THE EFFECTS OF CANNABINOID COMPOUNDS ON THE BRAIN AND BEHAVIOUR OF MOUSE MODELS OF ALZHEIMER'S DISEASE

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ABSTRACT

Compounds derived from the plant *Cannabis* demonstrate many therapeutic properties suggesting that they could delay the onset and progression of Alzheimer's disease (AD). The goal of the present experiment was to observe the effects of various dosages of cannabidiol (CBD) and Δ9-Tetrahydrocannabinol (THC) on the behaviour and brain pathology of an AD mouse model that expresses amyloid-beta (Aβ), $APP^{NL\text{-}G\text{-}F}$ mice. Additionally, we assessed the effects of 20mg/kg of CBD on the behaviour and brain pathology of a tauopathy mouse model, Tau P301S mice. Overall, various dosages of CBD and THC did not impact the behaviour or accumulation of Aβ and inflammation amongst $APP^{NL\text{-}G\text{-}F}$ mice. There was a dose dependent trend towards CBD, but not THC increasing the hippocampus (HPC) volume of $APP^{NL\text{-}G\text{-}F}$ mice. Similarly, CBD did not impact the behaviour or inflammation amongst Tau P301S mice. Finally, CBD did not restore neurodegeneration in the HPC of the Tau P301S mice.

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LIST OF ABBREVIATIONS

2-AG – 2-arachidonoylglycerol

APOE4 – Apolipoprotein E4 allele

Aβ – Amyloid-beta

AChEIs – Acetylcholinesterase inhibitors

AD – Alzheimer's Disease

APP – Amyloid precursor protein

CNS – Central nervous system

CBD – Cannabidiol

CB1 – Cannabinoid receptor 1

CB2 – Cannabinoid receptor 2

CA 1 – Cornu ammonis 1

CA 3 – Cornu ammonis 3

DG – Dentate gyrus

GSK3-β – Glycogen synthase kinase-3β

GFAP – Glial fibrillary acidic protein

FAD – Familial Alzheimer's disease

HPC – Hippocampus

Iba1 – Ionized calcium binding adaptor molecule 1

IR – Investigation ratio

i.p. – Intraperitoneal

SAD – Sporadic Alzheimer's disease

MTL – Medial temporal lobe

MWT – Morris water task

NMDA – N-methyl-D-aspartate

NPs – Neuritic plaques

NFTs – Neurofibrillary tangles

NTs – Neutrophil tangles

NOR – Novel object recognition

PNS – Peripheral nervous system

PFC – Prefrontal Cortex

PSEN-1 – Presinilin-1

PSEN-2 – Presinilin-2

PFA – Paraformaldehyde

PBS – Phosphate buffered saline

PP2A – Protein phosphatase 2A

THC – Δ9-Tetrahydrocannabinol

TBS – Tris buffered saline

CHAPTER 1 - GENERAL INTRODUCTION

Alzheimer's Disease

Alzheimer's disease (AD) is a debilitating neurodegenerative disease with the number of diagnoses growing at alarming rates as the "baby boomers" age. Along with the growing number of cases, the economic burden due to the disease will rise. Indeed, the cost of care for AD patients is estimated to surge from $321 billion dollars in 2022 to 1.1 trillion dollars in 2050 (Alzheimer's Disease Facts & Figures, 2022). AD is clinically characterized by memory impairments, behavioural changes, confusion, and an overall loss of cognitive abilities related to dysfunction of the medial temporal lobe (MTL) (Bekris, Yu, Bird, & Tsuang, 2010; Khachaturian, 1985; McKhann et al., 2011). The onset and progression of behavioural symptoms occurs over the span of years. However, once the symptoms become apparent, they continue to progress until the patients are no longer able to complete daily tasks, as a result AD patients require a primary care giver. The primary care givers of AD patients have been shown to have a reduced quality of life and overall well-being (George & Gwyther, 1986). There is little debate that AD negatively impacts the lives of patients, their caregivers, and the overall economy and the need to delay disease onset and progression is crucial.

The search for an appropriate treatment for AD has proven to be difficult due to the complexity and diversity of the disease (Gidyk, Deibel, Hong, & McDonald, 2015; McDonald, 2002). AD is frequently divided into two main categories, sporadic Alzheimer's disease (SAD) and familial Alzheimer's disease (FAD). SAD occurs in 99% of diagnosed individuals, whereas FAD only occurs in 1% (Cruchaga et al., 2018). Despite SAD being the most prevalent form of the disease, there is a large gap in the literature regarding the

genetic risk factors for SAD. Genome wide association studies have listed at least 40 gene loci that might be potential risk factors for SAD, with the apolipoprotein E4 allele (APOE4) being the largest known genetic risk factor for the disease (Andrews, Fulton-Howard, & Goate, 2020; Kunkle et al., 2019). Furthermore Kunkle et al. (2019) found variants of genes that are related to pathways involved in the pathology of AD, implicating that they could contribute to the progression of the disease. Although the genes are rare and not all individuals who carry the genes will develop the disease. In contrast to SAD, there are three known mutated genes associated with FAD: the amyloid precursor protein (APP), presenilin-1 (PSEN-1), and presenilin-2 (PSEN-2) (Weggen & Beher, 2012). Individuals who carry the familial genes are almost guaranteed to descend into dementia. APOE4, APP, PSEN-1, and PSEN-2 have been shown to upregulate pathologies related to AD and have helped researchers further our understanding of their role in the disease (Bateman et al., 2012; Cruchaga et al., 2018; Hatami, Monjazeb, Milton, & Glabe, 2017).

The pathological hallmarks of the disease include: the presence of intraneuronal abnormal tau protein and extracellular beta amyloid plaques (Aβ). The accumulation of abnormal tau protein contributes to the formation of more dispersed lesions, neutrophil tangles (NTs) and neurofibrillary tangles (NFTs), which are associated with the early stages of the disease (Braak & Braak, 1991; Braak, Thal, Ghebremedhin, & Del Tredici, 2011; Grundke-Iqbal et al., 1986). Aβ contributes to the formation of neuritic plaques (NPs), which are associated with late stages of the disease (Braak & Braak, 1991; Braak et al., 2011). Research suggests that the pathologies develop sequentially decades before symptom onset, during which patients are categorized to have “pre-clinical” AD (Braak &

Braak, 1991; Braak et al., 2011 Hanseeuw et al., 2019; Thal, Rüb, Orantes, & Braak, 2002). Specifically, the pathologies are believed to begin developing within the MTL.

The MTL is composed of the amygdaloid complex, the hippocampal complex, and parahippocampal cortices (Frankó, Insausti, Artacho-Pérula, Insausti, & Chavoix, 2014; Squire, Stark, & Clark, 2004). These brain regions have been implicated in various forms of learning and memory (Squire et al., 2004). Disruption to the structure and function of the MTL leads to memory impairments like those observed in AD (Duara et al., 2008; Squire et al., 2004). Moreover, the severity of pathologies within the MTL and its atrophy correlates with the onset of symptomatic AD (Cash et al., 2013; Duara et al., 2008; Juottonen et al., 1998; Jack et al., 2002; Jutten et al., 2019; Yilmaz et al., 2017). As such, treatments that target pre-clinical AD would provide patients with the most optimal quality of life by delaying dysfunction and neurodegeneration within the MTL.

Amyloid-Beta

Aβ is cleaved by APP, and it is released into the extracellular space. The release of Aβ monomers into the extracellular space is not in itself toxic (Cizas et al., 2010; De Felice et al., 2008). However, Aβ is capable of self assembling and can undergo various conformations (i.e., oligomers, protofibrils, and fibrils), which are believed to be toxic (Maezawa, Zimin, Wulff, & Jin, 2011; Reiss, Arain, Stecker, Siegart, & Kasselman, 2018). The oligomers, protofibrils, and fibrils can be present as soluble fractions, dispersible plaques, or mature plaques (Rijal Upadhaya et al., 2012; Rijal Upadhaya et al., 2014). Soluble oligomers and protofibrils are intermediates that develop during the formation of mature plaques (Reiss et al., 2018). The plaques themselves are primarily composed of insoluble fibrils and are not easily degraded in the brain. The various conformations of Aβ

begin aggregating during the pre-clinical stage and continue to progress into the symptomatic stage of AD, supporting their role in the disease (Rijal Upadhaya et al., 2014).

More specifically, the various conformations of Aβ are of interest to our understanding of the etiology of FAD and have been linked to the dysfunction of mitochondria, synapse loss, neuroinflammation, and oxidative stress, which have been further correlated to the descent into dementia (Koffie et al., 2009; Maezawa et al., 2011). Consequently, Aβ likely indirectly effects cognitive decline in AD by eliciting a variety of toxicities in the brain. In support of this hypothesis, Aβ has been linked to the formation and spread of NFTs, which are highly correlated to cognitive decline amongst AD cases (Giannakopoulos et al., 2003; Hurtado et al., 2010; He et al., 2018; Hanseeuw et al., 2019). Therefore, treatments that decrease or delay the aggregation of various conformations of Aβ are promising and might prevent mitochondrial dysfunction, synapse loss, neuroinflammation, oxidative stress, and the progression of NFTs in AD.

Tau Protein and NFTs

In a healthy adult brain, tau proteins are highly soluble and contain 6 isoforms consisting of 1 or 2 tandem repeats on the N-terminal and 3 or 4 tandem repeats on the C-terminal (Goedert, Spillantini, Jakes, Rutherford, & Crowther, 1989). The repeats promote microtubule stability and regularly undergo phosphorylation at 2-3 sites of each isoform (Goedert, 1993). Under pathological conditions tau will phosphorylate at multiple sites per isoform, which is considered hyperphosphorylated. All 6 tau isoforms hyperphosphorylate during AD, however only specific sites have been linked to the disease (Neddens et al., 2018).

The hyperphosphorylation of specific tau sites is of interest to AD researchers as it results in decreased microtubule binding, the destabilizing of microtubules, the aggregation of paired helical filaments (PHFs), and eventually the formation of NTs and NFTs. The aggregated forms of tau represent insoluble tau. The insolubility of the protein has been directly correlated to neurodegeneration and cognitive decline amongst AD patients (Giannakopoulos et al., 2003; Haroutunian, Davies, Vianna, Buxbaum, & Purohit, 2007; Hanseeuw et al., 2019; Petersen et al., 2019). More specifically, during the late stages of the disease intracellular NFTs change to extracellular NFTs. Extracellular NFTs also known as, ghost tangles, are characterized to lack cell bodies, dendrites, and axons, suggestive of cell dysfunction or loss (Augustinack, Schneider, Mandelkow, & Hyman, 2002; Braak, Braak, & Mandelkow, 1994). As the disease progresses the ghost tangles become more abundant and as a result there are numerous lesions in the brain. Neuronal loss is one of the largest known factors associated with cognitive decline among AD cases rendering the hyperphosphorylation of tau protein an appropriate therapeutic target (Cash et al., 2013; Gómez-Isla et al., 1997; Jutten et al., 2019).

Inflammation

In addition to the neurotoxicity's linked to Aβ and abnormal tau, these pathologies have also been associated with increased neuroinflammation. Neuroinflammation is normally an adaptive response in the brain and promotes the uptake and degradation of toxic species and maintenance of homeostasis in the CNS through the release of pro-inflammatory cytokines and anti-inflammatory cytokines (Piiranen et al., 2017; Sarlus & Heneka, 2017). However, chronic inflammation, such as that observed in AD can have negative consequences and contribute to the progression of the disease. Microglial and

astrocytes are inflammatory markers upregulated in the brains of AD patients. Many SAD genetic risk factors are inflammatory genes, further supporting their involvement in the onset and progression of the disease (Andrews et al., 2020; Cruchaga et al., 2018; Kunkle et al., 2019).

Microglial are the primary immune response in the central nervous system (CNS), they utilize their long dendritic processes to communicate with their environment (Piiranen et al., 2017; Sarlus & Heneka, 2017). In the presence of neurotoxicity, microglial will become active. Reactive microglial change in shape and can elicit phagocytosis (Thomas, 1992). However, when chronically activated or in the presence of disease, the normal function of microglial can be disrupted. The toxic effects of chronic microglial have been correlated to AD pathologies and neuronal damage in pre-clinical AD cases, further supporting their potential role in the descent into dementia (Ii, Sunamoto, Ohnishi, & Ichimori, 1996; Melah et al., 2016). Specifically, microglial induced by Aβ are believed to contribute to oxidative stress, synapse loss, and neuronal damage (Ii et al., 1996; Maezawa et al., 2011; Neniskyte, Neher, & Brown, 2011; Piirainen et al., 2017; Zhang, Jia, & Jia, 2020). In support of this, it is widely accepted that in neurodegenerative diseases chronic active microglial release excess amounts of pro-inflammatory cytokines thereby continuously pruning synapses and degrading neurons (Hong et al., 2016; Piirainen et al., 2017). Inflammatory inhibitors have been shown to reverse these negative effects, thereby supporting microglial as therapeutic targets (Yang, Liu, Xu, Xian, & Lin, 2020).

Furthermore, microglial are thought to signal the activation of astrocytes, another inflammatory response in the brain (Frost & Li, 2017; Liddelow & Barres, 2017). Once activated astrocytes are usually capable of phagocytosis, regulating microglial synaptic

pruning, and maintaining homeostasis in the CNS (Frost & Li, 2017; Preman, Alfonso-Triguero, Alberdi, Verkhratsky, & Arranz, 2021). However, it is believed that the function of astrocytes is disrupted in the presence of AD mutations (Gomez-Arboledas et al., 2018; Sanchez-Mico et al., 2021). Indeed, reactive astrocytes are closely linked to plaques and the soluble forms of Aβ appear to alter the ability of astrocytes to use phagocytosis on toxic species (Gomez-Arboledas et al., 2018; Sanchez-Mico et al., 2021). Additionally, like microglial, astrocytes have been shown to release pro-inflammatory cytokines, which have been shown to upregulate the APP gene and promote the cleavage of Aβ (Lesné et al., 2003). As such, the malfunction of astrocytes amongst AD brains in conjunction with the release of pro-inflammatory cytokines might contribute to progression of AD in the brain.

Overall, microgliosis and astrogliosis are upregulated in AD and might contribute to pathological progression of the disease. Treatments that target chronic neuroinflammation might improve the progression of the disease. However, multiple neuropathological changes occur throughout the course of AD. Targeting one pathology might not be as effective as treatment options that target multiple pathologies. Despite the multiple factors contributing to disease onset and prognosis, current treatment options often only target one pathology.

Current Treatments

The search for appropriate treatments for AD has been a challenging process. Countless drugs have been assessed over the last decade in attempt to improve the burden of the disease on patients, caregivers, and the economy. Many drugs have failed clinical trials due to the lack of improvement in symptoms and/or adverse side effects. Currently, the only food and drug administration approved drugs are acetylcholinesterase inhibitors

(AChEIs) (Donepzil, Galantamine, and Rivastigmine), a N-methyl-D-aspartate (NMDA) antagonist (Memantine), and recently the food and drug administration fast tracked approved a new drug which targets Aβ (Aducanumab) (Atri, 2019; Alzheimer's Disease Facts & Figures, 2022).

While it is promising that there are readily available treatment options, the majority simply mitigate symptoms rather than delay the progression of the disease (Alzheimer's Disease Facts & Figures, 2022). Furthermore, the treatments have reported side effects (i.e., diarrhea, nausea, and headaches) and it is recommended that the use of AChEIs not be terminated once treatments have begun (Atri, 2019; Doody et al., 2001). Indeed, Doody et al. (2001) found that patients who stopped Donepzil treatments for 6 weeks demonstrated a greater cognitive decline than that observed prior to the commencement of treatments. The recommencement of treatments was unable to fully restore their cognition. Therefore, for patients to experience clinical improvements the treatments cannot be terminated. This poses a particular concern regarding AD given the debilitating nature of the disease. Finally, AChEIs and NMDA antagonists are generally prescribed for mild to severe dementia symptoms (Atri, 2019). Treatment options, which target AD prior to symptom onset are the most optimal further illustrating the need of new treatment options to target the early stages of the disease.

Although it is important that there are some treatment options available, there is still a need for treatment options which will target early disease-stages, with fewer negative side effects, and that will delay or prevent the progression of various neuropathologies found in AD.

Cannabinoids

Recently excitement has emerged around the idea of using compounds from the plant, *Cannabis sativa*, to treat a variety of neurodegenerative diseases, including AD. Cannabinoid compounds exert a multitude of effects on the brain through direct and indirect action on the endogenous cannabinoid system (ECS).

Endogenous Cannabinoids

The ECS is found throughout the CNS and peripheral nervous system (PNS), it consists of cannabinoid receptors and their ligands, endocannabinoids (Howlett et al., 2002; Kilaru & Chapman, 2020; McPartland, Duncan, Di Marzo, & Pertwee, 2015). *Endocannabinoids:* The endocannabinoids bind to and activate cannabinoid receptors. Anandamide and 2-arachidonoylglycerol (2-AG) are the most widely researched endocannabinoids. Anandamide is a partial agonist for cannabinoid receptors, while 2-AG is a full cannabinoid receptor agonist (Kilaru & Chapman, 2020). *Cannabinoid receptors:* The most characterized cannabinoid receptors are, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 receptors are found in various cell types of higher order brain regions (Herkenham et al., 1990). CB1 receptors mediate a variety of functions in the brain, including maintaining homeostasis by influencing the release of neurotransmitters, exerting anti-inflammatory effects, through altering the function of astrocytes and microglial, and promoting neurogenesis (Albayram et al., 2011; Jin et al., 2004; Metna-Laurent & Marsicano, 2015; Pertwee, 2008). CB2 receptors are primarily located on immune cells, such as microglial and macrophages (Howlett et al., 2002; Kilaru & Chapman, 2020). CB2 receptors have been shown to be involved in the proliferation and migration of microglial in the PNS and CNS, thereby directly increasing inflammatory

responses (Carrier et al., 2004; Walter et al., 2003). Disruption to the release of neurotransmitters, decreased neurogenesis, and increased neuroinflammation are brain changes associated with the progression of AD. As a result, alterations to the ECS might be involved in the overall progression of the disease.

