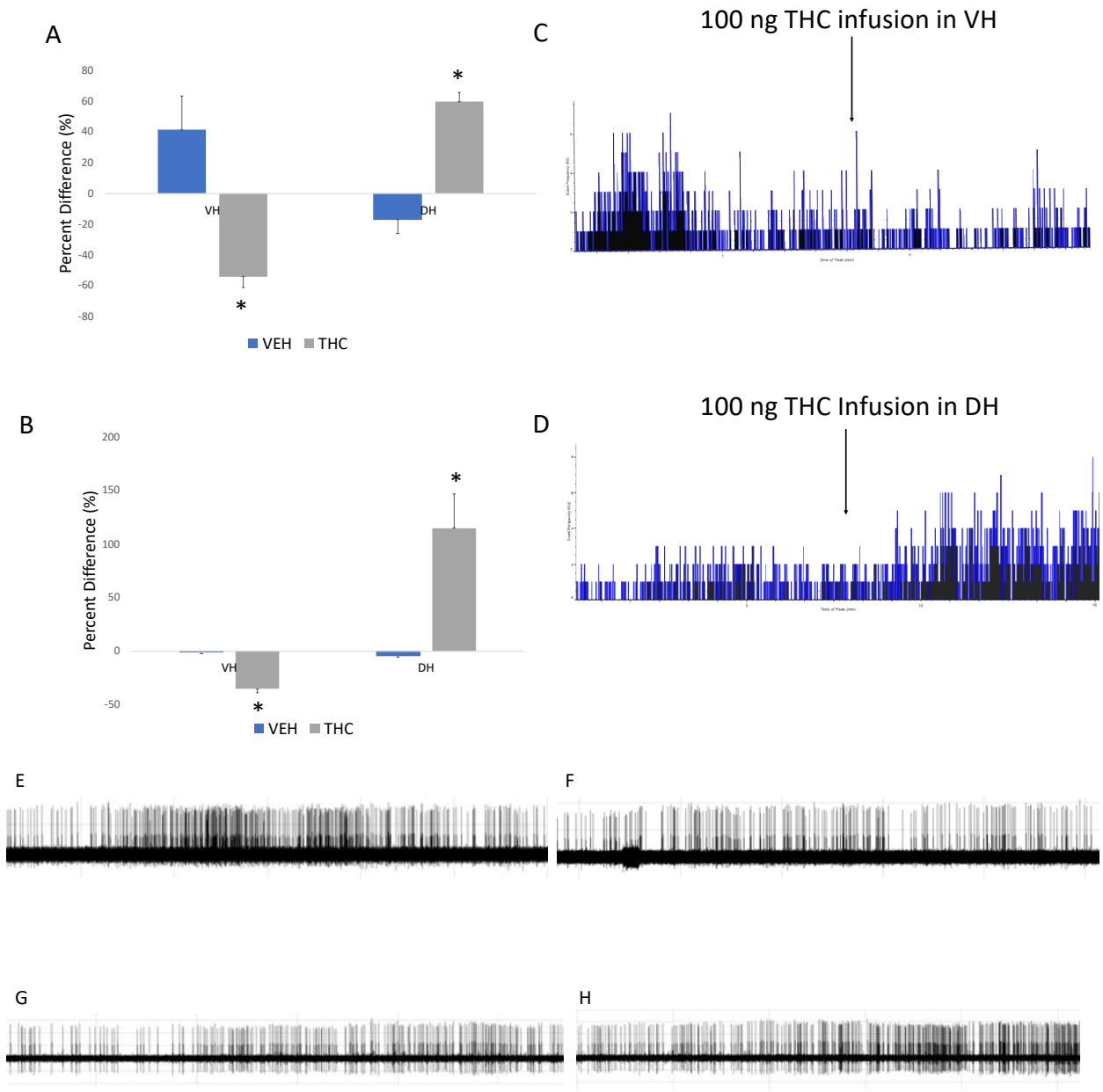

Figure 13 Single Unit In-vivo Electrophysiology in mPFC. A) Intra-VH THC treatment induced a significant decrease in cell firing and intra-DH THC treatment induced a significant increase in cell firing. B) Intra-VH THC treatment induced a significant decrease in cell bursting and intra-DH THC treatment induced a significant increase in cell firing. C) Representative histogram of mPFC cell decreasing cell firing after THC treatment in VH. D) Representative histogram of mPFC cell increasing firing after THC treatment in DH. E) Raw spike firing before intra-VH THC micro infusion. F) Raw spike firing after intra-VH THC micro infusion. G) Raw spike firing before intra-DH THC micro infusion. H) Raw spike firing data after intra-DH THC micro infusion. N: VH-VEH:19, VH-THC:13, DH-VEH:19, DH-THC:8; t-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Furthermore, two-tailed t-tests revealed cells that increased in firing after intra-VH THC treatment was not significant ($t_{(26)} = 0.469$, $p>0.05$) and neither were cells that decreased in firing after intra-DH THC treatment ($t_{(31)} = -2.033$, $p>0.05$). There was also no significance with cells that increased in burst firing after intra-VH THC treatment ($t_{(20)} = 2.012$, $p>0.05$) or cells that decreased bursting activity after intra-DH THC treatment ($t_{(25)} = -1.629$, $p>0.05$) (Figure 14).

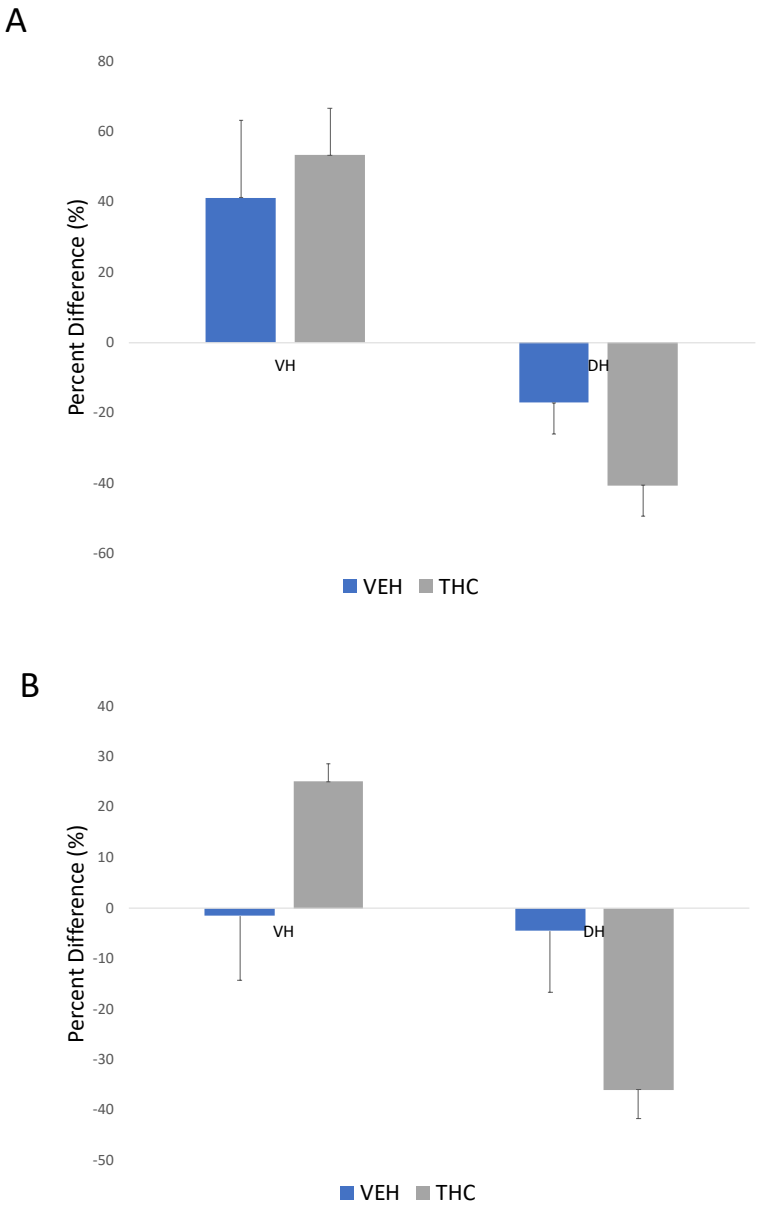

Figure 14 Single Unit In-vivo Electrophysiology in mPFC. A) No significant changes for cells that increased firing after intra-VH THC treatment or decreased firing after intra-DH THC treatment. B) No significant changes in cells

that increased in bursting activity after intra-VH THC treatment or decreased after intra-DH THC treatment. N: VH-VEH:19, VH-THC:11, DH-VEH:19, DH-THC: 6; t-tests, Error bars represent standard error of means (SEMs).

Synchronized Oscillatory Activity

Brain wave oscillatory activity is often disrupted after THC treatment as well as in various mental health disorders such as schizophrenia. Two-tailed t-test determined a significant decrease in beta oscillations in intra-VH THC treated animals compared to its VEH group ($t_{(55)}$ = -2.303, $p<0.05$) but no such difference between the DH treated animals ($t_{(20)}$ = -1.207, $p>0.05$). There was also a significant decrease in gamma oscillations in the intra-VH THC treated animals compared to the VEH group ($t_{(47)}$ = -2.465, $p<0.05$) but no such difference between the DH treated animals ($t_{(37)}$ = 0.616, $p>0.05$) (Figure 15).