Indeed, Bedse et al. (2014) found that AD mouse models have a decreased expression of CB1 receptors in the basolateral amygdala and dorsal hippocampus (HPC). Additionally, it has been shown that the expression of CB1 receptors is decreased in the brains of AD cases (Manuel et al., 2014; Ramírez, Blázquez, Gómez del Pulgar, Guzmán, & de Ceballos, 2005). Interestingly, aged mice that lack CB1 receptors demonstrate increased neuroinflammation and neuronal loss in the HPC (Albayram et al., 2011). Degeneration of the HPC has long been associated with the severity of AD further supporting the potential role of the ECS on the disease. CB2 receptors have been shown to be upregulated in glial cells near NPs of AD patients (Benito et al., 2003). The upregulation of CB2 receptors might contribute to neuroinflammation in AD patients by increasing the proliferation and migration of microglial. As previously discussed neuroinflammation is correlated with the progression and severity of AD. Overall, the decreased expression of CB1 receptors and the increased expression of CB2 receptors suggests that alterations to the ECS might be one of the many factors impacting AD. It is probable that targeting the ECS of AD patients can alleviate the pathological progression of AD.

Certainly, *in vitro* work suggests that CB1 and CB2 agonists can reduce inflammation and the release of nitric oxide from microglial cell cultures, directly decreasing toxicity (Ramírez et al., 2005; Martin-Moreno et al., 2011). Moreover, CB2 agonists have been shown to increase the reuptake of Aβ through phagocytosis in

microglial cell cultures, thereby directly decreasing the quantity of Aβ (Tolón et al., 2009). *In vivo* work supports the results of the *in vitro* studies, suggesting that CB1 and/or CB2 agonists can decrease inflammation, $A\beta_{1-42}$, and cognitive deficits in Aβ mouse models of AD (Haghani, Shabani, Javan, Motamedi, & Janahmadi, 2012; Martin-Moreno et al., 2012; Ramírez et al., 2005). Importantly the administration of low doses of CB1 and CB2 agonist do not impair the learning and memory of control mice (Martin-Moreno et al., 2012; Ramírez et al., 2005), suggesting that when administered at appropriate dosages CB1 and CB2 agonists are not toxic. Given the positive effects of cannabinoid agonists on AD related pathologies in the absence of memory disturbances it is likely that THC will benefit the brain in a similar manner. THC is readily available and now legal to the Canadian public, if proven to be an appropriate treatment for AD this could provide hope for many individuals.

Phytocannabinoids

Δ9-Tetrahydrocannabinol. Δ-9-Tetrahydrocannabinol (THC) is widely known as the main psychoactive component of the plant *Cannabis sativa*. THC behaves similarly to anandamide and is a partial agonist for CB1 and CB2 receptors, with a particular affinity for CB1 receptors (Petitet, Jeantaud, Reibaud, Imperato, & Dubroeucq, 1998; Pertwee, 2008). Activation of CB1 and CB2 receptors via THC and synthetic forms of THC have been shown to be neuroprotective, anti-inflammatory, reduce oxidative stress, reduce Aβ, reduce the hyperphosphorylation of tau protein, promote neurogenesis in the HPC, inhibit acetylcholinesterase, and improve cognitive deficits in AD mouse models (Cao et al., 2014; Eubanks et al., 2006; Martin-Moreno et al., 2011; Ramírez et al., 2005). The observed benefits of THC were in the absence of apparent adverse effects. Overall, THC directly acts

on the ECS and can benefit multiple pathologies related to the progression and severity of AD, strongly suggesting that THC is a promising treatment option for AD patients.

Despite the clear benefits of THC in absence of side effects, the daily use of THC has long been debated in society. High doses of THC can impair learning and memory and exert psychoactive effects through the activation of CB1 receptors (Calabrese & Rubio-Casillas, 2018). However, as clearly shown in the literature, low doses of THC are not harmful (Nidadavolu et al., 2021; Suliman, Taib, Moklas, & Basir, 2017). Nevertheless, considering the concerns associated with THC, CBD might be an alternative option for AD patients as it is not psychoactive at high or low dosages and exerts many benefits like that of THC.

Cannabidiol. Cannabidiol (CBD) is the main non-psychoactive component of the plant *Cannabis sativa*, its direct mode of action on the ECS is poorly understood. While, CBD has been shown to directly antagonize CB1 and CB2 receptors (Petitet et al., 1998; Thomas et al., 2007), it is more commonly believed that it indirectly affects the activation of the receptors (Pertwee, 2008). Research suggests that CBD can act as an inverse agonist for CB1 and CB2 receptors, likely by preventing the hydrolysis of anandamide (Fogaça, Campos, Coelho, Duman, & Guimarães, 2018; McPartland et al., 2015). In addition to interacting with the ECS, CBD can exert various effects through interacting with various neurotransmitter systems (i.e., glutamate, serotonin, and dopamine) and molecular mechanisms (i.e., adenosine A_{2A} receptors and peroxisome-proliferator activated receptor gamma (PPARγ)) (di Giacomo et al., 2020; Esposito et al., 2011; Martin-Moreno et al., 2011; McPartland et al., 2015). Through the various interactions of CBD in the CNS, it has been shown to be neuroprotective, enhance HPC neurogenesis, decrease the

hyperphosphorylation of tau protein, decrease the production of Aβ, reduce oxidative stress and reduce inflammation (di Giacomo et al., 2020; Esposito et al., 2006(A)(B); Esposito et al., 2007; Esposito et al., 2011; Fogaça et al., 2018; Iuvone et al., 2004; Martin-Moreno et al., 2011; Scuderi, Steardo, & Esposito, 2014). In summary, CBD acts on multiple AD related pathologies suggesting that like THC, it is a promising treatment option for AD patients.

Molecular Mechanisms. The mode of action of THC and CBD differs within the CNS, however despite this, the compounds share many promising properties. Interestingly, both THC and CBD share the ability to downregulate glycogen synthase kinase-3β (GSK3-β), which might be one of the underlying mechanisms associated with their observed benefits (Cao et al., 2014; Casarejos et al., 2013; Fogaça et al., 2018; Ozaita, Puighermanal, & Maldonado, 2007). GSK3-β is a kinase that is believed to be involved in the phosphorylation of tau protein (Planel, Sun, & Takashima, 2002). GSK3-β is also critical for the survival and death of cells, particularly its activation is believed to promote apoptosis (Jope & Johnson, 2004; Planel et al., 2002). Additionally, the inhibition of GSK3-β is correlated with the activation of the Akt pathway (Ozaita et al., 2007). The Akt pathway is associated with neuronal signalling and cell survival (Ozaita et al., 2007). Ultimately, through the activation of the Akt pathway while inhibiting GSK3-β, cannabinoids could delay AD onset and improve the burden of the disease by reducing the phosphorylation of tau protein, inhibiting neuronal death, and promoting cell survival.

Overall, it is indisputable that there are a vast number of studies which suggest that phytocannabinoids can improve a multitude of pathologies related to AD. AD is a multi-pathology disease and appropriate treatment options should target multiple pathologies. The

benefits of THC and CBD on AD related pathologies are further supported by their positive effects on observed learning and memory impairments in AD rodent models in the absence of negative side effects on the control subjects (Cheng, Low, Logge, Garner, & Karl, 2014(A); Cheng, Spiro, Jenner, Garner, & Karl, (2014(B)); Martin-Moreno et al., 2011). The lack of side effects on control subjects, supports the use of phytocannabinoids for individuals at risk of developing AD, but who are asymptomatic. Currently AD can only be diagnosed in the presence of symptoms, however providing treatment options pre-clinically will provide patients with the most optimal quality of life by delaying the progression of the disease in the brain. While the current research findings are promising, more research is still required to further our understanding of the ECS, phytocannabinoids, and AD. Understanding the effects of the chronic use of various dosages of THC alone, CBD alone, and how they interact with AD-related pathologies and learning, and memory impairments is crucial.

Purpose of the Present Experiments

Based on the previous findings the purpose of the present experiments was to observe the effects of cannabinoid treatments on mouse models of AD. The first experiment observed the effects of chronic administration of various dosages of CBD on mice that express Aβ. The second experiment assessed the effects of chronic administration of various dosages of THC on mice that express Aβ. Both treatment groups began receiving the cannabinoid compounds when they have been characterized to model “pre-clinical” AD (Mehla et al., 2018; Saito et al., 2014). A battery of behavioural tasks was used including object recognition, spatial navigation, and fear conditioning. These tasks were selected because of their sensitivity to dysfunction of neural networks centered on the perirhinal

cortex, HPC, and the amygdala, respectively (Bucci, Phillips, & Burwell, 2000; Ferbinteanu, Ray, McDonald, 2003; Goosens & Maren, 2001; Kochli, Thompson, Fricke, Postle, & Quinn, 2015; Morris, Garrud, Rawlins, & O'Keefe, 1982; Mumby, Piterkin, Lecluse, & Lehmann, 2007; Sutherland, Kolb, & Whishaw, 1982). Following behavioural testing the brains of the mice were analyzed to observe neurodegeneration in the HPC, as well as Aβ, astrocytes, and microglial in the HPC and prefrontal cortex (PFC). It was predicted that the cannabinoid compounds would improve any observed learning and memory impairments as well as the pathology in the brains of the APP^{NL-G-F} mice. It was also predicted that the higher dosages of CBD and THC would provide the animals with the most optimal benefits.

The third experiment utilized the highest dosage of CBD (20mg/kg) previously used along with a vehicle and chronically treated a tauopathy mouse model and their noncarrier littermates. The mice began treatments when they have been considered to model "pre-clinical" AD (López-González et al., 2015). At 6 and 9 months of age learning and memory functions were assessed as well as the sensory and motor functions as this tauopathy mouse model has previously been reported to develop hind limb weakness and paralysis as they age (López-González et al., 2015; Yoshiyama et al., 2007). Following the final set of behavioural tasks, the brains of the mice were analyzed for neurodegeneration, astrocytes, and microglial. It was predicted that the learning and memory of the Tau P301S mice would be impaired relative to the noncarrier littermates. CBD was expected to improve the observed learning and memory impairments. Similarly, it was predicted that HPC volume would be reduced amongst Tau P301S mice and that they would demonstrate increased neuroinflammation compared to the noncarrier littermates. CBD was predicted to increase

the volume of the HPC of the Tau P301S mice while decreasing the quantity of neuroinflammation. CBD was not predicted to impact the behaviour or pathology of the noncarrier littermates.

CHAPTER 2 – THE EFFECTS OF VARIOUS DOSAGES OF CANNABIDIOL AND Δ-9-TETRAHYDROCANNABINOL ON THE BEHAVIOUR AND BRAIN PATHOLOGY OF APP^{NL-G-F} MICE

Aβ, is a neural hallmark of the debilitating neurogenerative disease, AD. Aβ begins developing decades prior to symptom onset (Bateman et al., 2012) and has long been an intriguing therapeutic target for the disease. The growing interest in Aβ is related to the discovery of genes (i.e., APP, PSEN-1, PSEN-2) related to the onset of FAD (Weggen & Beher, 2012). The genes directly promote the release of Aβ peptides, strongly suggesting that the protein is involved in the progression and onset of AD (Hatami et al., 2017). However, despite the clear link between Aβ and AD from a diagnostic point of view, treatments which target the protein have been ineffective at mitigating the progression of AD (Cummings, Lee, Ritter, Sabbagh, & Zhong, 2020; Panza, Lozupone, Logroscino, & Imbimbo, 2019). The poor translatability between Aβ treatments and the progression of AD might be due to the simplistic approach of observing treatments that solely target Aβ. Alterations to the release of neurotransmitters, neuroinflammation, mitochondrial dysfunction, synapse loss, neurodegeneration, and the formation of NFTs might have to be targeted by treatments as well (Maezawa et al., 2011). The pathologies can act independently and instigate their own effects once initiated (Doig, 2018). Realistically, AD must be viewed as a multi pathology disease, as such treatments should target multiple AD related pathologies (Gidyk et al., 2015; McDonald, 2002). To determine appropriate treatment options, it is paramount to utilize appropriate animal models of the disease.

Animal Models

Unfortunately, no animal model accurately mimics AD as seen in humans, which might contribute to the poor translatability of potential treatment options from animal

research to clinical trials (Chin, 2011). However, that does not discount their use to further our understanding of the disease, rather researchers should be cautious of this. Currently, there are 213 animal models of AD (https://www.alzforum.org/research-models), each with their own strengths and shortcomings. The most used models have long been transgenic mouse models. Transgenic mouse models are genetically modified, and it is common for them to over express genes related to AD, particularly the APP gene (Jankowsky & Zheng, 2017; Sasaguri et al., 2017). The overexpression of the APP gene results in the mice expressing artificial phenotypes rendering them less than optimal as models of the etiology of AD (Saito et al., 2014; Sasaguri et al., 2017). However, the relatively newly developed knock-in mouse models of AD minimize this concern (Saito et al., 2014).

Knock-in mice contain the insertion of mutated genes without altering the natural structure of the gene (Jankowsky & Zheng, 2017; Sasaguri et al., 2017). A particularly exciting knock-in mouse model of FAD is the $APP^{NL\text{-}G\text{-}F}$ model. $APP^{NL\text{-}G\text{-}F}$ mice contain three humanized Aβ mutations: the Swedish mutation, the byeurian/Iberian mutation, and the artic mutation. The mutations lead to the development of Aβ by two months of age and astrogliosis and microgliosis by 4 months of age (Saito et al., 2014). Additionally, the mice demonstrate synaptic dysfunction. The observed pathologies correlate with an age-dependent loss of cognitive abilities (Mehla et al., 2018; Saito et al., 2014). As such, $APP^{NL\text{-}G\text{-}F}$ mice will allow researchers to observe the benefits of potential treatments on multiple AD related pathologies and learning and memory impairments in the absence of the overexpression of APP.

Cannabinoid Compounds

Remarkably, compounds from the plant *Cannabis sativa*, THC and CBD, demonstrate many properties suggesting that they could improve and delay the development of multiple brain changes related to AD. While THC and CBD can be used in combination, their mode of action in the CNS differs (Pertwee, 2008). Therefore, the present experiments will focus on understanding the benefits of the compounds independently.

Δ-Tetrahydrocannabinol

THC directly acts on the ECS and when administered at low dosages THC has a broad array of benefits, suggesting it could improve and delay the progression of AD. Cell cultures treated with low dosages of THC have been shown to reduce the aggregation of $A\beta_{1-40}$ (Cao et al., 2014). In support of this, THC has been shown to directly destabilize Aβ protofibrils and fibrils (Janefjord, Mååg, Harvey, & Smid, 2014; Kanchi & Dasmahapatra, 2021). Aβ protofibrils and fibrils are toxic and are associated with Aβ plaques, thus their destabilization would likely benefit AD patients. Furthermore, a CB1 and CB2 agonist, that behaves like THC has been shown to reduce pro-inflammatory cytokines upregulated due to Aβ (Martin-Moreno et al., 2011; Martin-Moreno et al., 2012). The benefits of THC administration on Aβ and Aβ related toxicities correlates with improved learning and memory impairments in mice. THC has been shown to improve object recognition (Aso, Sánchez-Pla, Vegas-Lozano, Maldonado, & Ferrer, 2015; Suliman et al., 2017) and spatial navigation (Nidadavolu et al., 2021) in Aβ mouse models of AD and in aged rodents. Moreover, THC was able to promote neurogenesis in rats in a dose dependent manner (Suliman et al., 2017). The authors administered acute and chronic treatments of THC at

0.75mg/kg, 1.5mg/kg, and 3.0mg/kg. No dosage of THC impaired the learning of the rats indicating that when administered at an appropriate dosage THC can promote learning and memory and enhance neurogenesis in the absence of certain side effects. Chronic administration of 1.5mg/kg of THC provided the most optimal benefits on neurogenesis and object recognition supporting the notion of using repeated administration of THC to treat AD. Overall, there are several benefits associated with the consumption of low doses of THC. Similarly, CBD also demonstrates a vast array of therapeutic properties suggesting that it could be used as a potential therapeutic target for AD patients.

Cannabidiol

It is believed that unlike THC, CBD does not directly act on the ECS (Pertwee, 2008). Despite the different modes of action in the CNS, CBD exerts many benefits like THC. *In vitro* work suggests that CBD can reduce the overexpression of APP, while decreasing the production of Aβ in cell cultures (Scuderi et al., 2014). CBD has also been shown to antagonize the compounds involved in microglial migration, thereby reducing neuroinflammation (Walter et al., 2003). In addition to reducing neuroinflammation, CBD decreases toxicities associated with chronic inflammation, such as, the release of nitric oxide and pro-inflammatory cytokines (Esposito et al., 2007; Esposito et al., 2011; Martin-Moreno et al., 2011). Furthermore, from a behavioural standpoint, CBD treatments have been shown to improve memory impairments amongst Aβ mouse models of AD (Cheng et al., 2014(A)(B); Martin-Moreno et al., 2011; Watt et al., 2020(A))). Finally, Esposito et al. (2011) demonstrated that 10mg/kg of CBD given to mice injected with Aβ reduced reactive astrocytes, and neurodegeneration in the HPC, while increasing neurogenesis.

Overall, there are numerous studies that support the use of THC or CBD to delay the pathological progression of AD in the absence of side effects. The reduced pathological progression of the disease is correlated with improved learning and memory impairments, further supporting the use of cannabinoid compounds to treat AD. While the results from previous experiments are novel and exciting, more research is required. Particularly it is important to further our understanding of the pre-symptomatic, chronic exposure of THC or CBD alone. Ideally, treatment options for AD patients will be safe to prescribe pre-clinically and provide long-term benefits. As a result, confirming the efficacy and safety of THC and CBD over prolonged periods of time prior to symptom onset is crucial. Additionally, it is important to observe the effects of various dosages of the compounds to determine the most optimal dosage to improve AD pathologies and learning and memory impairments. Finally, since the onset and progression of AD is associated with multiple factors, it is critical to further our understanding as to how THC and CBD impact a variety of AD related pathologies and if any observed effects occur in conjunction with improved cognition.

Purpose of the Present Experiments

Therefore, based on the findings from previous studies, two experiments were conducted to further our understanding on the effects of the pre-symptomatic, chronic administration of various dosages of CBD and THC on the behaviour and brain pathology of $APP^{NL\text{-}G\text{-}F}$ mice.