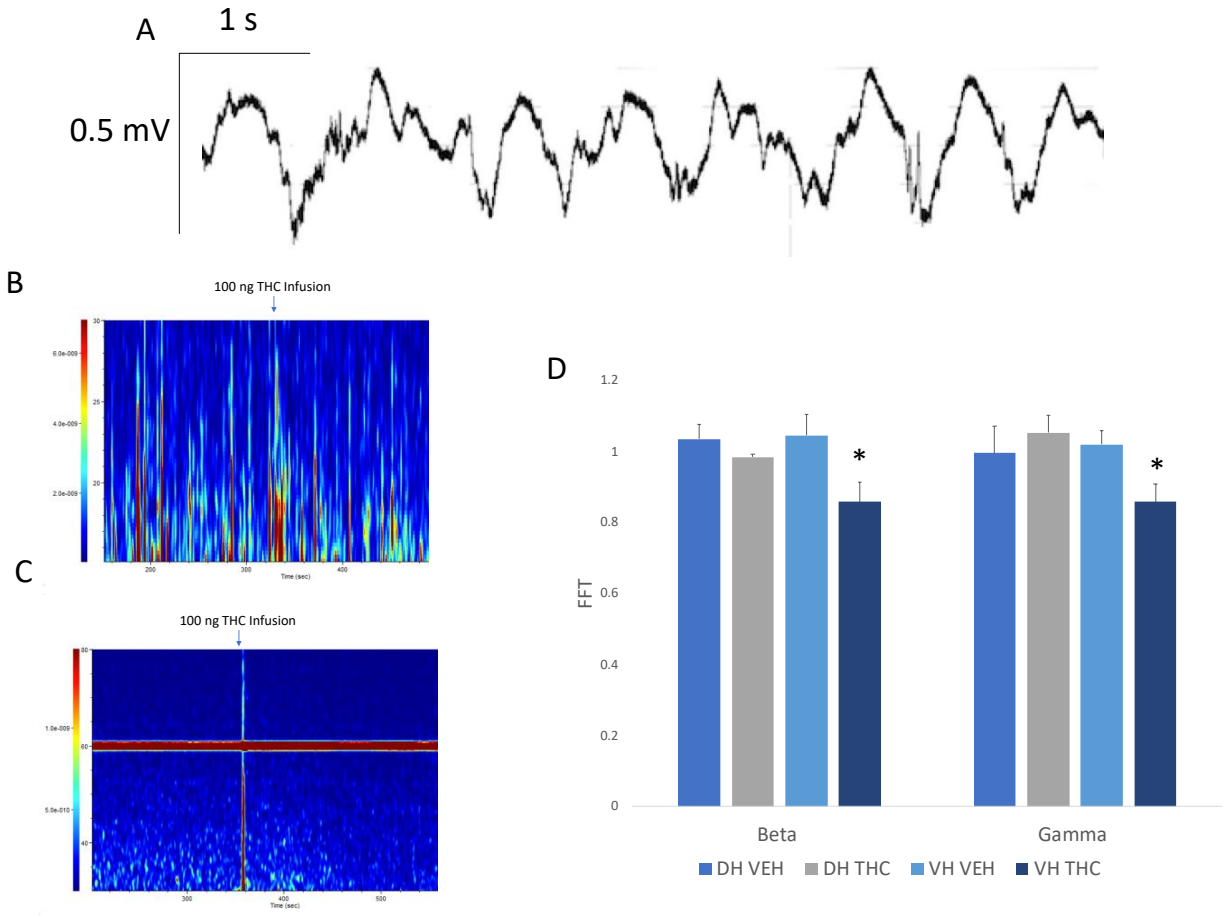

Figure 15 Synchronized oscillatory activity in mPFC. A) Synchronized waveform B) Representative heat map of beta oscillations decreasing after THC treatment in VH. C) Representative heat map of gamma oscillations decreasing after THC treatment in VH. D) Intra-VH THC treatment only caused a significant decrease in beta and

gamma oscillatory activity. N: VH-VEH: 29, VH-THC:18, DH-VEH: 21, DH-THC: 18; t-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

No significant differences in delta ($t_{(55)} = 1.230$, $p>0.05$), theta ($t_{(52)} = 0.527$, $p>0.05$), and alpha ($t_{(52)} = -0.706$, $p>0.05$) oscillations between the VH THC treated and VH VEH treated animals. Additionally, delta ($t_{(36)} = 0.748$, $p>0.05$), theta ($t_{(37)} = -0.649$, $p>0.05$), and alpha ($t_{(41)} = -0.666$, $p>0.05$) oscillations were not significantly different between the DH THC and DH VEH groups (Figure 16).

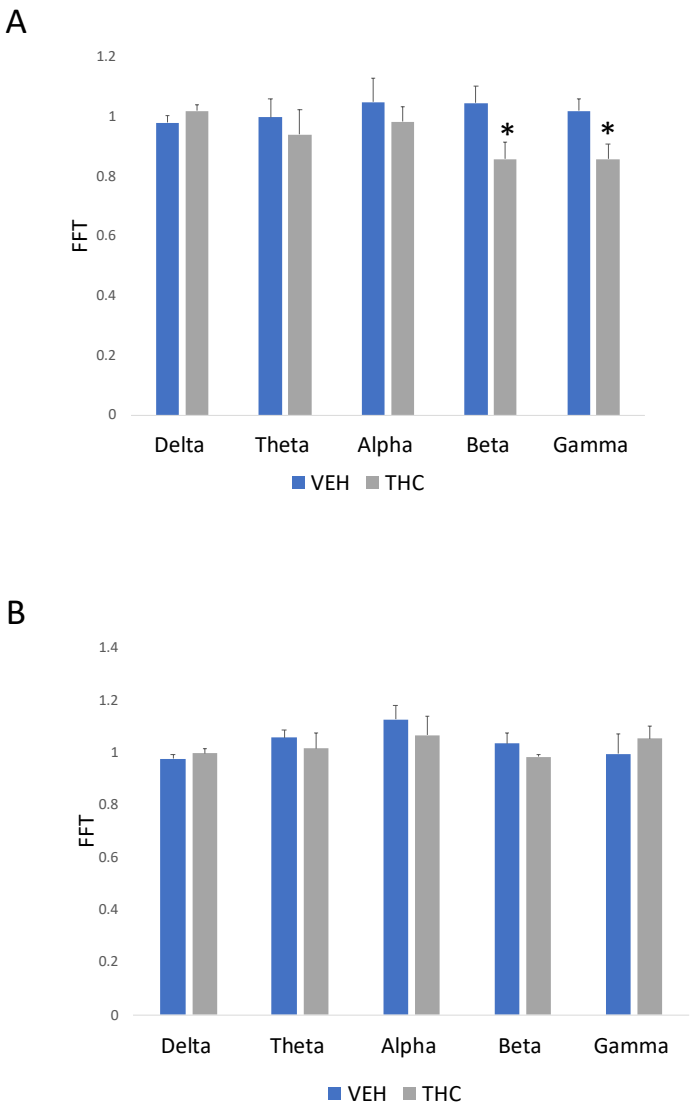

Figure 16 Synchronized Oscillations in mPFC. A) Significant decrease in beta and gamma oscillations only in intra-VH THC treated animals. B) No significant differences in oscillatory activity in intra-DH treated animals. T-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Desynchronized Oscillatory Activity

Two-tailed t-test revealed a significant decrease in alpha oscillations in the intra-DH THC group compared to the VEH group ($t_{(24)} = -2.207$, $p<0.05$), but no significant differences between the intra-VH THC and VEH groups ($t_{(34)} = 0.262$, $p>0.05$) (Figure 17).