Experiment One

The aim of experiment one was to observe the effects of chronic oral administration of 5mg/kg, 10mg/kg, and 20mg/kg of CBD on the behaviour and brain pathology of APP^{NL-G-F} mice. The dosages were chosen based on the observations of previous experiments (Esposito et al., 2007; Esposito et al., 2011; Hayakawa et al., 2007; Martin-Moreno et al., 2011) and pilot work. The mice began receiving the treatments at 3 months of age when they are considered to lack cognitive impairments (Mehla et al., 2018). After three months of treatments, when the mice were 6 months of age, they were behaviourally tested on tasks sensitive to the structure and function of brain regions within the MTL (Bucci et al., 2000; Ferbinteanu et al., 2003; Goosens & Maren, 2001; Kochli et al., 2015; Morris et al., 1982; Mumby et al., 2007; Sutherland et al., 1982). Following behavioural testing the mice were perfused and Nissl and immunohistochemistry were performed. Cresyl violet Nissl was used to assess the HPC volume of the mice. 82E1 assessed plaques at epitopes 1-16, which stains the N-terminal of Aβ (Aho et al., 2010). 4G8 was utilized to measure plaques at epitopes 7-24, which stains the mid proportion of Aβ (Aho, Pikkarainen, Hiltunen, Leinonen, & Alafuzoff, 2010; Wisniewski, Wen, & Kim, 1989). Glial fibrillary acidic protein (GFAP) is a well characterized marker of reactive astrocytes; therefore, it was used to detect the presence of astrocytes (Escartin et al., 2021). Finally, ionized calcium binding adaptor molecule 1 (Iba1) is a protein specifically found in microglial, the presence of the protein is believed to be suggestive of microgliosis (Hovens, Nyakas, & Schoemaker, 2014). Accordingly, Iba1 was used to visualize microglial in the brains of the mice. Furthermore, the markers used in the present experiment have previously been reported to be visualized in the brains of APP^{NL-G-F} mice

and progress in an age dependent manner (Mehla et al., 2018) suggesting that these measures could be used to assess the efficacy of various dosages of CBD on the progression of pathology in the brains of the mice.

Experiment Two

The goal of experiment two was to observe the effects of daily chronic oral administration of 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg of THC on $APP^{NL\text{-}G\text{-}F}$ mice. The dosages chosen were based on the findings from previous experiments, which have reported the dosages to be effective and non-psychoactive (Aso et al., 2015; Martin-Moreno et al., 2011; Martin-Moreno et al., 2012). Drug administration, behavioural tasks and immunohistochemistry were performed as in experiment one.

Hypotheses

It was hypothesized that CBD and THC would improve any observed learning and memory impairments of the $APP^{NL\text{-}G\text{-}F}$ mice on all behavioural tasks. The improved cognitive function was expected to correlate with a reduced quantity of Aβ, astrocytes, and microglial in the HPC and PFC. The treated mice were not expected to demonstrate neurodegeneration. The higher dosage of CBD, 20mg/kg, and the higher dosage of THC, 1.0mg/kg, were expected to be the most optimal dosages.

Experiment 1 – Method

Subjects

Twenty-one $APP^{NL\text{-}G\text{-}F}$ (male, n=14; female, n=7) mice were bred inhouse at the University of Lethbridge. The mice were housed with littermates in IVC cages, which contained corncob bedding, Crink-l'Nest, a cotton square, and a plastic tunnel for

enrichment. The housing room was kept on a 02h00-14h00 light/dark cycle and was temperature and humidity controlled (21°C). The mice were divided into three groups based on the given dosage of CBD (5mg/kg (n=7), 10mg/kg (n=8), 20mg/kg (n=6)). Beginning at three months of age the CBD was given to the mice daily via a palatable food (Nutella). Three hours before treatments the mice were food restricted, otherwise they had *ab libitum* access to food and water. All methods and procedures described in this experiment were in accordance with Canadian Council of Animal Care Guidelines and were approved by the University of Lethbridge Institutional Committee on Animal Care.

Drugs, Preparation, and Administration

The CBD powder was kept in a -20°C freezer prior to dilution. All dilutions took place in a biosafety fume hood. To prevent product contamination, materials were saturated in 100% Ethanol and placed under a UV light for 15 minutes except for the CBD and oil. The CBD and oil were saturated in 100% Ethanol and placed in the fume hood under a regular light for 15 minutes to maintain product stability. The CBD was diluted in PC Grapeseed oil (No Frills). The oil was filtered using a syringe and a sterile filter unit (FroggaBio). A stock solution of 20mg/kg was heated on a rotating plate to 40°C and left for 2 hours to allow the powder to dissolve. The stock solution was further diluted with more oil to achieve the 10mg/kg and 5mg/kg concentrations. The solutions were aliquoted in 1.5mL tubes and stored at -20°C. While the CBD oil was being used, it was stored in a 4°C fridge for up to 7 days.

The CBD oil was mixed in 0.25g of Nutella, daily. During treatments, the mice were transferred from their housing cages into individual holding cages, which contained a water bottle and a weigh boat with the Nutella/CBD mix. The mice were weighed every

second day to determine the amount of CBD (μL) that they would receive. Holding cages were cleaned daily with Virkon and water bottles were replaced weekly. The mice were placed in the holding cages for a maximum of one hour or until every mouse had finished eating their Nutella/CBD mix, any remaining Nutella was weighed and noted.

Apparatus, Materials, and Procedure

All behavioural tasks that were manually scored were done after behaviour was complete, by analyzing the videos with Videopad. The videos were watched on half speed to ensure appropriate times were noted. An experimenter who was blind to the treatment groups scored the behavioural tasks.

Novel Object Recognition

When the mice were around 5 months of age, they participated in the novel object recognition test (NOR) as previously described (Mehla et al., 2018; Vogel-Ciernia & Wood, 2014). The mice were subjected to 6 days of habituation, followed by one day of training, and one day of testing. Throughout habituation, training, and testing, the mice were brought from their housing room and into the experiment room with their cage mates. Upon entering the room each mouse was individually placed in a white plastic arena (46 x 48 cm) for their given trial. Corncob bedding was placed at the base of the arena and shuffled around between trials to eliminate olfactory cues. All males were tested prior to females. Prior to the females being placed in the context the arena was cleaned with 70% Ethanol and the corncob bedding was replaced to eliminate any odors from the males. *Habituation:* During habituation, the mice were placed in the context and given five minutes to freely explore, daily for 6 days. *Training:* Twenty-four hours after the last

habituation day the mice were subjected to training. During training two identical objects (A, A or B, B) were placed in the context, an equal distance from the walls. The mice were given 10 minutes to freely explore the context and objects. *Testing:* Twenty-four hours after training the mice were tested. One familiar object was replaced with a novel object (A, B or B, A). The mice were given 5 minutes to freely explore the context and objects. After each trial, the objects were cleaned with 70% Ethanol to eliminate olfactory cues. The behaviour of the mice was recorded in the contexts for further analysis. The videos were manually scored, and exploratory behaviour was defined as time (s) spent within 1 cm of the objects and facing the objects. Looking past the objects, sitting on the objects, and digging near the objects was not considered object-directed exploratory behaviour. The investigation ratio (IR) for the novel object was calculated by dividing the time spent exploring the novel object by the total exploration time for both objects, combined (Mehla et al., 2018). Mice are naturally exploratory, therefore increased exploration for the novel object is suggestive of recall for the familiar object (Vogel-Ciernia & Wood, 2014). Recall for the familiar object indicates that the mice retained object recognition thought to be dependent on the function of networks related to the perirhinal cortex (Kealy & Commins, 2011; Mumby et al., 2007).

Morris Water Task

Around 6 months of age the mice began Morris water task (MWT) training as previously described (Mehla et al., 2018). MWT consisted of eight spatial training days, followed by one probe day, and two visible platform days. Across all days, the temperature of the water was 22°C (+/- 1°C) and non-toxic white paint was placed in the pool to render the water opaque white. Each day the mice were individually placed in holding cages and

brought into the experiment room on a cart. Upon entering the room, the mice were left to sit for 30 minutes, so that they could adjust to the new environment. *Spatial training:* During training, a platform was placed in a fixed location, approximately 1 cm below the water. Distal cues were positioned around the pool to aid the animals in learning to locate the hidden platform. Each training day consisted of four trials, during each trial the mice were placed in the pool, facing the wall from a different start point (N, S, E, or W). The start points were counterbalanced across days. During each trial, the mice were given 60s to locate the hidden platform, if they failed to locate the platform an experimenter guided the mice with their hand. Once the mice were on the platform, they were left to sit for 10s prior to being removed. Once removed the mice were dried with a towel and placed back in their holding cage until their next trial. *Probe:* To assess the spatial recall of the mice, a single probe trial was performed 24 hours after the final training day. During the probe trial the mice were placed in the pool facing the wall, from one of the two start locations farthest from the previous platform location. The platform was removed, and the distal cues used during training remained fixed. The mice were left to swim in the pool for 60s prior to being removed. *Visible platform:* Twenty-four hours following the probe trial the mice were assessed on two visible platform tests, which were conducted over two days. The visible platform tests were performed to rule out any sensory, motor, or motivational deficits in the mice. During visible platform, the platform was placed 1 cm above the water, and a black cylinder was placed on top of the platform. During each visible platform test the platform and visible cues were moved to novel locations. The mice experienced four 60s trials each day, during each trial they were placed in the pool facing the wall from a different start location (N, S, E, or W). Once the mice located the platform they were removed from the pool. During training, probe, and visible platform a HVS software

package tracked the behaviour of the mice for analysis. A decreased path length to reach the hidden platform, across spatial training days indicates spatial learning. An increased time spent in the target quadrant relative to the alternate quadrants and an increased number of annulus crossings during the probe trial indicates spatial memory recall. Retained spatial learning and memory recall suggests that networks centered around the function of the HPC remained intact (Ferbinteanu et al., 2003; Morris et al., 1982; Sutherland et al., 1982). Finally, a decreased path length when the platform was visible is indicative of no sensory, motor, or motivational impairments.

Fear Conditioning

The mice were given a few days to rest after MWT training before beginning fear conditioning. Fear conditioning consisted of one day of conditioning, followed by context or tone recall performed over two days (Mehla et al., 2018). The order in which the mice experienced context or tone recall was counterbalanced across groups (5mg/kg, 10mg/kg, and 20mg/kg). During conditioning and tone/context recall the mice were individually placed in holding cages, which contained corncob bedding. The mice were not placed back in their housing cages until all cage mates were tested to eliminate any additional stressors. *Conditioning:* The mice were brought into the conditioning room one at a time and left to sit for two minutes prior to being placed in the context. The context was an acrylic square box (33 x 33 x 25 cm) that consisted of two black walls and two white walls, with grid flooring, which was attached to a shock generator. Once placed in the context a plexiglass cover was laid over the top to limit access to cues in the testing room and to prevent the mice from jumping out. A video camera was positioned to the right of the context to mimic the environment of tone and context recall; however, the behaviour of the mice was not

recorded. After a two-minute baseline period in the context a 20s, 2,000Hz tone was presented. The tone was co-terminated with a 2s, 0.5mA shock. The mice received five tone/shock pairings that were spaced two minutes apart. Following the last tone/shock pairing, the mice were left in the context for one minute prior to being removed. The mice were removed from the context and brought into an alternate experiment room in a separate holding cage until all cage mates had been tested. Once all cage mates were tested the mice were brought back to the housing room. Context or tone recall occurred 24 to 48 hours following conditioning. *Context recall:* During context recall the mice were individually brought into a novel experiment room and left to sit for two minutes. After two minutes the mice were placed in the same context used during conditioning, however no tone or shock was administered. The mice were given five minutes in the context to freely explore. *Tone recall:* During tone recall the mice were individually brought into a novel experiment room. The mice were left to sit for two minutes prior to being placed in a white triangular chamber (33 x 33 x 29 cm). Once placed in the chamber the mice were left to sit for an additional two minutes to explore the context. After a two-minute baseline period a 20s, 2,000Hz tone was presented 3 times at 2-minute intervals. Following the last tone, the mice were left for one minute prior to being removed. During context and tone recall the behaviour of the mice was recorded and their time spent freezing was manually scored. Freezing time was defined as the mice not moving. Rearing on the wall, remaining in a stretched-out positioning, and sniffing was not considered time spent freezing. The percent time spent freezing was calculated by dividing the total time the mice spent freezing by the total time being scored, times by 100. The first two minutes during tone recall was used as a baseline measure of freezing behaviour for the mice. For the remainder of the document, the baseline freezing measure will be referred to as novel context, when discussing context

recall, and before the tone, when discussing tone recall. Increased freezing in the presence of the context and tone indicates recall for the context/shock and tone/shock pairings and is suggestive of intact function of networks related to the amygdala (Goosens & Maren, 2001; Kochli et al., 2015).

Histology

Following all behavioural testing, around 7 months of age, the mice were given an intraperitoneal injection (i.p.) of sodium pentobarbital prior to being transcardially perfused with phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA) in PBS. The brains were extracted for histological purposes. The brains were kept in 4% PFA for 24 hours prior to being placed in 30% sucrose with PBS and sodium azide. The brains were stored in a 4°C refrigerator and were serially sectioned coronally with a 40µm freezing microtome.

Nissl

Cresyl violet Nissl was performed for a volumetric analysis of the HPC. Cells within the dentate gyrus (DG), cornu ammonis 1 (CA1), and cornu ammonis 3 (CA3) were counted by an experimenter who was blind to the treatment groups. Counting was performed using the optical fractionator on a stereology microscope at 20x magnification. The Allen brain mouse atlas was used to confirm the cells were being counted in the appropriate regions (https://mouse.brain-map.org/experiment/thumbnails/100048576?image_type=atlas).

Immunohistochemistry

Immunohistochemical procedures were performed as previously described (Mehla et al., 2018). The following stains were used: 4G8, stained for $A\beta_{7\text{-}24}$, 82E1, stained for

$A\beta_{1-16}$, GFAP, stained for astrocytes, and Iba1, stained for microglial. Sections were free float mounted on positively charged slides and left to dry for a few hours. Sections were then fixed with 4% PFA for 4 minutes, washes were performed in Tris-buffered Saline (TBS), incubated in 70% formic acid for 9-12 minutes, permeabilized with 0.1% Triton-X in TBS, and then blocked in 25% bovine serum albumin mixed with 0.1% Triton-X in TBS. Sections were incubated in the primaries (mouse anti-amyloid-β 4G8 (Biolegend, 800701, 1:1000); rabbit anti-GFAP (Abcam, Ab7260, 1:2000); anti-amyloid-β (N) (IBL, 10323, 1:1000); rabbit anti-Iba1 (Wako, 019-19741, 1:1000)), for 45 hours. Following the primaries, the sections were washed and placed in the secondaries (goat anti-mouse-alexa-488 (IgG [H + L], (A32723, ThermoFisher, 1:2000); (anti-rabbit-alexa-594 (IgG [H+L] goat, Invitrogen, A11037, Abcam, 1:1000)). To limit the amount of tissue used, 4G8 was double stained with GFAP and 82E1 was double stained with Iba1. The slides were coverslipped with Vectashield H-1900 (Vector Laboratory) and sealed with nail polish. Slides were imaged using Nanozoomer microscope (Nanozoomer 2.0-RS, HAMAMATSU, JAPAN) at 40x resolution.

Quantification

Immunohistochemical stains were quantified separately. Every slice of the individual stains (4G8, GFAP, 82E1, and Iba1) were exported from NDP view into individual Tiffs. Six full slices from various animals were trained on Ilastik for every stain (early PFC, middle PFC, late PFC, early HPC, middle HPC, and late HPC). When training Ilastik to quantify plaques (4G8 and 82E1), any object spherical in shape with a dense cell body was counted. When training Ilastik to quantify inflammation (Iba1 and GFAP), markers that appeared like active microglial or active astrocytes were counted. Active

microglial were characterized by enlarged cell bodies with short processes. Active astrocytes were characterized by enlarged cell bodies with long dendrites. Active microglial and active astrocytes were often found near plaques (See Figure 1). After Ilastik was sufficiently trained, the HPC and PFC of the exported images were cropped using ImageJ and the area of the HPC and PFC for each slice was obtained. The cropped images were then batched processed through Ilastik. Ilastik provided an excel file with the size of each marker counted for every brain slice. The excel files of every brain slice were combined to receive the data of the whole brain for each animal. The sum size of every marker counted for the whole brain was obtained and divided by the number of slices analyzed to receive the average size per slice. The average size of the markers per slice was then divided by the average volume of the brain region for each animal and times by 100 to receive the percent area covered (mm^2).

Results

Data Analysis

The statistical analysis was performed using SPSS statistical software package, version 27.0. Results are presented as mean +/- SEM. A p value < 0.05 was considered as statistically significant.

Novel Object Recognition

The training data was analyzed and any mouse that did not spend a total of 20s exploring the object's during training was excluded from the testing data. One mouse from the 20mg/kg group was excluded from the testing data. Therefore, the final group sizes for NOR analysis were as follows: 5mg/kg (n=7), 10mg/kg (n=8), 20mg/kg (n=5).

An IR value above 0.5 suggests that the mice explored the novel object more than the familiar object. While each group did explore the novel object more than the familiar object, their preference for the novel object was only slight, as evidenced by an IR value just above 0.5 (See Figure 2). The small preference for the novel object might be suggestive of dysfunction to the perirhinal cortex and related circuits. The 10mg/kg group had the highest IR value, as such 10mg/kg of CBD might be in part promoting object recognition function in $APP^{NL\text{-}G\text{-}F}$ mice around 5 months of age.

A two-tailed one-samples *t*-test revealed that none of the mice explored the novel object significantly above chance (0.5) (5mg/kg (t (6) = 0.817, $p < 0.445$), 10mg/kg (t (7) = 1.546, $p < 0.166$), 20mg/kg (t (5) = 0.878, $p < 0.420$)). An above chance IR value would statistically support that the mice recalled the familiar object and retained object recognition. A one-way between subjects analysis of variance (ANOVA) assessed if there was an effect of CBD dosage on the IR of the mice. There was no statistically significant effect of CBD dosage on the object recall of the mice (F (2, 17) = 0.462, $p < 0.638$). Overall, this analysis suggests that the $APP^{NL\text{-}G\text{-}F}$ mice did not maintain object recognition functions and various CBD dosages did not restore the object recognition of the mice.

Morris Water Task

Spatial Training

The results depicted in Figure 3 suggest that all the groups of mice were able to learn the location of the hidden platform as evidenced by a decreased path length to reach the platform from training day 1 to training day 8. There are no observable differences depending on the given dosage of CBD. A one-way within subjects repeated measures

ANOVA assessed if the path length to reach the hidden platform differed from day 1 to day 8 of training and if there was an effect of CBD dosage on the learning of the mice. There was a significant reduction in the path length of the mice to reach the hidden platform across training days ($F(7, 126) = 16.379$, $p < 0.001$). There was no significant effect of CBD dosage on the learning of the mice ($F(14, 126) = 1.067$, $p < 0.393$). Therefore, around 6 months of age the $APP^{NL\text{-}G\text{-}F}$ mice retained spatial learning function and various dosages of CBD did not enhance or impair the ability of the $APP^{NL\text{-}G\text{-}F}$ mice to learn.

Probe

Figure 4 (A) indicates the average time the mice spent in the target quadrant relative to the alternate quadrants during the probe trial. All the mice, regardless of the dosage of CBD given, spent more time in the target quadrant relative to the alternative quadrants. A one-way within subjects ANOVA was used to analyze the data. The results confirmed that within subjects there was a significant effect of quadrant preference ($F(1, 18) = 49.112$, $p < 0.001$). There was no significant effect of CBD dosage on the quadrant preference of the mice ($F(2, 18) = 0.727$, $p < 0.497$).

To assess how specific the spatial representation guiding mouse navigation during the probe trial was we assessed annulus crossings. This measure is a simple count of how many times the subject crosses the exact location of the target location during the probe trial. A high number of annulus crossings indicate that the subject had knowledge of the exact spatial position of the escape platform. The average number of annulus crossings for the previous platform location can be seen in Figure 4 (B). It appears that there is a trend towards a dose dependent increase in the number of annulus crossings. The 20mg/kg group had the highest number of annulus crossings, while the 5mg/kg group had the lowest

number of annulus crossings. Despite the appearance in the graph, a one-way between subjects ANOVA, suggests that there was no statistically significant effect of CBD dosage on the number of annulus crossings ($F(2, 18) = 2.285$, $p < 0.131$).

Overall, the probe results suggest that all the mice were able to recall the previous platform location, suggestive of retained spatial recall. While, various dosages of CBD did not impact the quadrant preference of the mice, there does appear to be a trend towards a dose dependent increase in the number of annulus crossing. However, the observed trend is not statistically supported.

Visible Platform

The path length of the mice to reach the platform during spatial training day 1, when the platform was hidden, and visible platform day 1, when the platform was visible, was compared. As seen in Figure 5 the $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, and 20mg/kg of CBD had a reduced path length to reach the platform when it was visible, relative to when it was hidden.

A one-way within subjects ANOVA revealed that, within subjects, there was a significant reduction in the path length when the platform was visible relative to when the platform was hidden ($F(1, 18) = 227.813$, $p < 0.001$). There was no significant effect of CBD dosage on the performance of the mice ($F(2, 18) = 1.844$, $p < 0.187$). This pattern of results suggests that the mice do not have sensory, motor, or motivational deficits and CBD had no impact on these supports of learning and memory.

Fear Conditioning

Figure 6 (A) shows a comparison of the percent time spent freezing in the presence of a novel context relative to the percent time freezing during context recall. The mice spent more time freezing in the novel context than during context recall. A decrease in freezing during context recall might be suggestive of impaired context recall. There are no observable differences depending on the given dosage of CBD. A one-way within subjects ANOVA suggests that, within subjects, the mice spent significantly more time freezing in the novel context relative to during context recall ($F(1, 18) = 9.973$, $p < 0.005$). There was no significant effect of CBD dosage on the freezing of the mice ($F(2, 18) = 0.358$, $p < 0.704$).

A comparison of the freezing of the mice prior to the tone relative to their freezing during tone recall can be seen in Figure 6 (B). The $APP^{NL\text{-}G\text{-}F}$ mice had a higher percentage of freezing during the tone compared to prior to the tone, which suggests that they were able to recall the tone/shock pairing. A one-way within subjects ANOVA analyzed the tone recall data. Within subjects, the mice spent significantly more time freezing during the tone compared to prior to the tone ($F(1, 18) = 87.770$, $p < 0.001$). There was no significant effect of CBD dosage on the freezing of the mice ($F(2, 18) = 1.330$, $p < 0.289$).

The fear conditioning data suggests that the mice were unable to recall the context/shock pairing, however they were able to recall the tone/shock pairing. Therefore, in some respect their amygdala and possibly perirhinal or postrhinal cortex function might be impaired.

Hippocampal Volume

Nissl-stained cells were counted in the DG, CA1, and CA3 to determine the HPC volumes of the mice. The group sizes for the volumetric analysis were as follows: 5mg/kg (n=6), 10mg/kg (n=7), 20mg/kg (n=6). Overall, the 20mg/kg group had the largest HPC volume, followed by the 10mg/kg group. The observed results demonstrate a trend in a dose dependent increase in HPC volume (see Figure 7). A one-way between subjects ANOVA was performed to determine if the HPC volumes significantly differed depending on the dosage of CBD given. The results suggest that there was a marginally significant effect of CBD dosage ($F(2, 16) = 3.117$, $p < 0.072$). Therefore, higher dosages of CBD might promote neurogenesis or prevent neurodegeneration in the HPC of the $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

Immunohistochemistry

The group sizes for immunohistochemistry analysis were as follows: 5mg/kg (n=6), 10mg/kg (n=6), 20mg/kg (n=6).

Amyloid-βeta

The results seen in Figure 8 (A) and (B) indicate that the average percentage of $A\beta_{1\text{-}16}$ (stained with 82E1) in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice does not differ depending on various dosages of CBD. A one-way between subjects ANOVA revealed no significant effect of CBD dosage on the percentage of $A\beta_{1\text{-}16}$ in the HPC ($F(2, 15) = 0.204$, $p < 0.818$) or PFC ($F(2, 15) = 0.889$, $p < 0.432$). Figure 9 (A) and (B) suggests that 5mg/kg, 10mg/kg, and 20mg/kg of CBD did not impact the quantity of $A\beta_{7\text{-}24}$ (stained with 4G8) in the HPC and PFC of the mice. Each group demonstrates a similar percentage of $A\beta_{7\text{-}24}$ in the HPC

and PFC. A one-way between subjects ANOVA assessed if there was an effect of CBD dosage. There was no significant effect of CBD dosage on the percentage of $A\beta_{7\text{-}24}$ in the HPC ($F(2, 15) = 1.291$, $p < 0.304$) or PFC ($F(2, 15) = 0.167$, $p < 0.848$).

Overall, various dosages of CBD do not appear to impact the percentage of Aβ in the HPC or PFC of $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

Inflammation

Figure 10 (A) and (B) show the percentage of Iba1 in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. There are no consistent trends observed between groups, suggesting that various dosages of CBD did not impact the quantity of Iba1 in the HPC or PFC of the mice. A one-way between subjects ANOVA confirmed that there was no significant effect of CBD dosage in the HPC ($F(2, 15) = 0.482$, $p < 0.627$) or PFC ($F(2, 15) = 0.320$, $p < 0.731$). However, unlike Iba1, the quantity of GFAP appeared to consistently decrease in the HPC and PFC in a dose dependent manner (see Figure 11 (A) and (B)). Despite the observed trend a one-way between subjects ANOVA suggests that there was no significant effect of CBD dosage on the percentage of GFAP in the HPC ($F(2, 15) = 2.395$, $p < 0.125$) or PFC ($F(2, 15) = 0.401$, $p < 0.676$).

Overall, CBD does not appear to impact the quantity of Iba1, a marker of microglial, in the HPC or PFC of the $APP^{NL\text{-}G\text{-}F}$ mice. However, it might be decreasing the quantity of GFAP, a marker of astrocytes, in a dose dependent manner.

Experiment 2 – Method

Subjects

Thirty-one APP^{NL-G-F} (male, n=14; female n=16) mice were bred inhouse at the University of Lethbridge. The mice were housed with littermates in IVC cages, which contained corncob bedding, Crink-l'Nest, a cotton square, and a plastic tunnel for enrichment. The housing room was kept on a 05h00-17h00 light/dark cycle and was temperature and humidity controlled (21°C). The mice were divided into three groups based on THC dosage (vehicle (n=7), 0.2mg/kg (n=8), 0.5mg/kg (n=8), 1.0mg/kg (n=8)). Beginning at 4 months of age the treatment was given to the mice daily via a palatable food (Nutella). The mice had *ab libitum* access to food and water. All methods and procedures described in this experiment were in accordance with Canadian Council of Animal Care Guidelines and were approved by the University of Lethbridge Institutional Committee on Animal Care.

Drugs, Preparation, and Administration

The THC was prepared and administered as described in experiment one, with a few alterations. The THC was diluted to a stock solution of 1.0mg/kg and was left on a rotating plate for one week to allow the product to properly dissolve. High-performance liquid chromatography was performed on the stock solution to confirm its concentration. The stock solution was then further diluted with more oil to achieve the 0.2mg/kg and 0.5mg/kg concentrations. The filtered grapeseed oil was used as the vehicle. The mice were no longer food restricted prior to treatments and the water bottles were removed in experiment two as they were not deemed necessary for the mice to eat the treatments. As in

experiment one the mice were left in the holding cage for a maximum of one hour, or until all their treatments were consumed. Following the hour any remaining treatments were weighed and noted.

Apparatus, Materials, and Procedure

When the mice were 6 months of age, they began behavioural testing. NOR, MWT, and the fear conditioning paradigm were performed identically to the procedures described in experiment one.

Histology

Following all behavioural testing, around 7 months of age, the mice were perfused. The perfusions, Nissl, immunohistochemistry, and quantification were performed as described in experiment one.

Results

Data Analysis

The statistical analysis was performed using SPSS statistical software package, version 27.0. Results are presented as mean +/- SEM. A p value < 0.05 was considered as statistically significant.

Novel Object Recognition

The training data was analyzed. Any mouse that did not spend a total of 20s exploring the object's during training was excluded from the testing data. One mouse from the 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg groups were excluded from the testing data analysis. The end group sizes were as follows: vehicle (N=7), 0.2mg/kg (N=7), 0.5mg/kg

(N=7), and 1.0mg/kg (N=7). The IR during testing was calculated for each individual animal as described in experiment one.

Figure 12 indicates that on average all the mice had an IR value slightly above 0.5. Suggesting that while the mice did explore to novel object more than the familiar object, their preference for the novel object was minimal. The vehicle animals had the lowest IR value followed by the 0.2mg/kg treated mice. The IR value of the 0.5mg/kg and 1.0mg/kg was the highest suggesting that THC might be partially improving the object recognition of the mice in a dose dependent manner. A statistical analysis of this data did not confirm this observation.

A two-tailed one-samples *t*-test revealed that none of the mice significantly explored the novel object above chance (vehicle (t (6) = 0.831, $p < 0.438$), 0.2mg/kg groups (t (6) = 1.822, $p < 0.118$), 0.5mg/kg (t (6) = 1.347, $p < 0.227$), 1.0 mg/kg (t (6) = 1.782, $p < 0.125$)). A one-way between subjects ANOVA was conducted to compare the IR value depending on the treatment group. There was no significant effect of THC dosage (F (3, 24) = 0.428, $p < 0.735$) on the novel object exploration of the mice. The statistical analysis suggests that the $APP^{NL\text{-}G\text{-}F}$ mice did not retain object recognition capabilities thought to be dependent on the function of networks centered around the perirhinal cortex. Various dosages of THC did not improve or impair the object recognition of the $APP^{NL\text{-}G\text{-}F}$ mice around 6 months of age.

Morris Water Task

Spatial Training

The spatial training data depicted in Figure 13, shows a reduced path length from training day 1 to training day 8 in all groups. Therefore, regardless of the treatment given the mice were able to learn the location of the platform. A one-way within subjects repeated measures ANOVA was conducted to determine if the decreased path length was significant across spatial training days within THC dosages. There was a significant effect of training day on the path length of the mice ($F(7, 189) = 32.429$, $p < 0.001$). There was no significant effect of THC dosage on the path length of the mice ($F(21, 189) = 1.460$, $p < 0.096$). The reduced path length across spatial training days suggests that at 6 months of age the $APP^{NL\text{-}G\text{-}F}$ mice learned and remembered the spatial location of the escape platform. Whether the mice received a vehicle, 0.2mg/kg, 0.5mg/kg, or 1.0m/kg of THC did not impact their spatial learning.

Probe

As seen in Figure 14 (A) the $APP^{NL\text{-}G\text{-}F}$ mice spent more time in the target quadrant relative to the alternate quadrants, regardless of the given dosage of THC. The observed results suggest that the mice were able to recall the location of the platform and various dosages of THC did not impact the spatial recall of the mice. A one-way within subjects ANOVA analyzed if the time spent in the target quadrant relative to the time spent in the alternate quadrants differed within subjects and if there was an effect of THC dosage on the quadrant preference of the mice. The results confirmed that all the mice spent significantly more time in the target quadrant compared to the alternate quadrants ($F(1, 27) = 47.848$, p

< 0.001). There was no significant effect of THC dosage on the recall of the mice (F (3, 27) = 0.143, $p < 0.933$).

Figure 14 (B) represents the annulus crossings of the mice during the probe trial. The 0.2mg/kg THC treated mice had the lowest number of annulus crossings, followed by the vehicle mice. The mice treated with 0.5mg/kg and 1.0m/kg of THC had the highest number of annulus crossings. This pattern of results suggests that higher dosages of THC might be improving the spatial recall of the precise location of the escape platform. However, a statistical analysis of this data set did not confirm this observation. A one-way between subjects ANOVA analyzed the results. There was no significant effect of THC dosage on the number of annulus crossings (F (3, 27) = 1.613, $p < 0.209$).

Overall, the probe data suggests that around 6 months of age the $APP^{NL\text{-}G\text{-}F}$ mice retained spatial recall evidenced by a preference for the target quadrant relative to the alternate quadrants for all the mice. Various dosages of THC did not impact the quadrant preference of the mice, nor did it significantly impact the annulus crossings of the mice.

Visible Platform

Figure 15 shows a comparison of the path length of the mice to reach the platform during training day 1, when the platform was not visible, relative to the visible platform test, when the platform was visible. Around 6 months of age the $APP^{NL\text{-}G\text{-}F}$ mice showed a reduced path length when the platform was visible. The vehicle, 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg treated mice showed similar levels of performance. A one-way within subjects ANOVA analyzed if the path length differed, within subjects, when the platform was hidden relative to when the platform was visible and if there was an effect of THC dosage

on the path length of the mice. There was a significant difference in path length depending on if the platform was hidden or visible ($F(1, 27) = 331.752$, $p < 0.001$). There was no significant effect of THC dosage on the path length of the mice ($F(3, 27) = 2.612$, $p < 0.072$). The pattern of results suggests that the $APP^{NL\text{-}G\text{-}F}$ mice did not demonstrate sensory, motor, or motivational impairments. Various dosages of THC did not impact the ability of the mice to swim to a visible escape platform.

Fear Conditioning

As seen in Figure 16 (A) the $APP^{NL\text{-}G\text{-}F}$ mice spent more time freezing during context recall compared to during the novel context. Increased freezing during context recall suggests that the mice remembered the context/shock pairing. The vehicle, 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg treated mice performed similarly. A one-way within subjects ANOVA suggests that within subjects, the time spent freezing during the novel context was significantly decreased relative to context recall ($F(1, 27) = 22.726$, $p < 0.001$). There was no significant effect of THC dosage on freezing ($F(3, 27) = 0.788$, $p < 0.511$).

Figure 16 (B) demonstrates that the mice spent more time freezing during tone recall compared to prior to the onset of the tone, suggesting that the mice were able to learn and remember the tone/shock pairing. There are no observable differences between the vehicle, 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg treated mice. A one-way within subjects ANOVA confirmed that the percent time spent freezing during tone recall was significantly increased compared to the freezing before the tone ($F(1, 27) = 457.106$, $p < 0.001$). There was no significant effect of THC dosage on the recall of the mice ($F(3, 27) = 1.295$, $p < 0.296$).

All the mice were able to recall the context/shock and tone/shock pairing. Various dosages of THC did not impact the fear associative behaviour of the mice, a learning and memory function supported by a neural network centered on the amygdala.

Hippocampal Volume

Nissl staining was performed, and cells in the DG, CA1, and CA3 were counted to measure the HPC volume of each animal. There were no consistent trends observed in the groups of mice treated with various dosages of THC impacting the HPC volume (see Figure 17). A one-way between subjects ANOVA supported the observed results. There was no significant effect of THC dosage on the HPC volume of the mice ($F(3, 27) = 0.979$, $p < 0.417$). Overall, THC did not alter the HPC volume of the $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

Immunohistochemistry

The group sizes for the 82E1 and Iba1 analysis were as follows: vehicle (n=5), 0.2mg/kg (n=5), 0.5mg/kg (n=5), 1.0mg/kg (n=5). The group sizes for 4G8 and GFAP analysis were as follows: vehicle (n=6), 0.2mg/kg (n=7), 0.5mg/kg (n=6), 1.0mg/kg (n=7).

Amyloid-βeta

The percentage of $A\beta_{1\text{-}16}$ (stained with 82E1) in the HPC and PFC can be seen in Figure 18 (A) and (B). There were no observable trends for a dosage of THC impacting the percentage of $A\beta_{1\text{-}16}$ in the HPC or PFC of the $APP^{NL\text{-}G\text{-}F}$ mice. A one-way between subjects ANOVA revealed no significant effect of THC dosage for the percentage of $A\beta_{1\text{-}16}$ in the HPC ($F(3, 16) = 2.508$, $p < 0.096$) and PFC ($F(3, 16) = 1.014$, $p < 0.412$) of the $APP^{NL\text{-}G\text{-}F}$ mice. Similarly, there were no observable differences in the percentage of $A\beta_{7\text{-}24}$

(stained with 4G8) in the HPC and PFC of the mice (See Figure 19 (A) and (B)). A one-way between subjects ANOVA confirmed that there was no significant effect of THC dosage on the percentage of $A\beta_{7\text{-}24}$ in the HPC ($F(3, 22) = 0.880$, $p < 0.467$) and PFC ($F(3, 22) = 0.521$, $p < 0.672$).