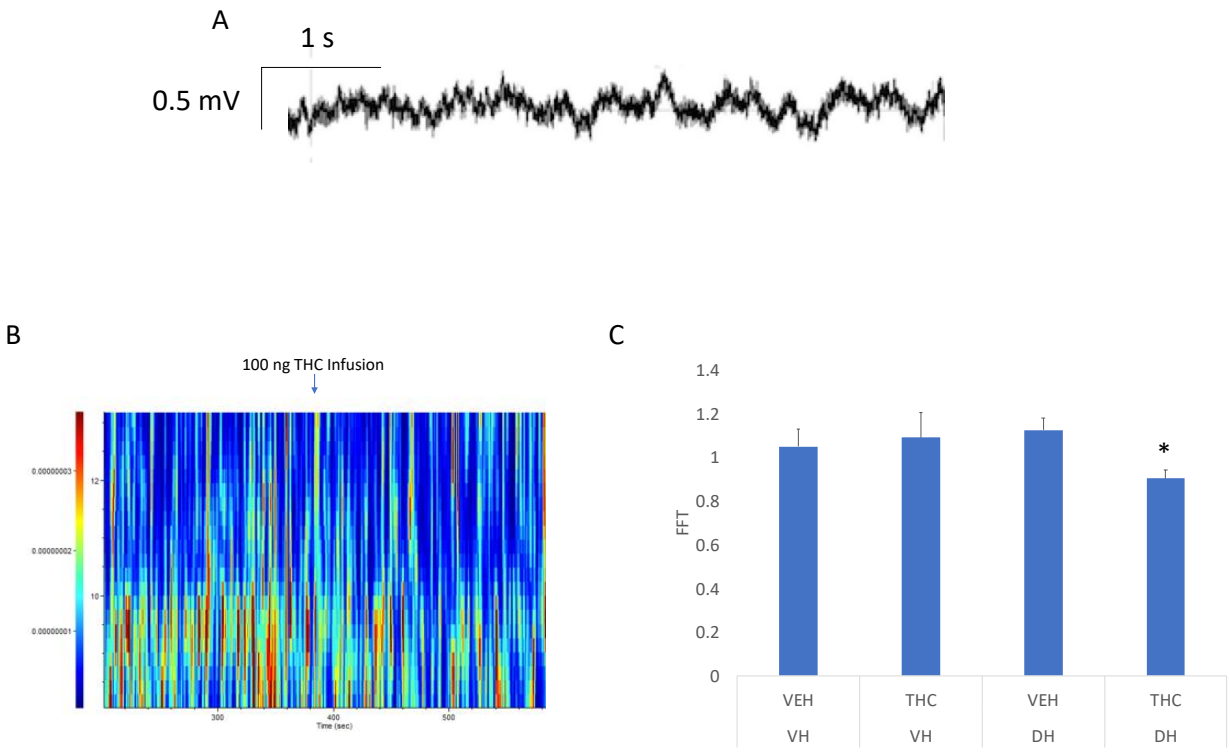

Figure 17 Desynchronized oscillatory activity in mPFC. A) Desynchronized waveform. B) Representative heat map demonstrating a decrease in alpha oscillations after intra-DH THC treatment. C) Intra-DH THC treatment only caused a significant decrease in alpha oscillatory activity. N: VH-VEH: 28, VH-THC: 8, DH-VEH:20, DH-THC: 6; t-test, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Furthermore, no significant differences were found in delta ($t_{(36)} = 0.558$, $p>0.05$), theta ($t_{(31)} = 1.631$, $p>0.05$), beta ($t_{(35)} = 0.650$, $p>0.05$), and gamma ($t_{(30)} = 0.041$, $p>0.05$) oscillatory activity between the intra-VH THC and VEH groups. There were also no differences found in the delta ($t_{(9)} = 0.182$, $p>0.05$), theta ($t_{(26)} = -1.985$, $p>0.05$), beta ($t_{(9)} = 1.216$, $p>0.05$) and gamma ($t_{(28)} = 1.262$, $p>0.05$) oscillations between the intra-DH THC and VEH groups (Figure 18).

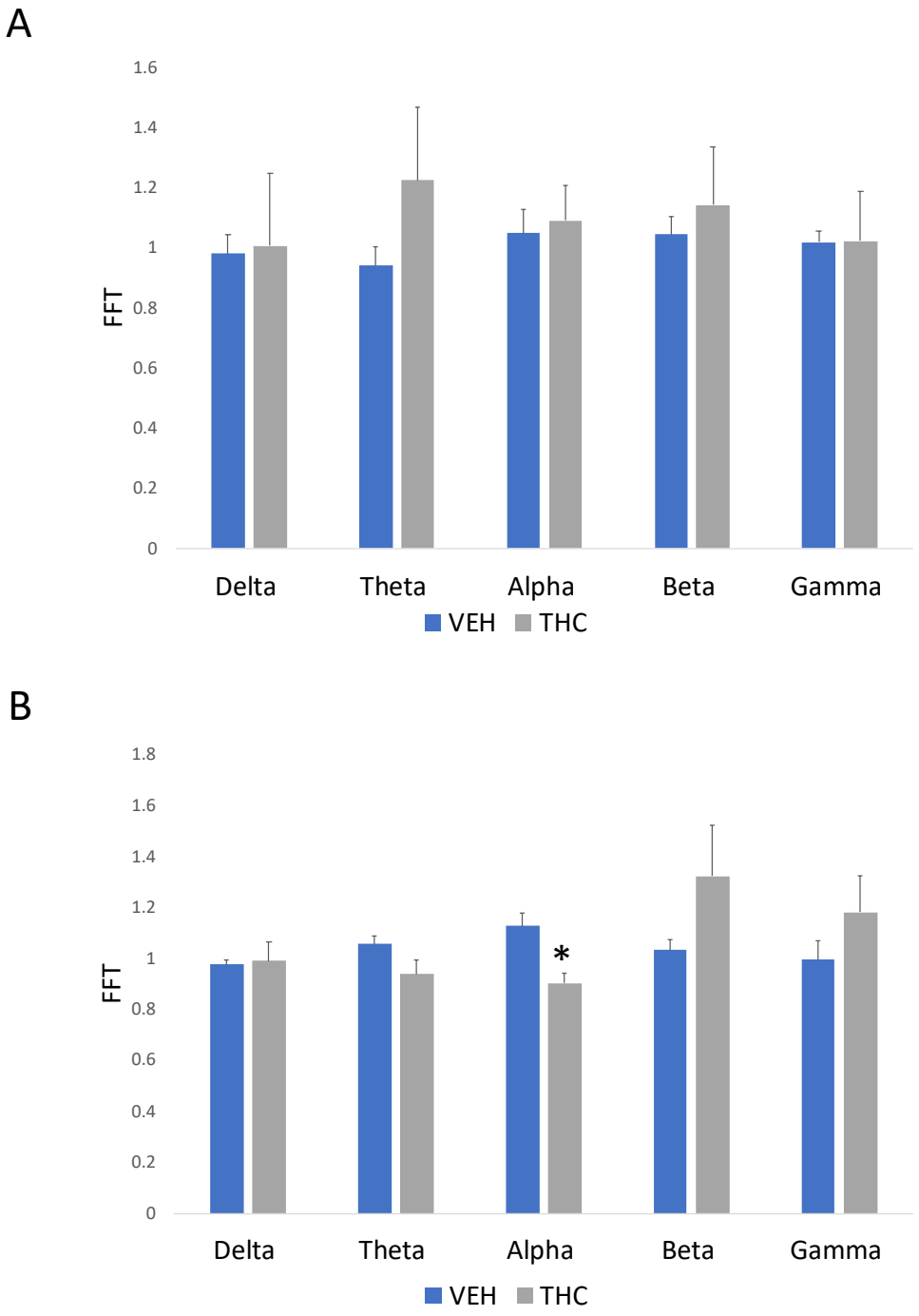

Figure 18 Desynchronized Oscillations in mPFC. A) No significant differences in oscillatory activity in intra-VH treated animals. B) Significant decrease in alpha oscillations only in intra-DH THC treated animals. T-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

3.3 Molecular Results

To determine any changes in protein expression that may have contributed to cognitive or emotional deficits, western blots on VH and DH tissue was performed. Two-tailed t-test revealed a significant increase in phosphorylated ERK 1 ($t_{(6)} = 2.542$, $p<0.05$) and ERK 2 ($t_{(6)} = 2.445$, $p<0.05$) expression in intra-VH THC treated animals compared to VEH. There were no significant differences in phosphorylated ERK 1 ($t_{(6)} = 0.374$, $p>0.05$) or ERK 2 ($t_{(6)} = 2.438$, $p>0.05$) levels between intra-DH THC and VEH groups (Figure 19).

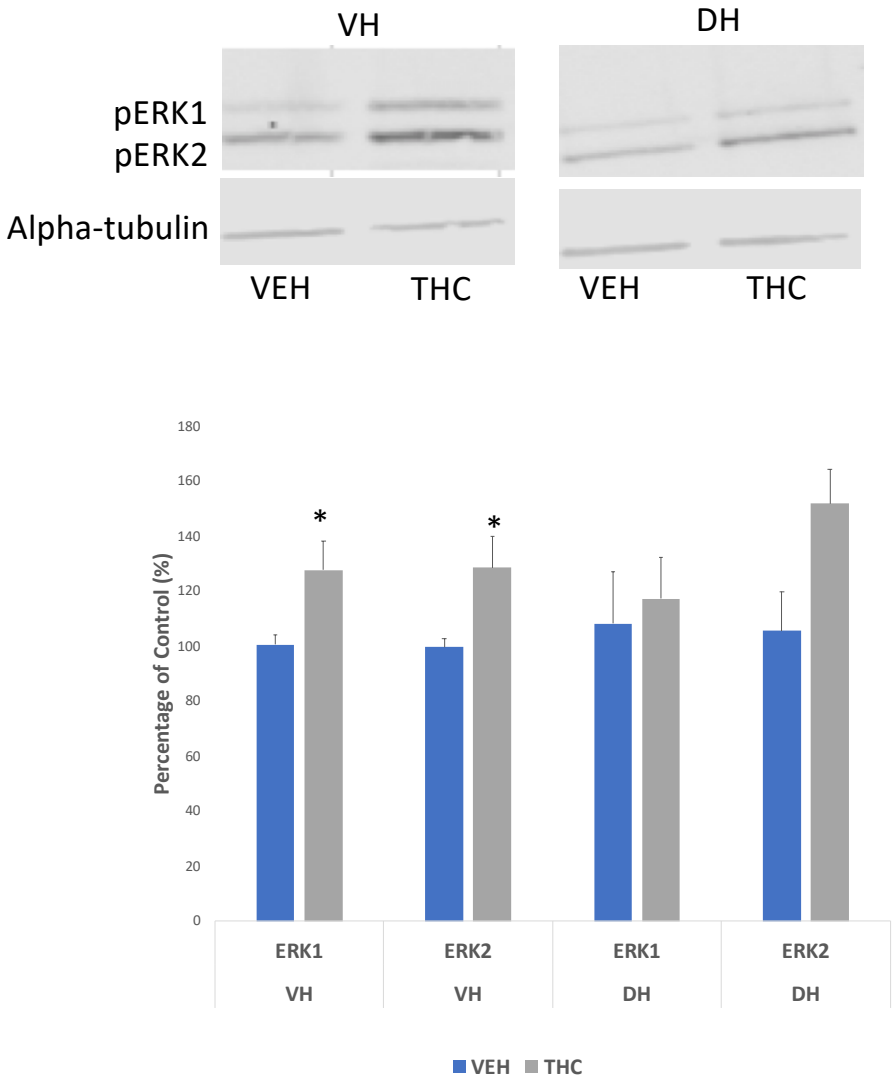

Figure 19 THC on phosphorylated ERK 1/2 levels. Representative western blot for pERK 1 (44 kDa) and pERK 2 (42 kDa) along with alpha-tubulin (55 kDa) expression in VH and DH tissue (top). A significant increase in pERK

1/2 levels in the intra-VH THC treated animals. N=4, t-tests, *Indicated p<0.05, Error bars represent standard error of means (SEMs).