Overall, various dosages of THC had no impact on the quantity of Aβ in the HPC and PFC of the $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

Inflammation

The percentage of Iba1 in the HPC and PFC of the $APP^{NL\text{-}G\text{-}F}$ mice treated with a vehicle, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC can be seen in Figure 20 (A) and (B). There were no observable trends indicating that any dose of THC impacts the percentage of Iba1 in the HPC and PFC of the $APP^{NL\text{-}G\text{-}F}$ mice. A one-way between subjects ANOVA confirmed that there was no significant effect of various THC dosages for the percentage of Iba1 in the HPC ($F(3, 16) = 1.145$, $p < 0.932$) and PFC ($F(3, 16) = 1.338$, $p < 0.297$) of the $APP^{NL\text{-}G\text{-}F}$ mice. As seen in Figure 21 (A) and (B) there were no observable differences in the percentage of GFAP in the HPC and PFC depending on the given dosage of THC. A one-way between subjects ANOVA revealed that there was no significant effect of THC dosage on the percentage of GFAP in the HPC ($F(3, 22) = 0.874$, $p < 0.470$) and PFC ($F(3, 22) = 1.565$, $p < 0.226$) of the mice.

Overall, various dosages of THC did not impact the quantity of Iba1, a marker for microglial, or GFAP, a marker of astrocytes, in the HPC and PFC of the $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

Discussion

The aims of experiment one and experiment two were three-fold. The experiments were conducted to (1) assess the effects of chronic, daily administration of various dosages of CBD and THC on APP^{NL-G-F} mice, (2) whether the pre-symptomatic administration of the treatments could improve the learning and memory of the mice, and (3) if the treatments would delay the progression of AD related pathologies in the HPC and PFC of the mice. Overall, around 6 months of age the APP^{NL-G-F} mice were impaired during object recognition, however they retained spatial learning and memory. The APP^{NL-G-F} mice treated with CBD were impaired during context recall, but not tone recall. The APP^{NL-G-F} mice treated with THC were unimpaired during context and tone recall. Overall, there were no significant effects of various dosages of CBD or THC on the learning and memory of the mice. Furthermore, the APP^{NL-G-F} mice did demonstrate Aβ accumulation and inflammation in the HPC and PFC. There was a marginal trend towards an effect of CBD dose dependently increasing the HPC volume of the mice. However, there was no effects of various dosages of CBD or THC on the accumulation of Aβ or inflammation in the HPC and PFC of the animals.

Impacts of Cannabinoid Compound Treatments on Learning and Memory of APP^{NL-G-F} Mice

The NOR results indicate that at 6 months of age the object recognition of the APP^{NL-G-F} mice was impaired. The impairment contradicts previous reports on APP^{NL-G-F} mice, which suggest that the object recognition of the mice is not impacted until 9 months of age (Mehla et al., 2018). Nevertheless, the impairment is consistent with those reported in humans with AD. Impaired object recognition is believed to be an early symptom that

occurs in patients with mild cognitive impairments and probable AD (Laatu, Revonsuo, Jaykka, Portin, & Rinne, 2003).

This impairment in object recognition of the APP^{NL-G-F} mice was not restored following exposure to various dosages of CBD or THC. However, the mice that received 10mg/kg of CBD and 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg of THC demonstrated a stronger preference for the novel object compared to the mice that received 5mg/kg and 20mg/kg of CBD, or the vehicle. Therefore, while not significant, the observed increased investigation for the novel object among the mice that received 10mg/kg of CBD and 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg of THC is promising and indicates that the cannabinoid compounds might promote learning and memory centered around networks related to the perirhinal cortex among AD patients. In support of this, previous research results suggest that THC and synthetic forms of THC were able to promote object recognition in rats and Aβ mouse models (Aso et al., 2015; Suliman et al., 2017). Similarly, previous experiments suggest the benefits of CBD on object recognition in mouse models of AD (Aso et al., 2015; Cheng et al., 2014(A); Coles, Watt, Kreilaus, & Karl, 2020; Khodadadi et al., 2021). As the sample sizes in the present study were small, larger sample sizes might be necessary to improve object recognition following cannabinoid treatments in this Aβ mouse model.

In contrast to the impairments observed during NOR, the mice did not demonstrate any spatial learning and memory impairments. The lack of observed spatial learning and memory impairments around 6 months of age contradicts the findings of Mehla et al. (2018), but it is supported by Latif-Hernandez et al. (2019). *Nevertheless, due to how well the mice performed during MWT* it is difficult to conclude whether CBD and/or THC can improve the performance of AD mouse models on this task sensitive to HPC dysfunction.

However, previous experiments have reported spatial learning improvements when CBD and THC were administered to AD mouse models and aged rodents (Martin-Moreno et al., 2011; Nidadavolu et al., 2021; Watt et al., 2020(A)). Therefore, it is likely that in the presence of impairments the drugs and dosages chosen would have improved spatial learning and memory in $APP^{NL\text{-}G\text{-}F}$ mice. Consistent with this, the number of annulus crossings during the probe trial support the potential protective effects of CBD and THC on spatial memory. While it was not a statistically significant finding, it appears as though CBD is dose dependently increasing the number of annulus crossings of the $APP^{NL\text{-}G\text{-}F}$ mice. Similarly, the 0.5mg/kg and 1.0mg/kg THC treated mice had higher annulus crossings relative to the vehicle and 0.2mg/kg treated mice. As such CBD and THC might be promoting the consolidation or retrieval of memories dependent on the HPC.

The fear conditioning data suggests that all the CBD treated $APP^{NL\text{-}G\text{-}F}$ mice were impaired during context recall, but not tone recall, whereas the vehicle and THC treated $APP^{NL\text{-}G\text{-}F}$ mice were able to recall the context and the tone. Mehla et al. (2018) found that at 6 months of age $APP^{NL\text{-}G\text{-}F}$ mice were impaired during context and tone recall, whereas Sakakibara, Sekiya, Saito, Saido, & Iijima (2018) found that the mice were unimpaired even at 15-18 months of age. Under normal circumstances the acquisition of delayed fear conditioning is dependent on amygdala functions (Goosens & Maren, 2001; Kochli et al., 2015). Interestingly, research suggests that the perirhinal cortex and postrhinal cortex might also be involved in delayed fear conditioning for the context, but not the tone (Bucci et al., 2000; Kealy & Commins, 2011). Indeed, Bucci et al. (2000) found that when the perirhinal cortex or postrhinal cortex was lesioned, rats were unable to recall the context but retained recall for the tone. As such, because the CBD treated mice were able to form the tone-shock

association, but not the context-shock association the impairment might be suggestive of dysfunction to circuits related to the perirhinal and/or postrhinal cortex rather than the amygdala. This finding is further supported by the NOR data, which is also believed to be dependent on retained function of the perirhinal cortex (Kealy & Commins, 2011; Mumby et al., 2007).

However, it is worth noting that the comparison utilized in the present experiment, was the freezing response of the mice prior to the tone. Previous experiments have shown that this "baseline" measure of freezing is contaminated as it is still a fear associative response (Jacobs, Cushman, & Fanselow, 2010). As such a better comparison to fully conclude if the mice recalled the context would have been their freezing response in the conditioning context prior to ever having been shocked. In support of this, Sakakibara et al. (2018) found $APP^{NL\text{-}G\text{-}F}$ mice to have increased locomotor activity during elevated plus maze, which is an anxiety-based task. In contrast to the hyperactivity during elevated plus maze, the activity of the mice prior to having been shocked during fear conditioning was decreased relative to healthy controls. The authors suggest that the $APP^{NL\text{-}G\text{-}F}$ mice might demonstrate increased activity in the presence of aversive stimuli. Future studies should use the freezing of $APP^{NL\text{-}G\text{-}F}$ mice prior to ever having been shocked as a "baseline" freezing measure as this would likely yield more reliable results.

Nevertheless, the age of the mice and the protocol used was identical for the CBD and THC treated $APP^{NL\text{-}G\text{-}F}$ mice, therefore the observed contextual impairment may be suggestive of a negative effect of CBD administration. Interestingly, Kreilaus, Przybyla, Ittner, and Karl, (2022) administered 100mg/kg of CBD for 7 weeks to a tauopathy mouse model and found that CBD decreased the context freezing response but increased the tone

freezing response of the mice. Whereas Cheng et al. (2014(B)) and Watt et al. (2020(A)) found no effects of CBD on fear associative memory amongst AβPP/PS1 mice. As such, more studies are required to further understand the effects of CBD on context and tone recall.

Despite the puzzling contextual recall results, it is important to emphasize that for the alternate behavioural tasks, there were no observable impairments because of CBD or THC administration. Indeed, the vehicle treated mice, the CBD treated mice, and the THC treated mice performed similarly. The lack of impairments supports that the dosages chosen were non-psychoactive and that their chronic consumption was safe and well tolerated amongst the $APP^{NL\text{-}G\text{-}F}$ mice.

Finally, overall, when comparing the behavioural results to other published experiments, there is variability as to when the $APP^{NL\text{-}G\text{-}F}$ mice begin to demonstrate cognitive impairments. While some researchers suggest that at 6 months of age the mice demonstrate cognitive impairments (Mehla et al., 2018; Saito et al., 2014), others have found that the mice retain cognitive functions at this age (Latif-Hernandez et al., 2019; Sakakibara et al., 2018). The inconsistent age of cognitive decline among $APP^{NL\text{-}G\text{-}F}$ mice is unlikely to be due to laboratory differences and protocol inconsistencies as the present experiment and that performed by Mehla et al. (2018) were performed in the same laboratory, using the same contexts, and protocols. Rather the discrepancies might be explained by experimenter sex differences (Sorge et al., 2014). The present experiment was performed by female researchers, whereas the experiments conducted by Mehla et al. (2018) were performed by male researchers. Mice have been shown to alter their behaviour in the presence of males compared to females (Sorge et al., 2014). As such future studies

should directly assess if APP^{NL-G-F} mice behave in a differing manner depending on the sex of the experimenter. If APP^{NL-G-F} are sensitive to experimenter sex differences, then moving forward laboratories should be cautious when choosing experimenters to perform behavioural experiments on this strain of mice. An alternative explanation for the varying results, might be due to a genetic drift, which could have altered the behavioural phenotype of the mice. Nevertheless, the reasons listed above are speculative. Importantly, the APP^{NL-G-F} mice did demonstrate pathology like Mehla et al. (2018) which is puzzling given the importance placed on this kind of pathology for defining and diagnosing this disease and assumed casual relationship to dementia.

Cannabinoid Compound Treatments and their Impacts on AD Related Pathologies in APP^{NL-G-F} Mice

Neurodegeneration has not been reported in APP^{NL-G-F} mice (Sasaguri et al., 2017). Likewise, in the present study, the appearance of the HPC was unaltered, from this we can conclude that around 7 months of age APP^{NL-G-F} mice do not demonstrate cell loss in the HPC. CBD and THC have been shown to inhibit GSK3-β and activate the Akt pathway (Cao et al., 2014; Casarejos et al., 2013; Fogaça et al., 2018; Ozaita et al., 2007). This mode of action would likely prevent cell loss and promote cell survival, therefore if neurodegeneration was observed, CBD and THC would likely delay the process. Future studies should observe a tauopathy mouse model, as NFTs have been directly correlated to neuronal loss (Giannakopoulos et al., 2003; Petersen et al., 2019).

Despite the absence of neurodegeneration in the HPC, CBD does appear to be dose dependently increasing the HPC volume of the mice. The increased HPC volume with increasing dosages of CBD might be suggestive of neurogenesis. Previous research studies

suggest that CBD can promote neurogenesis (Esposito et al., 2011; Fogaça et al., 2018). However, research also suggests that THC promotes neurogenesis (Suliman et al., 2017) and there were no clear trends towards THC impacting the HPC volume of the mice. Future studies should stain proliferating cells and immature neurons to directly assess the effects of CBD and THC on neurogenesis. Individuals with mild cognitive impairments and AD have reduced neurogenesis (Tobin et al., 2019), as such, if CBD and THC are promoting neurogenesis this would support their therapeutic potential.

While the mice did not demonstrate clear HPC atrophy, they did express Aβ in the HPC and PFC. There were no effects of various dosages of CBD or THC on the quantity of Aβ in the HPC and PFC of the $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age. Our findings are in support of select *in vivo* experiments, which have reported that CBD and THC do not reduce the quantity of Aβ in cortical areas (Aso et al., 2015) or soluble $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ (Cheng et al., 2014(B)). However, in contrast to our findings Khodadadi et al. (2021) found that i.p. injections of 10mg/mL of CBD, every second day for two weeks was able to reduce Aβ. Watt et al. (2020(A)) also found a marginal trend towards 50mg/kg of CBD decreasing insoluble $A\beta_{1\text{-}40}$ in the HPC, however they found no effects of CBD on insoluble $A\beta_{1\text{-}42}$ or soluble $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. Furthermore, *in vitro* studies support the use of CBD and THC to reduce the aggregation and formation of Aβ (Cao et al., 2014; Eubanks et al., 2006; Janefjord et al., 2014; Kanchi & Dasmahapatra, 2021; Scuderi et al., 2014) and to reduce Aβ fluorescence in cell cultures (Janefjord et al., 2014). As such, there is reason to believe that CBD and THC could reduce the overall quantity of Aβ.

Overall, while there is support for our findings that CBD and THC do not reduce the quantity of Aβ at various dosages (Aso et al., 2015; Cheng et al., 2014(B)), there is

contradicting literature suggestive of the benefits of the compounds on Aβ burden (Eubanks et al., 2006; Janefjord et al., 2014; Khodadadi et al., 2021; Watt et al., (2020(A)). Much of the literature has been collected on cell cultures, as such there is a need to further investigate the effects of CBD and THC on Aβ *in vivo*. Aβ has many conformations that are related to the progression and severity of AD (Chen et al., 2017). The present study assessed the quantity of Aβ through immunohistochemical staining, which allows for the detecting of Aβ and APP in the insoluble fraction (Aho et al., 2010). Future experiments should further assess the effects of the cannabinoid compounds on various Aβ conformations using ELISA's or western blots.

Finally, the $APP^{NL\text{-}G\text{-}F}$ mice did demonstrate positively stained Iba1 cells and positively stained GFAP cells, particularly surrounding plaques. This observation supports AD as seen in humans and AD mouse models (Benito et al., 2003; Gomez-Arboledas et al., 2018; Pike, Cummings, & Cotman, 1995). Positively stained Iba1 cells represent microglial and positively stained GFAP cells represent astrocytes. Previous experiments have also detected microgliosis and astrogliosis in the brains of $APP^{NL\text{-}G\text{-}F}$ mice at 6 months of age (Masuda et al., 2016; Mehla et al., 2018).

Despite the observation of neuroinflammation there were no effects of various dosages of CBD or THC in the quantity of Iba1 in the HPC or PFC. Although, while not significant, there does appear to be a dose dependent decrease in GFAP positive cells among the CBD, but not THC treated mice. Therefore, CBD might be reducing the quantity of GFAP positive cells in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age. In partial support of our findings Aso et al. (2015) did report that CBD and THC were able to reduce astrocytes, but not microglial. Similarly, Giuliano et al. (2021) found that CBD

reduced astrocytes, but not microglial in a mouse model of Parkinson's disease. Additionally, Cheng et al. (2014(B)) observed a trend towards a reduction of pro-inflammatory cytokines among CBD treated AD mouse models, however their observation was not statistically significant. Nevertheless, there is still a proportion of the literature that supports the use of CBD and THC to reduce inflammatory markers related to Aβ. Indeed, CBD administration *in vitro* and *in vivo* reduced microglial, astrocytes, and pro-inflammatory cytokines initiated by Aβ (Esposito et al., 2007; Martin-Moreno et al., 2011). Similarly, a CB1 and CB2 agonist, which behaves like THC was able to reduce microglial upregulated by Aβ (Ramírez et al., 2005). Therefore, while the present study did not find a significant reduction in neuroinflammatory markers after chronic administration of CBD and THC, there is still evidence supporting their anti-inflammatory use.

The lack of statistically significant effects of various dosages of CBD and THC on the quantity of Iba1 and GFAP in the HPC and PFC of our mice may be due to age the brains were assessed. Microglial and astrocytes are first detected in $APP^{NL\text{-}G\text{-}F}$ mice around 6 months of age and progress in an age dependent manner (Masuda et al., 2016; Mehla et al., 2018). Perhaps if the histology was performed when microgliosis and astrogliosis is more pronounced in $APP^{NL\text{-}G\text{-}F}$ mice, anti-inflammatory effects from CBD and THC administration would be observed. If CBD and THC do decrease inflammatory markers at a later AD stage this would provide hope for AD patients. While preclinical treatment is necessary to prevent or delay neurodegeneration in AD, inflammation during early disease stages might be protective (Albrecht et al., 2021; Frost & Li, 2017; Preman et al., 2021; Sarus & Heneka, 2017). As such if CBD and THC reduce inflammation during later disease

stages this would alleviate the burden of chronic inflammation without potentially removing the markers when they might be protective.

Concluding Remarks

Overall, our findings suggest that around 6 months of age the APP^{NL-G-F} mice were only partially impaired and various dosages of CBD and THC did not demonstrate clear benefits on the learning and memory of the mice. However, there were some observable benefits of the higher dosages of CBD and various dosages of THC on object recognition and spatial recall. Moreover, the histological analyses suggests that around 7 months of age the APP^{NL-G-F} mice did not demonstrate HPC cell loss. However, CBD appears to dose dependently increase the HPC volume of the APP^{NL-G-F} mice. There were no clear effects of various dosages of THC on the HPC volume of the mice. Furthermore, the HPC and PFC of the mice did express Aβ, Iba1 positive cells and GFAP positive cells. Various dosages of CBD and THC did not affect the quantity of Aβ or Iba1. CBD appears to dose dependently reduce the quantity of GFAP positive cells, suggestive of reactive astrocytes, but this observation was not statistically supported. Importantly, the data does not suggest that there are aversive effects due to chronic CBD and THC oral administration, at least on the brain functions and histological markers we assessed.