Two-tailed t-test revealed no significant differences in total ERK 1 ($t_{(6)} = 0.199$, $p>0.05$) and ERK 2 ($t_{(6)} = 0.183$, $p>0.05$) levels between intra-VH THC and VEH groups. There were also no significant differences in total ERK 1 ($t_{(6)} = -0.152$, $p>0.05$) and ERK 2 ($t_{(6)} = -0.234$, $p>0.05$) levels between intra-DH THC and VEH groups (Figure 20).

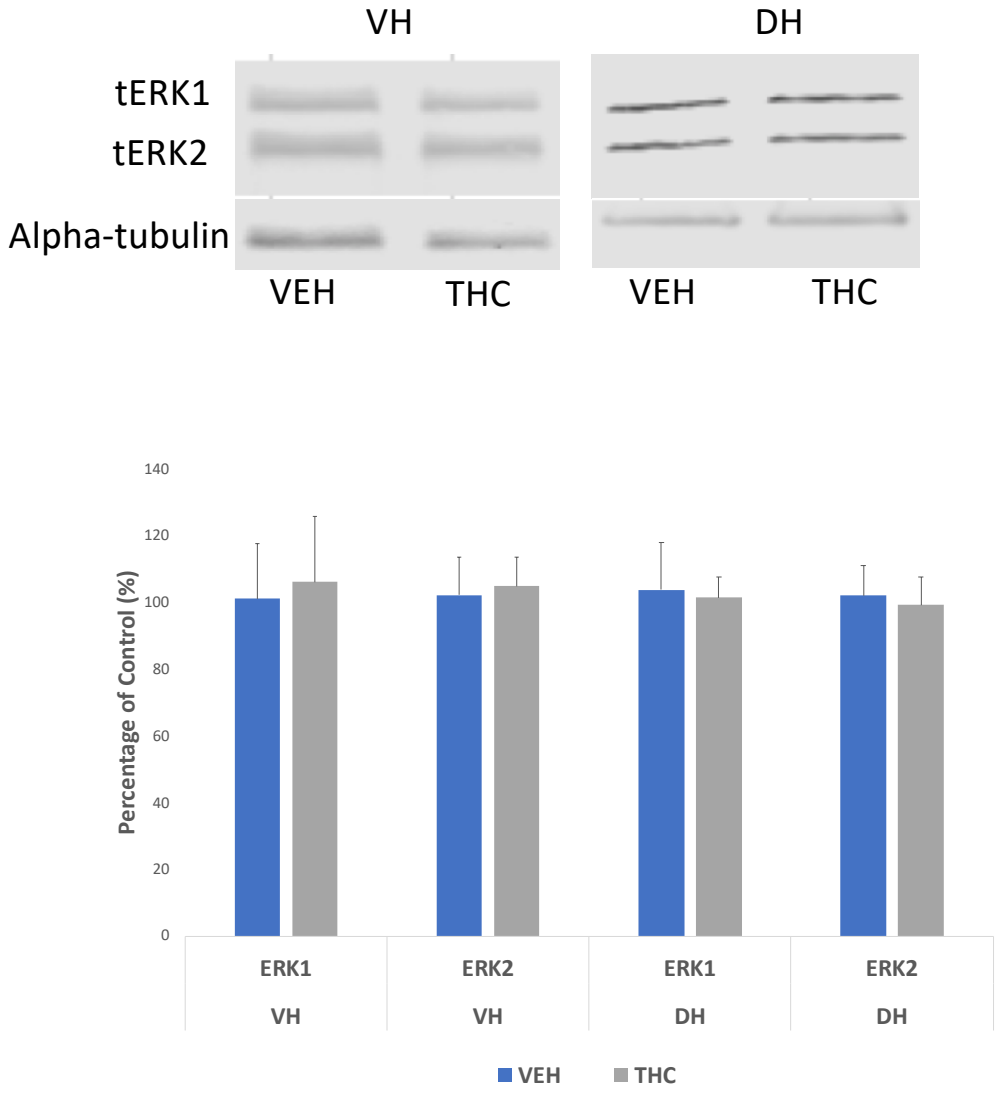

Figure 20 THC on total ERK 1/2 levels. Representative western blot for ERK 1 (44 kDa) and ERK 2 (42 kDa) along with alpha-tubulin (55 kDa) expression in VH and DH tissue (top). No significant differences in ERK 1/2 levels between VEH and THC treated animals. N=4, t-tests, *Indicated p<0.05, Error bars represent standard error of means (SEMs).

The ratios between phosphorylated ERK 1 and 2 with total ERK 1 and 2 was also taken (Figure 21). Two-tailed t-test revealed no significant difference between intra-VH THC and VEH groups with pERK1: tERK1 levels ($t_{(6)} = 0.676$, $p>0.05$) and pERK2:tERK2 levels ($t_{(6)} = 0.805$, $_p>0.05$). There were also no significant differences between intra-DH THC and VEH groups with pERK1: tERK1 levels ($t_{(6)} = 0.321$, $p>0.05$) and pERK2: tERK2 levels ($t_{(6)} = 2.113$, $_p>0.05$).

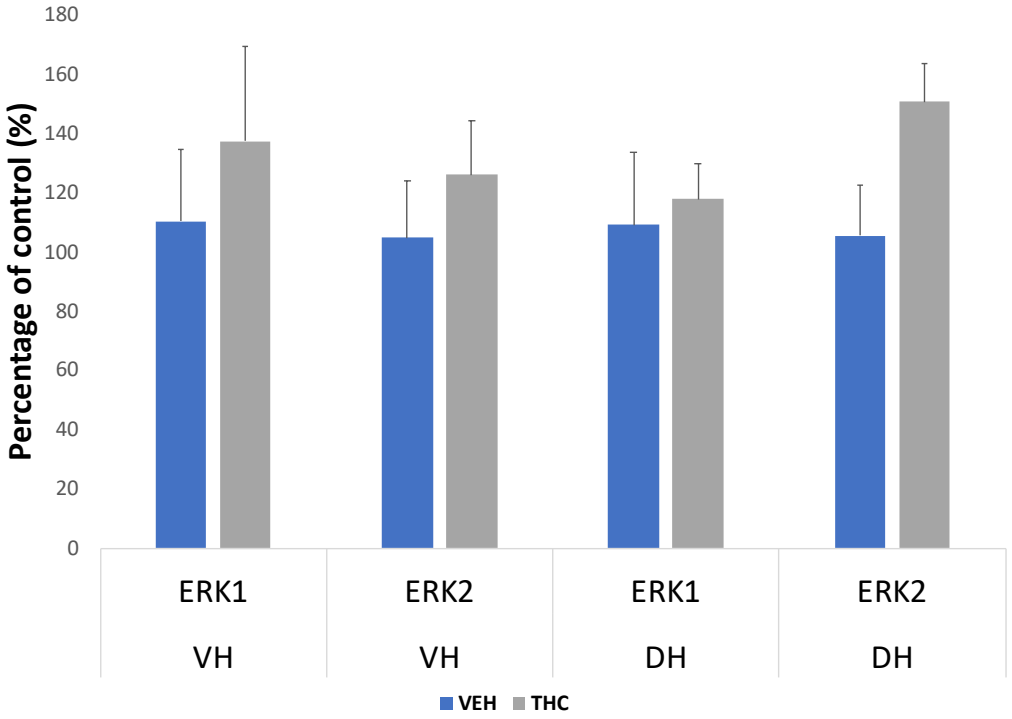

Figure 21 THC on pERK 1/2: tERK 1/2 levels. No significant differences between pERK 1/2: tERK 1/2 ratio between VEH and THC treated animals. N= 4; t-tests, Error bars represent standard error of means (SEMS).

Moreover, p70S6 kinase protein expression, involved in learning and memory was also measured (Figure 22). Two-tailed t-test revealed a significant increase in phosphorylated p70S6 kinase expression in intra-VH THC treated animals compared to the VEH group ($t_{(3)} = 3.177$, $p<0.05$). No significant difference in protein expression between the intra-DH groups ($t_{(6)} = 1.365$, $p<0.05$).

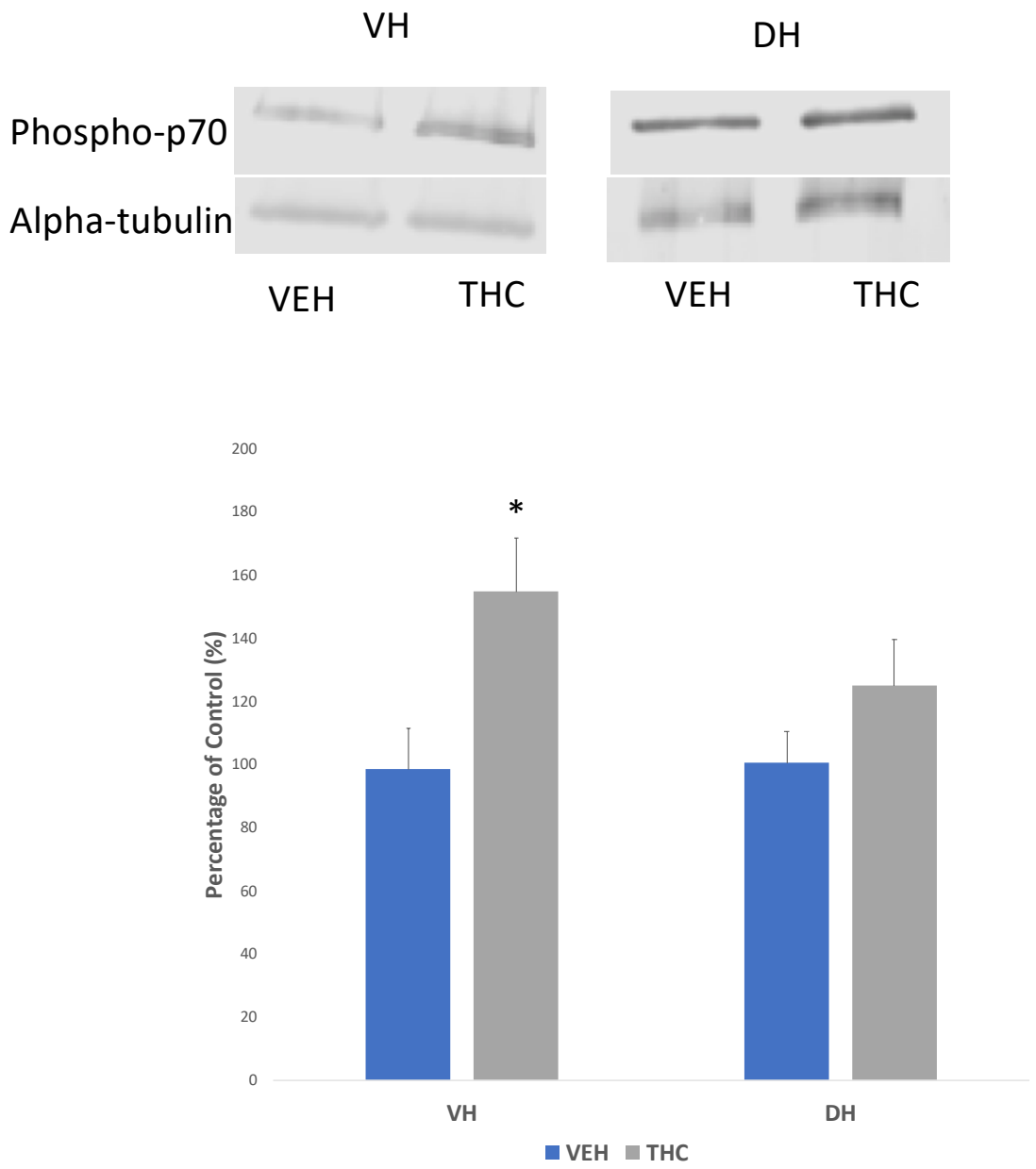

Figure 22 THC on phosphorylated p70S6 kinase levels. Representative western blot for phosphorylated p70S6 (70 kDa) and alpha-tubulin (55 kDa) expression in VH and DH tissue (top). Significant increase in phopho-p70S6 expression in intra-VH THC treated animals only. N=4; t-tests, *Indicated $p<0.05$, Error bars represent standard error of means (SEMs).

Two-tailed t-test revealed no significant differences in total p70S6 kinase between intra-VH VEH and THC groups ($t_{(6)} = 0.948$, $p>0.05$) as well as between intra-DH VEH and THC groups ($t_{(6)} = 0.784$, $p>0.05$) (Figure 23).

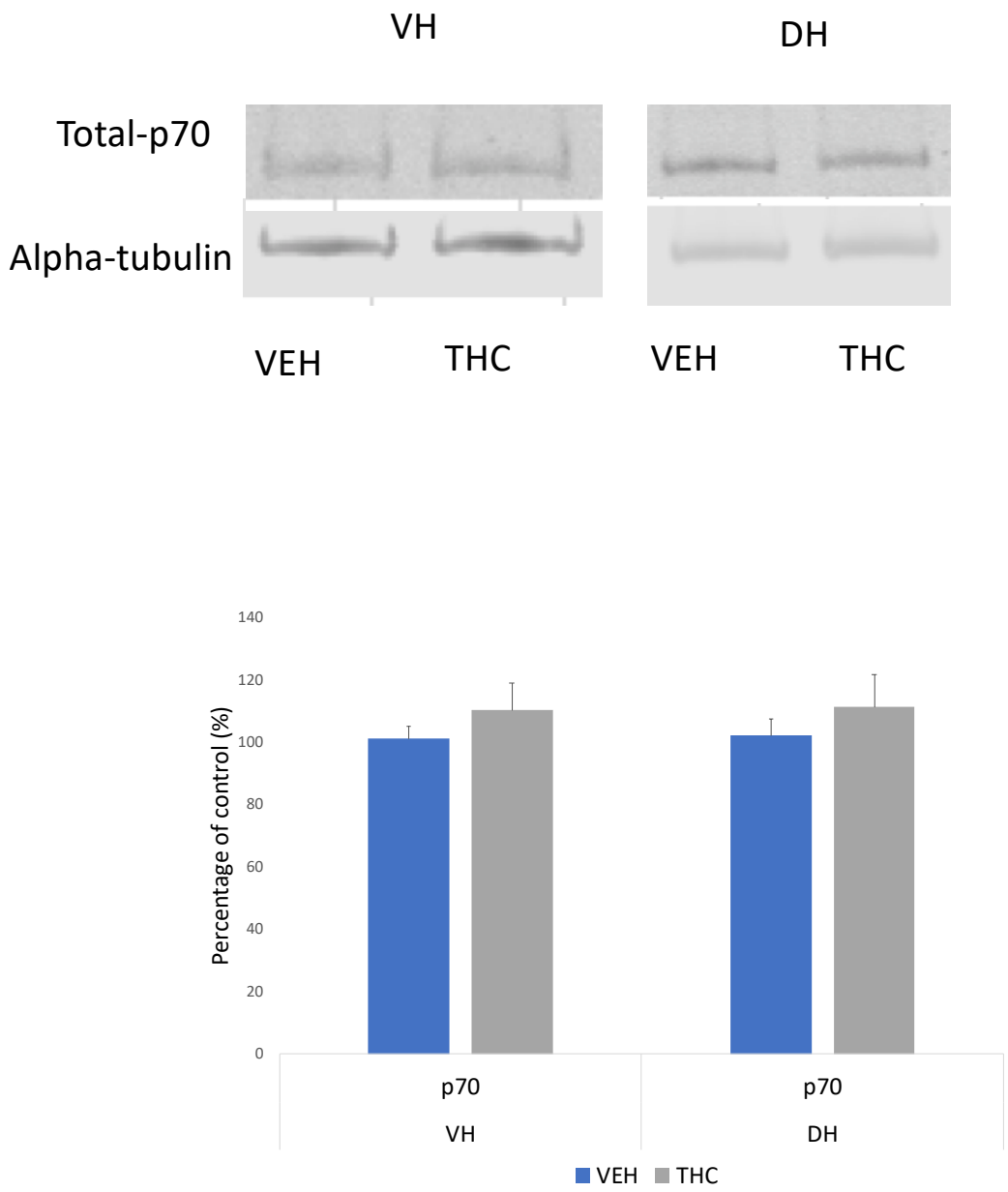

Figure 23 THC on total p70S6 kinase levels. Representative western blot for total p70 (70 kDa) and alpha-tubulin (55 kDa) expression in VH and DH tissue (top). No significant differences in p70 kinase levels between VEH and THC treated animals for both VH and DH groups. N=4, Error bars represent standard error of means (SEMs).

Similarly, with ERK the ratio of phosphorylated p70S6 kinase to total p70S6 kinase between the VEH and THC groups was compared (Figure 24). There were no significant differences in the ratio between intra-VH VEH and THC groups ($t_{(6)} = 2.176$, $p>0.05$) as well as no significant difference between intra-DH VEH and THC groups ($t_{(6)} = 0.860$, $p>0.05$).