CHAPTER 3 – THE EFFECTS OF CHRONIC CANNABIDIOL ADMINISTRATION ON THE BEHAVIOUR AND INFLAMMATION OF TAU P301S-LINE PS19 MICE

AD is classified by, amongst other things, the presence of NFTs, which are intraneuronal lesions related to the hyperphosphorylation of tau protein and PHFs. While NFTs are associated with a variety of neurodegenerative diseases, known as tauopathies (Bekris et al., 2010; Chung, Roemer, Petrucelli, & Dickson, 2021) they progress in a distinct manner during the pathological progression of AD. This aids to distinguish AD from the alternate tauopathies. Braak and Braak (1991) distinguished six distinct stages of the pathological progression of abnormal tau and NFTs. Specifically, the pathologies have been reported to begin developing in the transentorhinal region, progressing throughout the entorhinal cortex, the HPC, and eventually the isocortex (Braak & Braak 1991). Once a stage becomes apparent the progression of tau will not reverse (Harley, Walling, Yuan, & Martin, 2021). This is particularly concerning because NFTs are directly linked to synapse loss, neuronal loss, and ultimately cognitive decline among AD cases (Giannakopoulos et al., 2003; Haroutunian et al., 2007). As such, treatments that prolong the formation of NFTs would likely benefit AD patients by delaying the progression and accumulation of the lesions.

Specifically, treatments that prevent the hyperphosphorylation of tau are particularly intriguing. Hyperphosphorylated tau precedes PHFs and NFTs, suggestive of its early therapeutic potential (Augustinack et al., 2002; Bancher et al., 1989; Grundke-Iqbal, 1986). While the direct cause of hyperphosphorylated tau is unknown there are several factors believed to be involved in the process. Research suggests that an imbalance between tau kinases and phosphatases might contribute to its hyperphosphorylation

(Grundke-Iqbal et al., 1986; Gong, Singh, Grundke-Iqbal, & Iqbal, 1993). In support of this hypothesis, the activation of GSK3-β, a tau kinase, is increased in the brains of AD patients (Leroy, Yilmaz, & Brion, 2007). While protein phosphatase 2A (PP2A), a tau phosphatase has been shown to be decreased in AD brains (Gong et al., 1993). Furthermore, the phosphorylation of tau in microglial cell cultures has been correlated to increased GSK3-β activity and decreased PP2A activity (Wang et al., 2013). As such, treatments that decrease activated GSK3-β and/or increase PP2A activity might aid in preventing the hyperphosphorylation of tau, by regulating the phosphorylation and dephosphorylation of the protein.

In addition to an imbalance between tau kinases and phosphatases, alternate AD pathologies have further been correlated with the phosphorylation of tau. Indeed, increased Aβ (Chiarini, Armato, Gardenal, Gui, & Dal Prà, 2017; De Felice et al., 2008; Esposito et al., 2006(B)(C)), oxidative stress (Saez, Pehar, Vargas, Barbeito, & Maccioni, 2004), and inflammation (Li, Liu, Barger, & Griffin, 2003) have been shown to promote tau phosphorylation. Decreasing the pathologies might indirectly prolong the formations of NFTs by reducing abnormal tau. Overall, there are a plethora of factors that might be involved in tau phosphorylation, which would promote the formation of NFTs. Due to the multifactorial nature of AD, treatments that target several of the factors would likely be the most optimal therapeutic option (Gidyk et al., 2015; McDonald, 2002). As such animal models that express several AD related pathologies are necessary.

The Tau P301S-Line PS19 mouse model might be a promising mouse model to assess potential therapies that would ultimately reduce the formation of NFTs. The mice demonstrate synaptic dysfunction, mitochondrial dysfunction, oxidative stress,

neuroinflammation, hyperphosphorylated tau, NFTs, and neurodegeneration in an age dependent manner (Dumont et al., 2011; Hurtado et al., 2010; Hashem, Hu, Zhang, Gao, & Chen, 2021; Sun et al., 2020; Yoshiyama et al., 2007; Zhang et al., 2012). Research suggests that the progression of hyperphosphorylated tau in the Tau P301S mice progresses similarly to the stages of NFTs characterized by Braak and Braak (1991) with the exception that the brains of the Tau P301S mice progress at a quicker rate relative to the human stages (Hurtado et al., 2010). Furthermore, the hyperphosphorylation of tau amongst Tau P301S mice has been correlated with increased levels of GSK3-β (Dumont et al., 2011; Hashem et al., 2021). Finally, in addition to the development of AD related pathologies the mice demonstrate cognitive impairments (Dumont et al., 2011; Hashem et al., 2021; López-González et al., 2015; Takeuchi et al., 2011; Zhang et al., 2012). As such the mice can be used to further researchers understanding of NFTs and to assess the effects of potential therapies that might act on NFTs, their related toxicities, and cognitive impairments. Therapies that prolong the formation of NFTs should prolong neurodegeneration and ultimately delay symptom onset amongst AD cases.

CBD is a safe, readily available, and relatively cheap treatment option that has been shown to improve pathologies associated with AD, suggestive of its therapeutic potential. Certainly, in cell cultures, CBD has been shown to directly reduce the hyperphosphorylation of tau (Esposito et al., 2006(B)) and the aggregation of tau (Alali et al., 2021). The ability of CBD to reduce hyperphosphorylated tau is related to the inactivation of GSK3-β (Esposito et al., 2006(B)). Furthermore, CBD treated cell cultures have been shown to reduce Aβ, oxidative stress, and neuroinflammation (Chen et al., 2016; di Giacomo et al., 2020; Esposito et al., 2006(A); Esposito et al., 2007; Iuvone et al., 2004).

As previously mentioned Aβ, oxidative stress, and neuroinflammation promote tau phosphorylation, further supporting the use of CBD to delay the formation of NFTs. In addition, to directly and indirectly preventing the hyperphosphorylation of tau, CBD has been shown to prevent neurodegeneration, while promoting neurogenesis (Esposito et al. 2011). Neurodegeneration is correlated to hyperphosphorylated tau and NFTs (Giannakopoulos et al., 2003; Petersen et al., 2019). As such CBD might not only prevent the formation of the lesions themselves, but also delay the downstream toxic effects that result from the lesions. Finally, Kreilaus et al. (2022) found that CBD was able to promote spatial learning and memory in a tauopathy mouse model, further supporting the therapeutic potential of the cannabinoid compound.

Overall, CBD acts on many pathologies in the CNS that promote the hyperphosphorylation of tau protein and has been shown to improve select cognitive impairments in the presence of tau abnormalities. However, the need to further understand the effects of CBD on hyperphosphorylated tau and its related toxicities is still needed. Particularly, there is a need for research on the pre-symptomatic chronic administration of CBD in a tauopathy animal model and how the cannabinoid compound impacts associated pathologies *and* behavioural impairments. Ultimately, appropriate treatment options for AD patients will target the disease in the brain, pre-clinically, prior to severe neurodegeneration, which should delay symptom onset.

Purpose of the Present Experiment

Therefore, the present study sought to assess the effects of the pre-symptomatic chronic administration of 20mg/kg of CBD on the behaviour and brain pathology of Tau P301S-Line PS19 mice. The dosage chosen was based on the findings from Chapter 2

experiment 1, which found observable trends towards 20mg/kg of CBD promoting spatial recall, increasing the volume of the HPC, and decreasing astrocytes in the HPC of an AD mouse model that develops Aβ. Additionally, the findings from previous experiments support the therapeutic potential of 20mg/kg of CBD on mouse models of AD (Cheng et al., 2014(A)(B); Martin-Moreno et al., 2011). The Tau P301S mice and their noncarrier littermates were orally administered 20mg/kg of CBD or a vehicle, daily, beginning around 3 months of age. Tau P301S mice do not demonstrate cognitive deficits at this age (López-González et al., 2015), which allowed for the assessment of the pre-symptomatic administration of CBD. Around 6 months of age, when the mice have been reported to demonstrate cognitive deficits (Takeuchi et al., 2011), they were tested on a battery of tasks to assess object recognition, motor function, and spatial learning and memory. Due to a lack of observed impairments at 6 months of age, the mice were retested at 9 months of age. The fear conditioning paradigm was added when the mice were 9, but not 6 months of age. Following all behavioural testing, half the mice were perfused for histological purposes, and the other half were sacrificed for fresh tissue collection. The perfused brains were used to quantify the HPC volumes of the mice and to assess the quantity of Iba1, a microglial marker, and GFAP, an astrocytic marker, in the HPC and PFC. Increased levels of Iba1 and GFAP have previously been reported in the brains of Tau P301S mice supporting their use in the present study (Hashem et al., 2021; Sun et al., 2020; Vagnozzi, Giannopoulos, & Praticò, 2017).

Hypotheses

It was hypothesized that the pre-symptomatic administration of CBD would improve any observed learning and memory impairments amongst the Tau P301S mice.

CBD was not expected to impact the learning and memory of the noncarrier littermates. It was predicted that the Tau P301S mice would demonstrate reduced HPC volumes in conjunction with increased levels of Iba1 and GFAP in the HPC and PFC. CBD was expected to mitigate the observed pathologies. No histological abnormalities were expected to be observed in the noncarrier littermates and CBD was not expected to impact the brains of the noncarrier littermates.

Method

Subjects

Twenty-five heterozygous Tau P301S-Line PS19 (male (n=16); female (n=9) and twenty-two noncarrier littermates (male (n=8); female (n=14)) were bred inhouse at the University of Lethbridge on a C57BL/6N background. The mice were housed with littermates in IVC cages, which contained corncob bedding, Crink-l'Nest, a cotton square, and a plastic tunnel for enrichment. The housing room was kept on a 05h00-17h00 light/dark cycle and was temperature and humidity controlled (21°C). The mice were divided into four groups based on genotype and treatment: noncarrier littermates-CBD (n=11), noncarrier littermates-vehicle (n=11), Tau P301S-CBD (n=13), and Tau P301S-vehicle (n=12). Beginning at three months of age the CBD or vehicle was given to the mice daily via a palatable food (Nutella). The mice had *ad libitum* access to food and water. All methods and procedures described in this experiment were in accordance with Canadian Council of Animal Care Guidelines and were approved by the University of Lethbridge Institutional Committee on Animal Care.

Drugs Preparation and Administration

The CBD powder was dissolved, stored, and administered as described in Chapter 2 experiment 1. Following the dilution, high-performance liquid chromatography was performed to confirm the concentration of the CBD oil was 20mg/kg. As in Chapter 2 experiment 2, the mice were no longer food restricted and water bottles were no longer placed in their holding cages, as it was not deemed necessary for the mice to consume the treatments. The mice were weighed every second day to ensure they received to appropriate amount of CBD or vehicle (μL).

Procedure, Apparatus and Materials

Around 6 and 9 months of age NOR and MWT were performed as previously described (see Chapter 2). A probe retention was added to the MWT task at 6 months of age and will be described in detail below. Balance beam and tail suspension were added to the present experiment to assess the sensory and motor functions of the mice at 6 and 9 months of age. The motor coordination and hind limb strength of the Tau P301S has been reported to decline as they age (López-González et al., 2015; Yoshiyama et al., 2007). Therefore, the tasks were added to rule out motor impairments as the result of any behavioural abnormalities. Fear conditioning was performed at 9, but not 6 months of age to avoid any training effects from the task (see Chapter 2). Behavioural tasks that were manually scored were done using Videopad editor. All videos were scored at half speed by an experimenter who has blind to the genotype and treatment groups.

Novel Object Recognition

NOR was performed identically as in Chapter 2, with the exception that the mice were retrained and tested at 9 months of age. The mice were trained and tested with different objects at 6 and 9 months of age to avoid potential training effects. The exploration of the mice during training and testing was manually scored. Exploration was defined as time spent engaging with the object at a maximum distance of 1 cm. Digging near the object, looking past the object, and sitting on the object were not considered object-directed exploratory behaviour. The IR value for the novel object was calculated as described in Chapter 2. Increased exploration for the novel object would suggest that the mice retained object recognition thought to be dependent on the function of networks centered around the perirhinal cortex (Kealy & Commins, 2011; Mumby et al., 2007).

Balance Beam

Following NOR, balance beam assessed the motor coordination of the mice (Luong, Carlisle, Southwell, & Patterson, 2011). During balance beam there was one training day followed by one testing day. Training and testing were performed using a beam that was elevated 50cm above the table, 100cm in length, and 2mm wide. At the base of the beam there was a black tunnel for the mice to traverse to. *Training:* During training, the mice were placed on the beam from different start points that varied in distance from the end of the beam (10cm, 50cm, and 100cm). The mice did not move onto the farther start points until they were able to successfully traverse the beam. If the mice began to slip during their given trial an experimenter assisted them back onto the beam. A trial was considered successful if the mice did not fall off prior to reaching the tunnel. Training was complete when the mice successfully traversed the beam from each start point and successfully

completed three trials from 100cm. *Testing:* Twenty-four hours after training the mice were tested. During testing the mice were placed 100cm from the end of the beam and given 3 consecutive trials. When the head of the mouse was inside the tunnel the trial was considered successfully complete. If the mice fell off the beam, the trial was not considered successful. The behaviour of the mice was recorded during testing for analysis. The average latency of the mice to traverse the beam during successful trials and the distance travelled across all three trials were manually scored. A decreased latency and an increased distance travelled was suggestive of intact normal motor coordination.

Tail Suspension

Immediately following balance beam, tail suspension assessed the hind limb clasping of the mice. During tail suspension the mice were suspended by the middle of their tail for 10s. The behaviour of the mice while suspended was recorded for analysis. The clasping behaviour of the mice was scored on a four-point scale as previously described (Guyenet et al., 2010; Miedel, Patton, Miedel, Miedel, & Levenson, 2017). Two experimenters scored tail suspension. Any scores that varied between experimenters were observed by a third party. A score of 0 indicated a normal splay of limbs, a score of 1 indicated one limb slightly retracted forward, a score of 2 indicated two limbs slightly retracted forward with toes splayed, a score of 3 indicated two limbs slightly retracted forward with occasional clasping and toes clasped, a score of 4 indicated limbs completely retracted forwarded and clasped along with the clasping of toes. The scores were given based off the clasping behaviour of the mice for 50% of the video. A higher clasping score was indicative of hind limb weakness.

Morris Water Task

MWT was performed as described in Chapter 2, with the addition of a retention probe test performed at 6 but not 9 months of age. The retention probe test was performed to assess the long-term memory of the mice. The test was not replicated at 9 months of age due to the spatial training impairments already observed amongst the Tau P301S mice at the latter age. Furthermore, at 9 months of age the mice were trained to locate a hidden platform in a different quadrant relative to that of 6 months of age to avoid training effects. *Retention test:* Immediately following the initial probe trial, the mice were retrained for three trials. During the retraining, the platform was placed 1cm below the water in the fixed location used during spatial training. Distal cues remained fixed. The mice were placed in the pool from three start points and given 60s to locate the hidden platform. Once the mice located the platform, they were left to sit for 10s prior to being removed. One week following the initial probe trial and retraining, the retention test took place. During the retention test the platform was removed from the pool and the mice were placed in the pool for 60s from one of the two start locations farthest from the previous platform location. Distal cues remained fixed. A single visible platform test was performed 24 hours after the retention test at 6 months of age and 24 hours following the initial probe trial at 9 months of age. During training, probe, and visible platform a HSV software package recorded the behaviour of the animals for analysis. As in Chapter 2 a decreased path length across spatial training days was indicative of spatial learning. An increased percentage of time spent in the target quadrant relative to the alternate quadrants and an increased number of annulus crossings during the probe trial and during the retention probe test was indicative of spatial recall. Retained spatial learning and spatial recall suggests that the mice learned

and remembered the location of the escape platform and that the function of the HPC and related circuits was not compromised (Ferbinteanu et al., 2003; Morris et al., 1982; Sutherland et al., 1982). A decreased path length during the visible platform test, when the platform was visible, relative to spatial training day 1, when the platform was hidden, indicated that the mice did not have sensory, motor, or motivational impairments.

Fear Conditioning

At 9 months of age the mice were given a few days to rest after MWT before beginning fear conditioning. Fear conditioning was performed identically to the procedures described in Chapter 2. The percent time spent freezing during context and tone recall was calculated. The percent time spent freezing during the first two minutes of tone recall, prior to the onset of the tone was used as the baseline freezing measure. An increased percentage of freezing during context recall and tone recall relative to the baseline freezing measure was considered retained memory for the context/shock and tone/shock pairing and that a neural network centered on the amygdala was functional (Goosens & Maren, 2001; Kochli et al., 2015). The baseline freezing measure will be referred to as novel context, when discussing context recall, and before the tone, when discussing tone recall throughout the remainder of the document. Time spent freezing was manually scored and defined as time spent completely still. Remaining in a stretched-out position, sniffing, and rearing on the walls was not considered time spent freezing.

Histology

Perfusions

Around 10 months of age 27 mice were given an i.p. injection of sodium pentobarbital and transcardially perfused with PBS and 4% PFA in PBS. The brains were extracted for histological purposes. The brains were kept in 4% PFA for 24 hours prior to being placed in 30% sucrose with PBS and sodium azide. The brains were stored in a 4°C refrigerator and were serially sectioned coronally with a 40µm freezing microtome.

Fresh Tissue Collection

Around 10 months of age 20 mice were sacrificed for fresh tissue collection. The mice were deeply anesthetized with Isoflurane. While anesthetized, blood was collected from their heart through the exterior of their chest cavity. The mice were then immediately decapitated. Following decapitation, the adrenal glands, kidney, spleen, liver, colon, lung, heart, testes/ovaries were collected on ice. The organs were immediately flash frozen in liquid nitrogen except for the colon, which was stored in a 4°C refrigerator in 10% formaldehyde. The brain was collected, and the following sections were isolated on ice: PFC, neocortex, HPC, striatum, pineal glands, and brainstem. All regions of the brain were flash frozen in liquid nitrogen. Following the fresh tissue collection, the organs were placed in a -80°C freezer. The blood was centrifuged for 10 minutes, and the serum was collected and stored in a -80°C freezer.

Nissl

Nissl was performed on all perfused brains for a volumetric analysis of the HPC. The cells in the DG, CA1, and CA3 were counted using the optical fractionator on a

stereology microscope at 20x magnification. The Allen mouse brain atlas was used to ensure the proper regions were being counted (https://mouse.brain-map.org/experiment/thumbnails/100048576?image_type=atlas).

Immunohistochemistry

Immunohistochemical procedures were performed on all perfused brains for neuroinflammation. Microglial was stained with Iba1, and astrocytes were stained with GFAP. The brain slices were free float mounted in PBS onto positively subbed slides. Washes were done in TBS. To prevent nonspecific staining, sections were blocked in TBS with 0.3% Triton-X and 3% goat serum for two hours. Following the blocking sections were washed and incubated in the primaries (rabbit anti-GFAP (Abcam, Ab7260, 1:2000); rabbit anti-Iba1 (Wako, 019-19741, 1:1000)) for 24 hours. Following the primaries, the sections were washed and placed in the secondaries (rabbit-anti-alexa 594 (IgG [H+L], (A11037, Invitrogen, 1:1000)) for 24 hours. Finally, sections were counterstained with Dapi. A stock solution of 20ug/mL of Dapi was diluted at a 1:2000 concentration in TBS. The diluted solution was pipetted onto the slides and were coverslipped for 1 hour in a dark humidity chamber. Following the incubation period in Dapi, slides were washed and coverslipped. GFAP stained slides were coverslipped with a fluorescent mounting medium made in our laboratory. Vectashield H-1000 (Vecta Laboratory) was used to coverslip the Iba1 stained slides. Slides were imaged using Nanozoomer microscope (Nanozoomer 2.0-RS, HAMAMATSU, JAPAN). The HPC and PFC were quantified by an experimenter who was blind to the treatment groups. Quantification was performed using Ilastik and ImageJ, as described in Chapter 2.

Results

Data Analysis

The statistical analysis was performed using SPSS statistical software package, version 27.0. Results are presented as mean +/- SEM. A p value < 0.05 was considered as statistically significant.

Novel Object Recognition

The training data was analyzed and any mouse that did not spend a total of 20s exploring the object's during training was excluded from the testing data. No mice were excluded from the testing data at 6 months of age. However, 3 noncarrier littermates treated with CBD were excluded from the testing data at 9 months of age. Therefore, the end group sizes at 9 months of age were as follows: noncarrier-vehicle (n=11), noncarrier-CBD (n=8), Tau P301S-vehicle (n=12), Tau P301S-CBD (n=13).

The IR for each mouse was calculated for the novel object during NOR. An IR above 0.5 indicates that the mice explored the novel object more than the familiar object. As seen in Figure 22 (A) all the mice, regardless of genotype (Tau P301S and noncarrier littermates), had an IR value well above 0.5 at 6 months of age. Whether the mice received CBD, or the vehicle did not impact object recognition of the mice. A two-tailed one-samples t-test was conducted for the novel investigation of each group of mice to determine if the exploration for the novel object was significantly above chance (0.5). An above chance value would statistically support that the mice retained object recognition memory. The results suggest that the noncarrier-vehicles (t (10) = 3.467, $p < 0.006$), noncarrier-CBD (t (10) = 3.859, $p < 0.003$), Tau P301S-vehicles (t (11) = 4.694, $p < 0.001$), and Tau

P301S-CBD (t (12) = 4.622, $p < 0.001$) explored the novel object significantly above chance. A two-way between subjects ANOVA assessed the effect of genotype or treatment on the object recognition of the mice. The results suggest that there was no significant effect of genotype (F (1, 43) = 0.129, $p < 0.721$) or treatment (F (1, 43) = 0.003, $p < 0.955$) on the object recognition of the mice. As such, at 6 months of age the Tau P301S mice retained object recognition and CBD did not impact the object recognition of the mice.

The NOR data collected from the groups of mice around 9 months of age can be seen in Figure 22 (B). The IR value of the Tau P301S-CBD mice was the lowest followed by the Tau P301S-vehicle mice. The performance of the noncarrier littermates was similar. CBD might be impairing the object recall of the Tau P301S mice, but CBD does not appear to impact the memory of the noncarrier littermates. A two-tailed one-samples t-test revealed that the novel object exploration of the noncarrier-vehicles (t (10) = 5.665, $p < 0.001$), noncarrier-CBD (t (7) = 2.669, $p < 0.031$), and Tau P301S-vehicles (t (11) = 2.925, $p < 0.014$) was significantly above chance, but the novel object exploration for the Tau P301S-CBD mice (t (12) = 0.374, $p < 0.715$) was not significantly above chance. A two-way between subjects ANOVA suggested that between subjects there was a significant effect of genotype (F (1, 40) = 4.794, $p < 0.034$). There was no statistically significant effect of treatment (F (1, 40) = 1.170, $p < 0.286$). Therefore, at 9 months of age the Tau P301S mice are impaired relative to the noncarrier littermates. CBD might be further impairing the object recall of the Tau P301S mice. However, CBD did not impact the object recognition of the noncarrier littermates.

Balance Beam

The average latency to traverse the beam during successful trials was assessed. Figure 23 (A) demonstrates the latency of the mice depending on genotype and treatment. The CBD treated mice had a shorter latency relative to the vehicle mice. There were no observable differences between genotypes. A two-way between subjects ANOVA suggested that there was no statistically significant effect of genotype ($F(1, 43) = 0.003$, $p < 0.959$), but there was a significant effect of treatment ($F(1, 43) = 4.168$, $p < 0.047$) on the latency of the mice. The average distance travelled across the three trials is depicted in Figure 23 (B). There were no observable differences depending on treatment. The Tau P301S mice appear to have a slight shorter distance travelled relative to the noncarrier littermates, suggesting that they successfully completed fewer trials than the noncarrier littermates. However, a two-way between subjects ANOVA revealed no significant effect of genotype ($F(1, 43) = 0.623$, $p < 0.434$) and no significant effect of treatment ($F(1, 43) = 0.159$, $p < 0.692$) on the average distance travelled. In summary, at 6 months of age the motor coordination of the Tau P301S mice was unimpaired. CBD, in part, appears to be promoting the motor coordination of the mice, independent of genotype at this age.

At 9 months of age the average latency to traverse the beam during successful trials does not appear to differ depending on the treatment and genotype of the mice (See Figure 23 (C)). A two-way between subjects ANOVA confirmed that there was no significant effect of genotype ($F(1, 43) = 0.077$, $p < 0.783$) or treatment ($F(1, 43) = 0.857$, $p < 0.360$) on the average latency of the mice. As seen in Figure 23 (D) the Tau P301S mice do have a shorter distance travelled across all three trials. Therefore, the Tau P301S mice successfully traversed the beam fewer times relative to the noncarrier littermates, suggesting that their

motor coordination may have been beginning to decline at this age. CBD does not appear to impact the average distance travelled. A two-way between subjects ANOVA analyzed the data and despite the observed results there was no significant effect of genotype ($F(1, 43) = 3.034$, $p < 0.089$) or treatment ($F(1, 45) = 0.180$, $p < 0.673$). Overall, the Tau P301S mice maintained intact motor coordination at 9 months of age and CBD treatments did not impact the motor coordination of the mice at this age.

Tail Suspension

The hind limb clasping of the mice was scored on a four-point scale (0-4). At 6 months of age there were no observable differences in the hind limb clasping of the mice depending on genotype or treatment (See Figure 24 (A)). A two-way between subjects ANOVA revealed that at 6 months of age there was no significant effect of genotype ($F(1, 43) = 0.004$, $p < 0.952$) or treatment ($F(1, 43) = 2.314$, $p < 0.136$) on the clasping of the mice. However, as seen in Figure 24 (B), at 9 months of age, the Tau P301S mice did demonstrate increased clasping relative to the noncarrier littermates. There are no observable differences in the clasping of the animals receiving CBD or a vehicle. A two-way between subjects ANOVA confirmed that there was a significant effect of genotype ($F(1, 43) = 10.644$, $p < 0.002$), but there was no significant effect of treatment ($F(1, 43) = 0.915$, $p < 0.344$) on the clasping of the mice. The results suggest that the Tau P301S mice demonstrate hind limb weakness at 9, but not 6 months of age. CBD did not improve nor impair the hind limb weakness of the Tau P301S mice at either age.

Morris Water Task

At 9 months of age one Tau P301S-vehicle mouse and one Tau P301S-CBD mouse were excluded from the MWT data due to the inability to swim. The final group sizes at 9 months of age were as follows: noncarrier-vehicle (n=11), noncarrier-CBD (n=11), Tau P301S-vehicle (n=11), Tau P301S-CBD (n=12).

Spatial Training

The path length of the mice to reach the hidden platform during spatial training was plotted from day 1 to day 8 as seen in Figure 25 (A). At 6 months of age all the mice were able to learn the location of the hidden platform, evidenced by a decreased path length across training days. The performance of the Tau P301S mice and noncarrier littermates was similar. There does not appear to be a consistent effect of CBD on the spatial learning of the mice. A two-way within subjects repeated measures ANOVA analyzed the path length of the mice from training day 1 to training day 8. There was a significant decrease in the path length of the mice across days ($F(7, 301) = 58.070$, $p < 0.001$). There was no significant effect of genotype ($F(7, 301) = 0.565$, $p < 0.784$) or treatment ($F(7, 301) = 0.793$, $p < 0.593$) on the path length of the individual mice.

As seen in Figure 25 (B), at 9 months of age, the path length of the Tau P301S mice to reach the hidden platform was increased relative to the noncarrier littermates, suggesting impaired spatial learning. CBD does not appear to impact the spatial learning of the mice. A two-way within subjects repeated measures ANOVA analyzed the observed results. Within subjects there was a significant effect of day ($F(7, 287) = 40.473$, $p < 0.001$). Additionally, there was a significant effect of genotype on the path length of the mice

across training days (F (7, 287) = 5.055, $p < 0.001$). A pairwise comparison with Bonferroni corrections assessed on which days did the path length of the Tau P301S and noncarrier littermates differ. The results suggest that the path length of the mice differed on day 1 ($p < 0.004$), day 5 ($p < 0.034$), day 6 ($p < 0.000$), day 7 ($p < 0.011$), and day 8 ($p < 0.006$). There was no significant effect of treatment on the path length of the mice across days (F (7, 287) = 0.929, $p < 0.485$).

Ultimately, at 9, but not 6 months of age the spatial learning of the Tau P301S mice was impaired relative to the noncarrier littermates, particularly from day 5 to day 8. Suggesting that the function of networks centered around the HPC were beginning to decline in this group of mice. CBD did not impair or restore the spatial learning of the mice.

Probe

Figure 26 (A) demonstrates the time spent in the target quadrant relative to the time spent in the alternate quadrants for the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD and vehicle at 6 months of age. All the mice spent more time in the target quadrant relative to the alternate quadrants. There were no observable differences in the quadrant preference of the mice depending on genotype or treatment. A two-way within subjects ANOVA revealed a significant difference in the time spent in the target quadrant relative to the alternate quadrants, within subjects (F (1, 43) = 121.304, $p < 0.001$). There was no effect of genotype (F (1, 43) = 2.441, $p < 0.126$) or treatment (F (1, 43) = 0.101, $p < 0.752$) on the quadrant preference of the mice.

Despite the quadrant preference results, the annulus crossing of the Tau P301S mice were reduced relative to the noncarrier littermates at 6 months of age (see Figure 26 (B)). Therefore, the spatial recall of the Tau P301S mice may be beginning to decline. CBD does not appear to impact the spatial recall of the mice. A two-way between subjects ANOVA revealed that there was a significant effect of genotype ($F(1, 43) = 4.542$, $p < 0.039$), but there was no significant effect of treatment ($F(1, 43) = 0.605$, $p < 0.441$) on the annulus crossings of the mice.

Probe retention: Figure 26 (C) depicts the quadrant preference of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD and a vehicle during the probe retention, performed one week after training. The noncarrier littermates treated with CBD demonstrate the strongest preference for the target quadrant. The performance of the noncarrier littermates treated with a vehicle and the Tau P301S treated with CBD and a vehicle was similar. A two-way within subjects ANOVA revealed a significant effect of quadrant preference, within subjects ($F(1, 43) = 102.818$, $p < 0.001$) and a strong trend towards an effect of genotype on quadrant preference ($F(1, 43) = 4.014$, $p < 0.051$). There was no significant effect of treatment on quadrant preference ($F(1, 43) = 1.128$, $p < 0.294$). As seen in Figure 26 (D) the Tau P301S mice treated with the vehicle have the lowest number of annulus crossings during the probe retention. The performance of the Tau P301S mice treated with CBD and the noncarrier littermates treated with vehicle was similar, while the noncarrier littermates treated with CBD have the highest number of annulus crossings. A two-way between subjects ANOVA revealed no significant effect of genotype ($F(1, 43) = 3.339$, $p < 0.075$) or treatment ($F(1, 45) = 2.928$, $p < 0.094$) on the annulus crossings during the probe retention.

The quadrant preference of the mice depending on genotype and treatment during a 24-hour probe trial, at 9 months of age, can be seen in Figure 26 (E). It appears that only the noncarrier littermates treated with CBD were able to recall the previous platform location. As such CBD might be improving the spatial learning of the noncarrier littermates. The performance of the noncarrier-vehicle, Tau P301S-CBD, and Tau P301S-vehicle mice was similar. A two-way within subjects ANOVA suggests that despite the slight quadrant preference observed in the noncarrier-vehicle, Tau P301S-vehicle, and Tau P301S-CBD mice, there was a significant effect of quadrant for all groups ($F(1, 41) = 4.348$, $p < 0.043$). There was no significant effect of genotype ($F(1, 41) = 1.1615$, $p < 0.211$) or treatment ($F(1, 41) = 0.884$, $p < 0.353$) on the quadrant preference of the mice. As seen in Figure 26 (F) there does not appear to be any observable trends towards an effect of genotype or treatment on the number of annulus crossings at 9 months of age. A two-way between subjects ANOVA confirmed that there was no significant effect of genotype ($F(1, 41) = 2.257$, $p < 0.141$) or treatment ($F(1, 41) = 1.720$, $p < 0.197$) on the annulus crossings of the mice.

Overall, the probe data suggests that the spatial recall of the Tau P301S mice began to decline at 6 months of age, as evidenced by a significant effect of genotype on the number of annulus crossings during the 24 hours probe. While nonsignificant there was a strong trend towards an effect of genotype on the quadrant preference during the probe retention, further supporting that the spatial recall of the Tau P301S mice was beginning to decline. There was no significant effect of genotype or treatment at 9 months of age. However, the quadrant preference of each group of mice was weak.

Visible Platform

Figure 27 (A) demonstrates the path length of the mice during spatial training day 1, when the platform was hidden, relative to their path length during the visible platform test, when the platform was visible, at 6 months of age. The Tau P301S mice and noncarrier littermates had a shorter path length when the platform was visible relative to when it was hidden. CBD does not appear to impact the performance of the mice. A two-way within subjects ANOVA confirmed a significant decrease in path length during the visible platform test compared to spatial training day 1 ($F(1, 43) = 743.317$, $p < 0.001$). There was no significant effect of genotype ($F(1, 43) = 2.860$, $p < 0.098$) and there was no significant effect of treatment ($F(1, 43) = 1.285$, $p < 0.263$) on the performance of the mice.

As seen in Figure 27 (B), at 9 months of age, all the mice show a drastic decrease in path length when the platform was visible relative to when it was hidden. It does not appear as though genotype or treatment are impacting the sensory, motor, or motivational behaviour of the mice at this age. A two-way within subjects ANOVA revealed a significant decrease in path length during the visible platform test relative to training day 1 for all groups ($F(1, 41) = 414.799$, $p < 0.001$). Surprisingly, there was a significant effect of genotype on the performance of the mice ($F(1, 41) = 10.100$, $p < 0.003$). A pairwise comparison with Bonferroni corrections further analyzed the results and revealed that there was a significant effect of genotype during training day 1 ($p < 0.005$) and there was a significant effect of genotype during the visible platform test ($p < 0.010$) (see supplemental Figure 1). There was no significant effect of treatment on the performance of the mice ($F(1, 41) = 2.250$, $p < 0.141$).

Overall, the visible platform data suggests that the motor, motivational, and sensory behaviour of the Tau P301S is declining at 9, but not 6 months of age. Importantly, the Tau P301S mice were still able to see and locate the platform when it was visible, their performance was just subpar relative to the noncarrier littermates. CBD did not impact the sensory, motor, or motivational behaviour of the mice.

Fear Conditioning

One Tau P301S-vehicle mouse was perfused prior to fear conditioning due to the onset of paralysis, characterized by a hunched back posture comorbid with weight loss. See Figure 28 for a demonstration of the posture of the Tau P301S mice as they descend into the final stages of the disease. The final group sizes during fear conditioning were as follows: noncarrier-vehicle (n=11), noncarrier-CBD (n=11), Tau P301S-vehicle (n=11), Tau P301S-CBD (n=13).

As seen in Figure 29 (A) the Tau P301S mice demonstrate an increased freezing response during the novel context. Furthermore, the freezing response of the Tau P301S in the presence of the conditioning context was slightly lower than the noncarrier littermates. As such the freezing difference is less pronounced for the Tau P301S mice. There were no observable differences between the mice treated with CBD or vehicle. A two-way within subjects ANOVA assessed the percent time spent freezing during the novel context compared to during context recall. Within subjects there was a significant increase in the freezing response of the mice during context recall compared to in the novel context ($F(1, 42) = 19.747$, $p < 0.001$). There was a significant effect of genotype on the freezing of the mice ($F(1, 42) = 10.916$, $p < 0.002$). However, a pairwise comparison with Bonferroni corrections revealed that the significant effect of genotype was only applicable to the

freezing of the mice in the novel context ($p < 0.012$). There was no effect of genotype on the context recall freezing ($p < 0.114$). There was no significant effect of treatment on the freezing of the mice ($F(1, 42) = 0.057$, $p < 0.813$).

Figure 29 (B) depicts the tone recall data of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle. The freezing of the Tau P301S mice before the tone and during tone recall is higher relative to the noncarrier littermates. However, each group of mice regardless of genotype and treatment appear to be able to recall the tone/shock paring. Evidenced by an increased freezing response during the tone relative to before the tone. A two-way within subjects ANOVA revealed that there was a significant difference in the percent time spent freezing during the tone, relative to before the tone ($F(1, 42) = 378.463$, $p < 0.001$). There was no significant effect of genotype ($F(1, 42) = 1.509$, $p < 0.226$) or treatment ($F(1, 42) = 0.743$, $p < 0.394$) on tone recall.