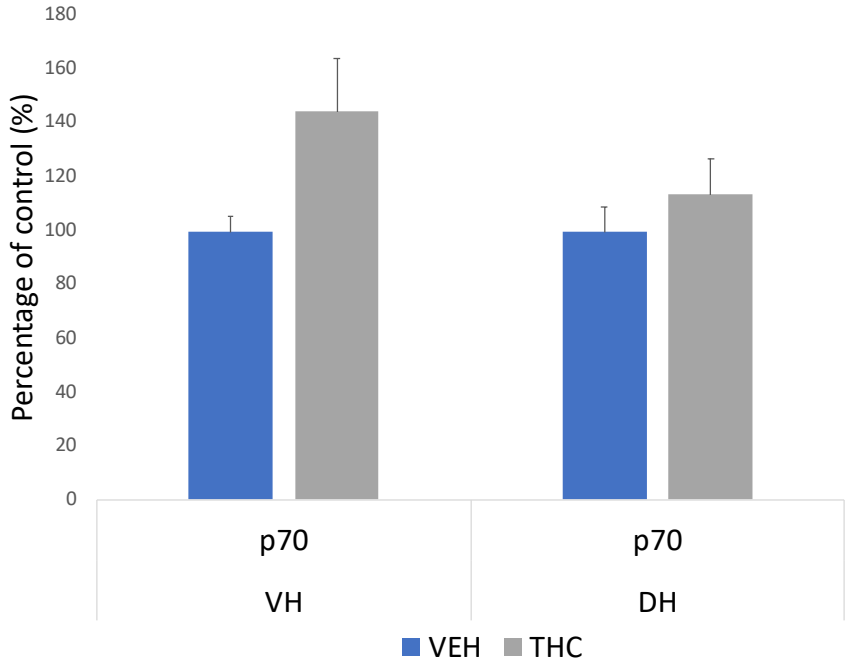

Figure 24 THC on p-p70: p70 kinase levels. No significant differences between p-p70: p70 kinase ratio between VEH and THC treated animals. N= 4; t-tests, Error bars represent standard error of means (SEMS).

4 Discussion

The current findings demonstrate, for the first time several dissociative effects of THC within distinct ventral vs. dorsal hippocampal sub-regions (Figure 25). This data improves our understanding of the precise brain mechanisms involved in the modulation of THC-induced cognitive and emotional side-effects, and how this in turn may lead to various mental health-related complications. Specifically, it was observed that THC directly in the dorsal hippocampus primarily induced short-term cognitive learning and memory deficits, whereas THC in the ventral hippocampus induced several deficits in affective/emotional processing as well as deficits in higher order cognitive functioning. Given my initial hypothesis that the effects of THC within the hippocampus would dissociate along cognitive vs. emotional impairments across dorsal vs. ventral regions, respectively, these findings suggest a more expansive role for both the dorsal and ventral hippocampus in terms of THC-induced cognitive processing deficits, and a selective role

for the ventral hippocampus specifically in the context of affective/emotional-related side-effects.

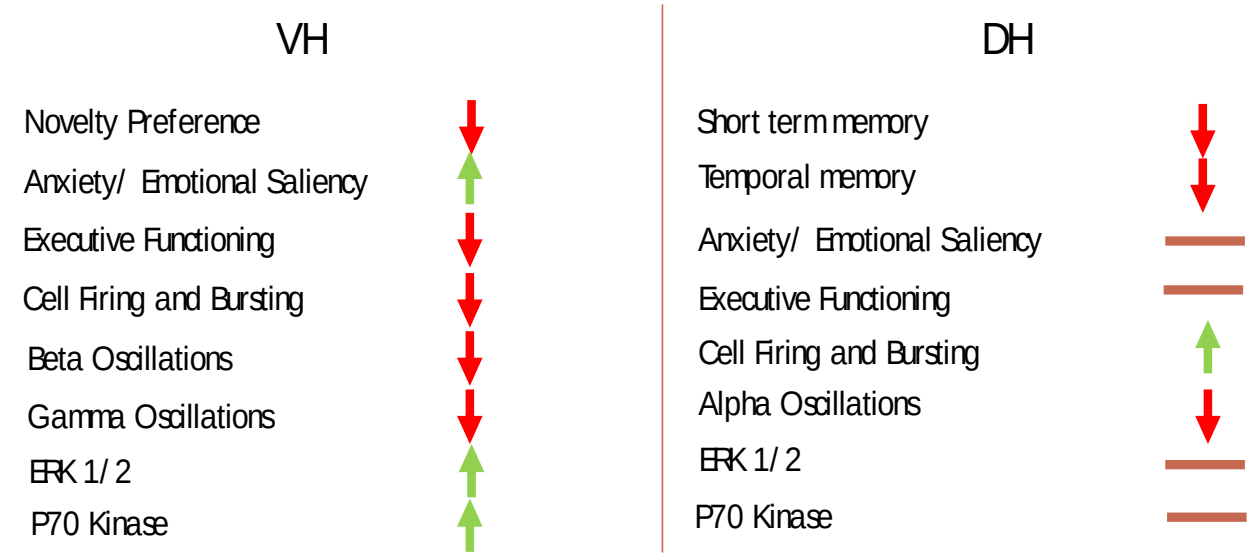

Figure 25 Summary of research findings

Intra-DH THC treatment caused deficits in short-term memory and cognition

Behavioural results demonstrated that THC infusions into the DH caused deficits in short-term memory, both spatial and non-spatial. These findings are consistent with the current literature, which has suggested that acute THC exposure causes deficits in short-term memory. Specifically, systemic injections of THC are known to cause deficits in novel object recognition (Kasten et al., 2017). Thus, it can be concluded that short-term memory impairments from THC exposure is likely related to effects directly within the DH.

Similarly, JWH-018 a synthetic cannabinoid agonist, impaired cognitive functioning, shown in a novel object recognition paradigm (Barbieri et al., 2016). *In-vitro* electrophysiology, using JWH-018 exposure in hippocampal slices negatively evoked LTP and synaptic transmission, thereby demonstrating that interference of hippocampal memory transmission causes deficits in cognitive functioning (Barbieri et al., 2016). While the LTP was not recorded in the hippocampus, similar behavioural deficits as JWH-018 was observed and it can be speculated that there could be a decrease in LTP after THC treatment in the DH.

Moreover, the medial temporal lobe, specifically the hippocampus, is critical for memory encoding. The hippocampus is involved in episodic memory consolidation and hippocampal

neural hyperactivity resulting from marijuana consumption has been associated with deficits in episodic memory (Ilan et al., 2004, Nestor et al., 2008). Results from the temporal order paradigm demonstrated deficits in episodic memory after acute infusions of THC in the DH. Therefore, cognitive deficits related to exposure to high concentrations of THC might be linked to the effects of THC within the DH.

The hippocampus is involved in spatial working memory deficits mediated by THC (Mishima et al., 2002, Wise et al., 2009). In the present project, significant deficits in working memory during intra-DH THC treatment was not observed, however this deficit has been reported previously in the literature. For example, using a radial maze paradigm, intra-DH THC treatment has been shown to cause deficits in working memory (Mishima et al., 2002, Wise et al., 2009). Given that a more simplistic spontaneous alternations paradigm was used in the present study, it is possible that more complex tasks (such as the radial arm maze) may be necessary to observe these subtle deficits.

Additionally, there was an observation of a decrease in alpha desynchronization after intra-DH THC treatment. Desynchronized alpha oscillations in the frontal lobe has been shown to predict successful memory encoding and retrieval (Parish et al., 2018). Hence, it can be speculated that intra-DH THC treatment causes deficits in short term memory through reduced desynchronization in alpha oscillatory activity. A major caveat is the total N for desynchronized alpha oscillations is low, a large amount of desynchronized activity during electrophysiology was not witnessed. Additionally, all recordings were taken from anaesthetized animals and there is a possibility that our results may have differed had recordings been taken in awake animals. Therefore, a correlational relationship between our alpha oscillatory results with the behavioural results as opposed to causational is assumed.

Nevertheless, the reduction in desynchronized alpha activity may prove to be detrimental should it occur chronically. Abnormal desynchronized alpha activity over areas of the prefrontal cortex can pose as a risk factor for Alzheimer's disease (AD) (del Val et al., 2016). This may indicate that chronic exposure to THC may increase risk of developing AD later in life.

With regards to protein expression, addictive and non-addictive drugs alike activate the ERK signaling pathway (Valjent et al., 2004). While it was not significant, there was a trend of an increased phosphorylated ERK2 after intra-DH THC treatment but not ERK1. While there are many similarities between the isoforms, ERK 2 is more prevalent in the brain and is often

involved in spatial learning and memory, more so than ERK 1 (Indrigo et al., 2010). This may indicate a role for ERK2 in the modulation of cognitive deficits seen during THC micro infusions to the DH. Total ERK 1/2 levels had no changes after acute THC treatment in the DH, meaning acute doses of THC may have post-translational effects on ERK levels. There were no significant differences in the pERK 1/2: tERK 1/2 ratio however, there is a notable increase in ERK2. Perhaps if the N was increased it could decrease the variability seen in order to give a significant p value.

Interestingly, ERK activity may differ depending on the method of activation. ERK 1/2 activation in the hippocampus, via an excitatory neuropeptide known as orexin-A, can reverse impairments in spatial learning and memory (Zhao et al., 2014). However, Zhao et al., (2014) specifically looked at protein expression in the dentate gyrus while the current study looked at protein expression in the CA1 region of the hippocampus. This may suggest potential differential effects dependent on the specific hippocampal region.

Lastly, there were no significant deficits in emotional learning and memory during intra-DH THC treatment. However, there was a slight increase in locomotion after THC micro infusions in the DH, which could indicate a slight decrease in anxiety. As stated earlier, the DH is more involved in cognitive functioning, however CB1R activation in the DH has been noted to cause anxiolytic effects (Lisboa et al., 2015). No significant anxiolytic effects were found during CA1 dorsal THC micro infusions, yet Lisboa et al., (2015) found anxiolytic effects during CB1R activation specifically in the dentate gyrus, demonstrating potentially functional differences between the CA1 and dentate gyrus regions of the DH.

Interestingly, in addition to potential anxiolytic effects, increased activation of CB1 receptors in the DH can also elicit anti-depressant effects (McLaughlin et al., 2007). Therefore, the DH may be a therapeutic target to alleviate depressive-like symptoms. The present study did not utilize a paradigm like the forced swim test, which is often used to model depression. Regardless, the current study targeted the CA1 region whereas McLaughlin et al., (2007) targeted the dentate gyrus. Therefore, if there are differences between the CA1 and dentate gyrus, the present study may not have seen anti-depressive effects during the forced swim test paradigm.

Additionally, the DH interacts with the mPFC in the formation of fear and reward-related memories (Han et al., 2014, Lisboa et al., 2015, Yan et al., 2014). Yang et al., (2014) determined

that interactions between the DH and mPFC are required for fear memory formation during a context dependent fear conditioning paradigm. In regard to proteins, P70S6 kinase is activated in the dorsal hippocampus after context dependent fear retrieval (Gafford et al., 2013). There were no significant differences in p70S6 kinase protein expression between the VEH and THC DH treated groups. Behaviourally, there were no significant deficits of intra-DH THC treatment in the context-independent fear conditioning paradigm. This signifies that different neural mechanisms may be required for context dependent vs. independent fear-memory processing. Our results revealed that the DH may not be involved in context independent fear conditioning, while previous studies suggest its involvement in modulating context dependent fear conditioning (Gafford et al., 2013, Yang et al., 2014).

It is also entirely possible that the dorsal hippocampal- prefrontal cortex pathway is not involved in the acquisition of fear memories. Activation of dorsal hippocampal neurons that directly project to the prefrontal cortex strengthen the reconsolidation of fear memories (Ye et al., 2017). From our electrophysiology results, there was a significant increase in cell firing and bursting activity after intra-DH THC treatment. Prefrontal bursting activity has been shown to modulate emotional learning and memory (Laviolette and Grace, 2006) and as such an increase should have strengthened fear memories. However, this effect was not witnessed behaviourally, possibly because THC was infused into the DH right before the acquisition phase of the fear memory. Thus, intra-DH THC treatment had no effect on the acquisition of fear memories but it may modulate fear memory consolidation. Future studies are required to resolve these questions.

Intra-VH THC treatment caused deficits in emotional learning and higher order cognitive functioning

Acute injections of THC in mice have been reported to cause an increase in anxiogenic behaviours (Kasten et al., 2017). This increase in anxiety is modulated by CB1 receptors in areas of the limbic cortex like the amygdala and hippocampus (Bhattacharyya et al., 2017). High doses of THC infusions in the VH specifically have been shown to cause anxiogenic responses (Rubino et al., 2008). Using the light/dark box paradigm, it was observed that intra-VH THC treatment caused an increase in anxiety, in line with current literature. Additionally, through our fear conditioning paradigm there was a significant increase in emotional saliency to aversive stimuli

during intra-VH THC treatment. Therefore, in line with the current literature, acute local infusions of THC in the VH caused an increase in anxiety-like behaviours.

While the present study found anxiogenic effects during the light/dark box and fear conditioning paradigms, there weren't any significant deficits during the open field maze. Intra-VH THC treated rats did display a slight decrease in overall locomotor activity and a slight increase in distance travelled in the periphery compared to its vehicle group, which would be signs of an increase in anxiety. Yet, the lack of significance may have been due to methodological issues. All rats were experimentally naïve; thus, they had not undergone a different behavioural paradigm before the open field maze. Trained rats typically are less anxious compared to untrained rats (Martin et al., 2001). Therefore, anxiogenic responses may not have been detected since the control groups were untrained, prior to the open field maze task, and may have demonstrated an unusual increase in anxiety.

With regards to single neuron and oscillatory activity, when THC was infused in the VH, there was a significant decrease in cell firing and bursting activity in the PFC. This predicted aberrant anxiety-like behaviours, as the PFC cell bursting activity is involved in the modulation of emotionally salient behaviours (Laviolette and Grace, 2006). Furthermore, a decrease in synchronized gamma oscillatory activity was observed. Gamma oscillations have been seen to play a role in translating emotionally salient events into memories (Caliskan et al., 2018). Additionally, during slow wave sleep, synchronized gamma oscillations bind the hippocampus and prefrontal cortex, which is required for memory consolidation (Ferraris et al., 2018). Therefore, THC, which caused a desynchronization of gamma waves and decrease in single neuron bursting activity altered memory encoding and consolidation of emotionally salient events. This in turn led to an exaggeration of emotionally salient memories.

Dysregulated emotional learning not only can exaggerate emotionally salient cues but can also cause disruptions in stimuli that would otherwise be significant, such as sociability. Hippocampal CB1 receptor activation via WIN-55 can induce deficits in normal social behaviour (Loureiro et al., 2015). Thus, similarly to synthetic CB1 agonists THC micro-infused into the VH caused deficits in social recognition memory, however they were not as adverse as WIN-55 treatment. Local WIN-55 infusions into the VH caused an attenuation in social motivation as well as social recognition; even the effects of social recognition were more adverse as WIN-55 treated rats had a strong preference to the familiar rat as opposed to the stranger rat (Loureiro et

al., 2015). THC treatment did not cause a strong preference or aversion to either stranger or familiar rat, but it did diminish preference for social novelty. This is often a rewarding behaviour for rodents. While synthetic and phytocannabinoids produce similar behavioural effects, the former are full CB1 agonists while the later are partial CB1 agonists (Tai & Fantegrossi, 2014). THC treatment in the VH causes deficits in reward memory formation, however the effect is not as potent as WIN-55.

What was not expected was THC micro infusions in the VH to cause deficits in many of the cognitive paradigms. However, there was also an increase in anxiety and emotional saliency to aversive stimuli, therefore instead of displaying cognitive deficits, THC directly infused in the VH may result in a decrease in novelty preference. Though it was not significant there is a slight decrease in time exploring the novel object versus the familiar within the THC treated groups, in all the object recognition paradigms. Additionally, THC has been shown to decrease exploratory behaviour, also resulting in a decrease in novelty (Schreiber et al., 2018). Beta oscillations are naturally tuned for novelty detection (Franca et al., 2014). Thus, a decrease in beta oscillations in the mPFC following intra-VH THC treatment may have caused deficits in novelty recognition during these tasks.

Besides a decrease in novelty detection capacity, this study observed deficits in spatial working memory after intra-VH THC micro-infusions, despite observing no working memory deficits during intra-DH THC treatment. Oscillatory activity in the mPFC plays a crucial role in spatial working memory. Indeed, O'Neil et al., (2013) reported that theta oscillations in the mPFC synchronized with theta oscillations in the VH during the modulation of spatial working memory. In fact, the VH plays a major role in synchronizing theta oscillations in the mPFC and VH. While significant changes in the theta band was not observed, there was a slight increase in desynchronized theta oscillations in mPFC after THC micro-infusions in the VH. This may suggest THC in the VH but not DH interferes with theta synchronization between the hippocampus and mPFC, thereby causing deficits in spatial working memory. However, due to the nature of our THC micro-infusions, recording oscillatory activity in the VH was not possible thus, no conclusions can be drawn with regards to how THC interferes with oscillations within the VH. Moreover, a potential limitation to our study is that electrophysiological recordings were taken on anaesthetized animals rather than awake animals. Thus, this study may not have

witnessed significant changes in theta oscillatory activity because the recordings were not taken from awake animals performing a working memory paradigm.

Furthermore, there was a significant decrease in cognitive flexibility in the social motivation task, during intra-VH THC treatment. Cognitive flexibility is another executive function modulated by the mPFC and requires beta and gamma oscillatory functioning (Brockett et al., 2018). Deficiency in gamma oscillatory activity in prefrontal interneurons has been hypothesized to cause cognitive flexibility impairments (Cho et al., 2015). Though pyramidal cells were targeted for our single unit recordings, interneurons are often dispersed within prefrontal granule layers, and may have been a part of the LFP recordings. As such the cognitive inflexibility observed during the social motivation task may have been correlated to the deficiency in gamma oscillatory activity.

Moreover, there was an observation of an upregulation in phosphorylated ERK1 and 2 levels after THC treatment in the VH. As stated previously, increases in ERK activation is common with many drugs of abuse. Additionally, ERK is involved in upregulating long-term potentiation (LTP), required for the formation of new memories. Specifically, it is involved in modulating emotional learning and memory and as such, it can be correlated with the abnormalities in emotional processing. An upregulation of phosphorylated ERK 1/2 via CB1R activation, in the VH, is correlated with an increase in emotionally salient behaviours. This study did not witness a significant increase in total ERK levels after an acute dose of THC in the VH. Therefore, consistent with the effects observed with acute treatments in the DH, THC locally infused in the VH causes post-translational effects on ERK 1/2 levels.

Finally, the present study also observed a significant increase in phosphorylated p70S6 kinase protein expression after intra-VH THC treatment. P70S6 kinase is part of the mTOR pathway, which is highly affected by THC via CB1R activation (Puighermanal et al., 2009). An increase in p70S6 kinase in particular is associated with an increase in fear expression (Zubedat & Akirav, 2017). Hence, it is speculated that the upregulation of p70S6 kinase from intra-VH THC treatment is involved with the anxiogenic effects and an increased expression of fear.

Intra-VH THC treatment can produce a schizophrenia-like pathophysiology

These deficits can also lead to developing a psychiatric disorder such as schizophrenia. Marijuana consumption results in an increased risk of developing a psychiatric disorder (Renard et al., 2016, Renard et al., 2018). The mediotemporal brain region is often implicated in the modulation of memory deficits as well as the induction of psychotic symptoms (Bhattacharyya et al., 2009). This makes it a region of interest for therapeutic interventions for many psychiatric disorders. Hyperactivity, particularly in the hippocampus has been characterized to produce a schizophrenia like pathophysiology (Lodge et al., 2009). In particular, it is the hyperactivity within the ventral hippocampus that produces a similar pathophysiology to schizophrenia (Grace, 2010). Moreover, THC also affects cortical activity in the prefrontal cortex, which results in developing or exacerbating symptoms of schizophrenia (Aguilar et al., 2016, Renard et al., 2017). The current study has shown the hippocampus modulates neural activity in the prefrontal cortex. Thus, the hippocampus specifically is a region of interest in finding a pharmacological intervention for treating the symptoms of schizophrenia.

Oscillatory abnormalities in schizophrenia include a global decrease in alpha band power but an increase in alpha band power over prefrontal areas. Both effects are linked to the positive and negative symptoms of schizophrenia (Knyazeva et al., 2008). There were no observations in abnormalities in alpha oscillations after intra-VH THC treatment possibly because it was only an acute treatment, but there was a decrease in alpha power after intra-DH THC treatment. If an increase in alpha oscillatory activity is associated with symptoms of schizophrenia, a decrease could alleviate or prevent such symptoms. As stated previously, CB1R activation in the DH has been seen to cause anti-depressant effects (McLaughlin et al., 2007), thus, it could be possible for DH CB1R activation to also have anti-psychotic effects. However, further investigation is required.

Disruptions in prefrontal gamma and beta oscillations are often seen to underlie symptoms of schizophrenia (Uhlhaas et al., 2013). This study found reduced synchrony in gamma and beta oscillations in the PFC, during intra-VH THC treatment which may suggest the VH having a role in modulating schizophrenia-like symptoms. High gamma band oscillations in particular are involved in the cognitive deficits of schizophrenia, as impairments in task performance are accompanied by deficits in gamma oscillatory power (Grutzner et al., 2013).

While beta oscillatory activity in relation to schizophrenia has been less explored, there is evidence to suggest deficient beta activity in patients with schizophrenia. Synchronized beta

activity maintains proper sensorimotor and cognitive functioning (Engel and Fries, 2010). Patients with schizophrenia have impairments in synchronized beta activity, causing failure to accurately predict causes of sensory perceptions, resulting in auditory hallucinations (Ford et al., 2007). THC may increase the risk of developing schizophrenia through disrupting high frequency oscillations in the gamma and beta range.

The present study also witnessed behavioural abnormalities in working memory and emotional saliency, which are also common symptoms of schizophrenia. This is further evidence that THC can increase the risk of developing the cognitive symptoms of schizophrenia. Further behavioural paradigms are required to better test whether THC can model schizophrenia-like symptoms. As an example, sensorimotor gating alternations like a loss of normal prepulse inhibition is a characteristic symptom of schizophrenia and systemic injections of THC have been shown to cause these deficits (Ibarra-Lecue et al., 2018). Prepulse disinhibition is a positive symptom of schizophrenia and as such would be a useful measurement to obtain to determine whether THC locally infused into the VH or DH has a role in the pathophysiology of schizophrenia.

Finally, the current study also did not witness the appearance of any negative symptoms of schizophrenia, from THC exposure such as social withdrawal. Slamberova et al., (2016) demonstrated acute systemic injections of THC having no effect on social interaction. However, this does not rule out the potential effects of THC on negative symptoms of schizophrenia. Chronic THC exposure during adolescence has shown a decrease in social motivation (Renard et al., 2017), which furthers the risk of developing schizophrenia in the future. All in all, it can be speculated that the VH, but not DH playing a role in the increased risk of developing schizophrenia from THC exposure but targeting the DH may also help to alleviate schizophrenia-like symptoms.

General Conclusions and Future Directions

With the impending legalization of marijuana in Canada, it is more important than ever to determine the potential psychopathological effects of THC exposure and understand the specific neural mechanisms underlying these phenomena. In this thesis, it has been demonstrated that THC, a CB1R agonist, is capable of inducing cognitive deficits in the DH whereas it causes a

broader range of both cognitive and emotional deficits in the VH. This may indicate a larger role of the VH for the pathophysiology of psychiatric and addictive disorders, however further research is required to more precisely dissociate these symptom clusters.

Moving forward, future studies should work on challenging these deficits pharmacologically. As there was an upregulation in ERK 1/2 levels, ERK inhibitors combined with THC could be infused into the VH and DH, to determine whether THC deficits are alleviated. A similar experimental design can be performed with p70S6 kinase inhibitors, as there was an increase in p70S6 kinase protein levels after THC treatment in the VH.

In addition to ERK and p70S6 kinase, CB1R activation activates a variety of protein cascades that also might be affected by THC. For example, AKT is also a protein activated through CB1 receptors and modulates acute effects of psychosis and cognition in marijuana users (Morgan et al., 2017). As such, changes in AKT protein expression may have been implicated in the cognitive deficits of THC.

As stated previously the mTOR pathway is linked to the neuropsychiatric effects of THC exposure (Renard et al., 2016), and studies have shown that blocking mTOR activation alleviates the anxiolytic effects of THC (Puighermanal et al., 2009, Puighermanal et al., 2013). However, mTOR activation via THC has not previously been differentiated between the VH and DH. Additionally, whether mTOR attenuates cognitive deficits induced by THC has not been determined. mTOR related mutations which cause an overexpression of the protein can cause intellectual disabilities (Reijinders et al., 2017). While the study observed changes in p70S6 kinase, a downstream protein in the mTOR pathway, it would be interesting to determine whether THC modulates mTOR expression at both a protein and/or genetic level.

While THC is a CB1R agonist, it can also activate other receptors. Chronic THC exposure causes supersensitive coupling of serotonin 2A receptors (5-HT2A) towards inhibitory G-proteins as well as inducing a pro-hallucinogenic molecular conformation of the receptor (Ibarra-Lecue et al., 2018). Functional selectivity of 5-HT2A is involved in modulating the positive symptoms of schizophrenia (Ibarra-Lecue et al., 2018). Serotonin networks are also involved in emotion regulation (Wolf et al., 2018). Therefore, it may be possible that some of the THC-induced behavioural deficits observed could have been due to activation of 5-HT2A receptors and therefore, the potential role of serotonin receptor signaling is another promising avenue for future research studies.

Additionally, marijuana is comprised of a variety of phytocannabinoids, some of which have opposing effects to THC. For example, CBD which is another major phytochemical found in marijuana, has been shown to have opposite effects compared to THC and as such, may represent a promising counteractive agent to the negative effects of THC exposure. For example, CBD has been reported to have strong anti-psychotic properties in both clinical (Corroon & Phillips, 2018) and pre-clinical studies (Renard et al., 2016). Moreover, it would also be interesting to examine possible dissociable effects of CBD between the ventral and dorsal hippocampal sub-regions. CBD has been shown to alleviate symptoms of anxiety and is noted to be a potential treatment for anxiety disorders (Blessing et al., 2015, R de Mello Schier et al., 2014).

The present study explored the acute effects of THC in terms of affective and cognitive deficits in the dorsal versus ventral hippocampal sub-regions. However, it did not examine potential effects of long-term, chronic THC exposure within these distinct hippocampal sub-regions. This is of significant relevance, as marijuana is initially consumed during adolescence and can cause persistent deficits lasting into adulthood. As a potential experimental design adolescent rats can be given chronic injections of THC for 10 days. A month later when they are adults, cannulations can be surgically implanted to the VH or DH and the same battery of behavioural paradigms can be performed. Electrophysiology can also be performed to determine whether the brain pathophysiology closely resembles that of schizophrenia.

Due to the nature of our infusions, cell activity and oscillatory states in the hippocampus could not be recorded. Synchronous theta waves are often required in the acquisition phase of learning and memory (Parish et al., 2018). Future studies should determine whether irregular theta oscillations in conjunction with irregular oscillatory states in the prefrontal cortex, resultant of THC, is responsible for the deficits in learning and memory.

In summary, the data presented in this thesis sheds important new light on how THC can differentially impact distinct anatomical regions of the hippocampal formation in the context of marijuana-induced psychiatric side-effects. Results of these studies will have important implications for understanding how and why chronic marijuana exposure may selectively damage distinct regions within the hippocampus and the resulting neuropsychiatric pathology. In addition, results from the molecular studies performed in this thesis may inform future studies as

to the potential importance of the ERK and p70S6K signaling pathways as potential biomarkers for increased mental health risks following marijuana exposure.

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Khan, D., Laviolette., SR (SONA, 2018). Differentiating the Cognitive and Emotional Effects of THC in distinct hippocampal sub-regions

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