Therefore, overall, the context recall and tone recall of the Tau P301S mice did not differ from the noncarrier littermates. These results are suggestive of retained fear associative memory, an assay of learning and memory supported by neural networks centered on the amygdala. The Tau P301S mice did freeze more prior to the tone relative to the noncarrier littermates. The potential cause for these results will be explored in the discussion.

Hippocampal Volume

The group sizes for the volumetric analysis were as follows: noncarrier-vehicle male (n=3), noncarrier-vehicle female (n=3), noncarrier-CBD male (n=3), noncarrier-CBD

female (n=3), Tau P301S-vehicle male (n=4), Tau P301S-vehicle female (n=3), Tau P301S-CBD male (n=5), Tau P301S-CBD female (n=3).

The brains of the perfused mice were stained with Nissl and HPC volumes were quantified. As seen in Figure 30 (A), overall, the noncarrier littermates have a larger HPC volume relative to the Tau P301S mice. The HPC volume of the male noncarrier littermates was slightly larger than the female noncarrier littermates. In contrast to this the HPC volume of the female Tau P301S mice is much larger compared to the male Tau P301S mice. CBD does not appear to impact the HPC volume of the animals. A three-way between subjects ANOVA was conducted to determine any effects of genotype, treatment, and sex on the HPC volume of the animals. The results suggest that there was a significant effect of genotype ($F(1, 19) = 40.419$, $p < 0.001$). There was no significant effect of treatment ($F(1, 19) = 1.115$, $p < 0.304$) or sex ($F(1, 19) = 0.893$, $p < 0.357$). There was a significant interaction of genotype X sex ($F(1, 19) = 8.837$, $p < 0.008$). A pairwise comparison with Bonferroni corrections analyzed the significant interaction, and the results suggest that the significant effect of sex was only applicable to the Tau P301S mice ($p < 0.009$), but not the noncarrier littermates ($p < 0.185$). The size difference of the HPC depending on genotype and sex can very clearly be observed by looking directly at the stained brains. Refer to Figure 30 (B) for a demonstration of the HPC volume of the male and female Tau P301S mice and noncarrier littermates.

Inflammation

Microglial

There was a visibly larger quantity of Iba1 in the HPC of the Tau P301S mice relative to the noncarrier littermates (see Figure 31(A)). The difference in the quantity of Iba1 in the PFC, depending on genotype, was less pronounced when observing the stained images (see Figure 31(B)). A consistent difference depending on the treatment and sex of the mice can not be observed by simply looking at the stained images.

The collected data on the percentage of Iba1 in the HPC of the male and female Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle can be seen in Figure 32 (A). The Tau P301S mice treated with the vehicle have the highest percentage of Iba1 in the HPC, followed by the male and female Tau P301S mice treated with CBD. The percentage of Iba1 is similar in the male and female noncarrier littermates treated with the vehicle or CBD, with the CBD mice having a slightly reduced quantity of Iba1. A three-way between subjects ANOVA confirmed the observed results. There was a significant effect of genotype ($F(1, 19) = 76.741$, $p < 0.001$). There was no significant effect of treatment ($F(1, 19) = 3.003$, $p < 0.099$) and there was no significant effect of sex ($F(1, 19) = 1.459$, $p < 0.242$). *A priori* assumptions were made that CBD is reducing the quantity of Iba1 in the HPC of the Tau P301S mice. A pairwise comparison with Bonferroni corrections revealed a marginal trend towards the CBD treated Tau P301S mice having a reduced quantity of Iba1 compared to the Tau P301S mice treated with a vehicle ($p < 0.070$). There was no trend towards a significant effect of treatment for the noncarrier littermates ($p < 0.560$). Overall, the Tau P301S mice had more Iba1 stained cells in their HPC relative to the noncarrier littermates. There was a marginal trend towards CBD

decreasing the quantity of Iba1 in the HPC of Tau P301S mice. Sex did not statistically impact the quantity of Iba1 in the HPC of the mice.

Figure 32 (B) represents the percentage of Iba1 in the PFC of the male and female mice depending on their genotype and treatment group. The male Tau P301S-CBD mice and the female Tau P301S-vehicle mice have the highest percentage of Iba1 in the PFC, followed by the male Tau P301S-vehicle mice and the female Tau P301S-CBD mice. Amongst the noncarrier littermates the males consistently have more Iba1 relative to the females. Overall, the Tau P301S mice have more Iba1 in the PFC relative to the noncarrier littermates. A three-way between subjects ANOVA revealed that there was a significant effect of genotype ($F(1, 19) = 25.487$, $p < 0.001$). There was no significant effect of treatment ($F(1, 19) = 1.157$, $p < 0.296$) and there was a marginal trend towards an effect of sex ($F(1, 19) = 3.577$, $p < 0.074$). Therefore, Iba1 was increased in the PFC of the Tau P301S mice relative to the noncarrier littermates. CBD did not impact the quantity of Iba1 in the PFC. Sex might be in part impacting the quantity of Iba1 in the PFC.

Astrocytes

When observing the staining of GFAP in the HPC of the mice, the male Tau P301S mice followed by the female Tau P301S have a visibly larger quantity of the inflammatory marker relative to the noncarrier littermates (see Figure 33(A)). The quantity of GFAP in the PFC varied amongst male and female Tau P301S mice and there was no GFAP observed in the PFC of the noncarrier littermates (see Figure 33(B)). No effect of treatment can be observed when looking at the stained images.

The collected data on the quantity of GFAP in the HPC of the male and female mice depending on their genotype and treatment can be seen in Figure 34 (A). The male and female Tau P301S mice treated with the vehicle have a higher percentage of GFAP in the HPC, relative to the male and female Tau P301S mice treated with CBD. Overall, the Tau P301S males and noncarrier littermate males demonstrate a higher percentage of GFAP relative to the female mice, however the sex difference between the noncarrier littermates is slight. A three-way between subjects ANOVA confirmed the observed results. There was a significant effect of genotype ($F(1, 19) = 27.729$, $p < 0.001$). There was no significant effect of treatment ($F(1, 19) = 0.101$, $p < 0.754$). There was a significant effect of sex ($F(1, 19) = 4.747$, $p < 0.042$). Due to the appearance of the graph an *a priori* assumption was made that the effect of sex was only applicable to the Tau P301S mice. A pairwise comparison with Bonferroni corrections confirmed that there was only a significant effect of sex amongst the Tau P301S mice ($p < 0.018$) there was no significant effect of sex between the noncarrier littermates ($p < 0.564$). Ultimately, the results suggest that the Tau P301S mice have a higher percentage of GFAP in the HPC. Additionally, the male Tau P301S mice have a higher percentage of GFAP relative to the female Tau P301S mice. The effect of sex was not seen in the noncarrier littermates. CBD was unable to delay the accumulation of GFAP in the HPC of the mice.

The quantity of GFAP in the PFC of the mice depending on genotype, treatment, and sex can be seen in Figure 34 (B). Amongst the vehicle treated Tau P301S mice the females demonstrate a higher percentage of GFAP relative to the males, however amongst the CBD treated mice the males demonstrate a higher percentage of GFAP relative to the females. Overall, the Tau P301S have a higher percentage of GFAP in the PFC relative to

the noncarrier littermates. A three-way between subjects ANOVA assessed the observed results and revealed that there was a significant effect of genotype ($F(1, 19) = 13.507$, $p < 0.002$). There was no significant effect of treatment ($F(1, 26) = 0.239$, $p < 0.630$) or sex ($F(1, 19) = 0.062$, $p < 0.806$). The results suggest that the Tau P301S mice have a higher quantity of GFAP positively stained cells in the PFC, suggestive of reactive astrocytes. CBD does not reduce the quantity of GFAP in the PFC of the mice. Sex did not impact the quantity of GFAP in the PFC.

Discussion

The aims of the present experiment were as follows: (1) to observe the effects of chronic, pre-symptomatic administration of CBD on Tau P301S mice and their noncarrier littermates; (2) to observe if CBD would improve any observed learning and memory or motor impairments in the Tau P301S mice; (3) to observe if CBD would prevent neurodegeneration in the HPC and reduce the quantity of Iba1 or GFAP in the HPC and PFC of the Tau P301S mice; (4) to assess the safety and tolerability of chronic CBD administration in noncarrier littermate mice.

Ultimately, the results suggest that the spatial recall of the Tau P301S mice began to decline around 6 months of age, while the object recognition, spatial learning, and hind limb strength began to decline around 9 months of age. In the present experiment the Tau P301S mice retained motor coordination and fear associative memory. No cognitive impairments were reported for the noncarrier littermates. CBD did not restore the cognitive functions of the Tau P301S mice. Importantly, CBD was well tolerated in the noncarrier littermates as no impairments on a variety of learning and memory tasks was observed. These results are important because it shows that long-term exposure to CBD does not

negatively impact the neural networks that support various cognitive functions as well as the neural machinery that support these processes including sensory, motor, and motivational systems. Further, CBD was well tolerated regardless of genotype, suggesting its chronic oral consumption was safe at 20mg/kg, in normal subjects as well as those suffering from an age-related neurodegenerative disease. In addition to the cognitive impairments, the Tau P301S mice had pronounced HPC volume loss, as well as increased inflammation in the HPC and PFC relative to the noncarrier littermates around 10 months of age. Neurodegeneration and the percentage of astrocytes in the HPC of the male Tau P301S mice was more severe than the female Tau P301S mice. The pathology in the PFC varied amongst all Tau P301S mice. CBD was unable to restore the severe HPC volume loss observed in the Tau P301S mice. Furthermore, CBD did not affect the quantity of astrocytes in the HPC and PFC of the mice. However, there was a marginally significant trend towards CBD decreasing the quantity of microglial in the HPC of the Tau P301S mice, suggesting potential anti-inflammatory effects of the cannabinoid compound. CBD did not differently impact the pathology of the mice depending on sex. Finally, CBD did not impact the brains of the noncarrier littermates, which did not demonstrate HPC cell loss or pronounced astrogliosis and microgliosis.

CBD on the Learning and Memory of Tau P301S Mice and their Noncarrier-littermates

The NOR results demonstrate that at 9, but not 6 months of age the Tau P301S mice had an overall decreased preference for the novel object relative to the noncarrier littermates. Suggesting impaired object recognition and dysfunction to networks centered around the perirhinal cortex. Previous authors have found Tau P301S mice to be impaired

during NOR at 7 (Hashem et al., 2021), 8 (Fan et al., 2020), 10, and 12 months of age (Vagnozzi et al., 2017). However, in contradiction to these reports, López-González et al. (2015) found that the object recognition of the mice was not impaired at 9 months of age, but it was impaired at 10 months of age. Therefore, there is variability as to when Tau P301S mice demonstrate object recognition impairments.

In addition to the overall impairment amongst the transgenic mice, the 9-month-old Tau P301S mice treated with CBD were the only group that did not statistically explore the novel object above chance. Importantly, there were no observed impairments on the object recall of the noncarrier littermates treated with CBD. Furthermore, there were no negative effects of CBD on any group of mice at 6 months of age. As such CBD might be impairing the object recall of the Tau P301S mice as they age, and the declined object recognition correlated to the cannabinoid compound might be specific to the tauopathy mutation. To our knowledge there are no reports of the effects of CBD on the object recognition of a tauopathy mouse model. Previous experiments have found that CBD was able to improve object recall on Aβ mouse models of AD (Aso et al., 2015; Cheng et al., 2014(A); Coles et al., 2020; Khodadadi et al., 2021). As Aβ and NFTs occur in conjunction during the course of the disease (Thal et al., 2002), future studies should assess the effects of CBD on a mouse model that develops Aβ and NFTs to further observe if the benefits of the cannabinoid compound on Aβ mouse models persists in the presence of tau abnormalities.

Similarly, the spatial learning of the Tau P301S mice was impaired at 9, but not 6 months of age. Previous experiments have reported that the Tau P301S mice learn the location of the platform at a slower rate relative to noncarrier littermates at 6 (Takeuchi et al., 2011), 7 (Hashem et al., 2021), 10, and 12 months of age (Vagnozzi et al., 2017).

However, in contrast to these reports Dumont et al. (2011) only found a trend towards a spatial learning impairment at 10 months of age, but not 7 months of age. Importantly Dumont et al. (2011) and Vagnozzi et al. (2017) only trained their mice for 4-5 days. In the present experiment we trained our mice for 8 days, and the learning of the Tau P301S mice was only significantly increased from that of the noncarrier littermates on day 5 to 8.

Despite the intact spatial learning, the spatial recall of the Tau P301S mice began to decline at 6 months of age. While the quadrant preference of the mice was unaffected 24 hours after training, the Tau P301S mice had a reduced number of annulus crossings relative to the noncarrier littermates. Furthermore, during the probe retention, performed one week after training there was a strong trend towards an effect of genotype on the quadrant preference of the mice. CBD did not restore or impair the spatial recall of the mice at 6 months of age. However, to my surprise, while not statistically significant, it appears as though only the noncarrier littermates treated with CBD demonstrated a preference for the target quadrant relative to the alternate quadrants at 9 months of age. As such CBD might be promoting spatial recall in the noncarrier littermates as they age.

Previous experiments have found Tau P301S mice to have impaired spatial recall at 6 (Takeuchi et al., 2011) and 7 (Hashem et al., 2021) months of age. Wild type mice are generally unimpaired during spatial recall tasks at 9 months of age. The impairments observed in the present experiment might be a result of retraining. While there was a three-month delay between the 6-month MWT test and the 9-month MWT test it is plausible that at 9 months of age the mice retained recall for the platform they were trained to locate at 6 months of age, resulting in potential interference between competing memories acquired in that training context.

CBD did not impact the spatial learning and memory of the Tau P301S mice at 6 or 9 months of age. Interestingly, CBD might be promoting spatial recall in the noncarrier littermates at 9 months of age. Previous papers have reported the benefits of 20mg/kg of CBD on the spatial learning of an Aβ mouse model (Martin-Moreno et al., 2011). Furthermore, Kreilaus et al. (2022) found that 100mg/kg of CBD improved the spatial learning and recall in a tauopathy mouse model. Perhaps in the presence of tau mutations higher dosages of CBD are needed to observe behavioural benefits of the drug. In support of this theory, Watt et al. (2020(A)) found that 50mg/kg of CBD was sufficient to improve cognitive impairments in an Aβ mouse model of AD. However, when the same laboratory assessed the effects of 50mg/kg of CBD on a tauopathy mouse model they found no benefits from the cannabinoid compound (Watt et al., 2020(B)). As such future studies should observe the effects of higher dosages of CBD on tauopathy mouse models. Finally, none of the studies found alterations of the spatial learning and memory of the noncarrier littermates. However, as previously mentioned the probe data collected at 9 months of age may be contaminated due to retraining, as it is abnormal for 9-month-old healthy mice to demonstrate impaired spatial recall.

Additionally, there was a significant effect of genotype during the visible platform performance of the mice at 9, but not 6 months of age. Importantly, the Tau P301S mice did have a significantly reduced path length to locate the platform when it was visible, their path length was simply longer than that of the noncarrier littermates. The effect of genotype during visible platform might be suggestive of a decline in motor, motivational, and/or sensory behaviours. In contrast to our findings a previous experiment did not report an effect of genotype during the visible platform test at 10 months of age (Dumont et al.,

2011). Dumont et al. (2011) utilized a 2-day version of the visible platform test, whereas the visible platform test in the present experiment was conducted over one day. As such, the Tau P301S mice might require a second visible platform test as they age.

Furthermore, the balance beam data disputes that the mice had motor impairments. Indeed, there was no effects of genotype during balance beam at 6 or 9 months of age. In support of this, Dumont et al. (2011) did not find motor impairments during balance beam in Tau P301S mice at 2, 7, or 10 months of age. Interestingly, the CBD treated mice had a reduced latency to traverse the beam at 6 months of age, suggesting CBD might be improving the motor coordination of the animals. At 9 months of age there was no longer an influence of CBD on the performance of the mice.

In contrast to our findings, Watt et al. (2020(B)) tested the effects of 50mg/kg of CBD on Tau58/2 mice, the tau mice used in their experiment demonstrated motor impairments on balance beam and the CBD treated mice had an increased number of paw slips compared to the vehicle mice. In the present study CBD did not impact the paw slips of the mice (data not shown). However, Giuliano et al. (2021) found that when CBD was administered to a mouse model of Parkinson's disease the motor coordination of the mice was improved on a cylinder task, but there was no effect of CBD on the rotarod task. Like the present experiment Giuliano et al. (2021) did not find any negative effects of CBD on the motor coordination of the animals. More studies are needed to further observe the potential benefits or detriments of CBD on the motor coordination of mice, particularly with varying dosages and on various tasks.

While the Tau P301S mice did not demonstrate motor impairments they did demonstrate hind limb weakness at 9, but not 6 months of age. The hind limb weakness

was assessed by suspending the mice from their tail and observing their clasping behaviour. Increased clasping was suggestive of limb weakness. Yoshiyama et al. (2007) have previously reported that Tau P301S mice demonstrate increased clasping behaviour beginning at 3 months of age, while Dumont et al. (2011) did not find muscular weakness assessed with a grip test at 10 months of age. The varying results further suggests variability as to when the behavioural abnormalities commence within this mouse model. Regardless, CBD did not restore the hindlimb weakness of the Tau P301S mice.

Finally, the fear conditioning data suggests that the Tau P301S mice did not demonstrate contextual recall or tone recall impairments around 10 months of age. However, the freezing response of the Tau P301S mice before the tone, in a novel context, was increased relative to the noncarrier littermates. Due to the increased freezing in the novel context, the contextual recall of the Tau P301S mice was less pronounced than the noncarrier littermates. Interestingly Takeuchi et al. (2011) also found that the freezing of Tau P301S mice during context recall was less pronounced, but not statistically different from controls. Furthermore, previous experiments have found that the Tau P301S mice are significantly impaired during context recall, but not tone recall, at 8 months of age (Fan et al., 2020).

The increased freezing response of the Tau P301S mice during the novel context does not necessarily discount the fear conditioning data. Previous papers have found a correlation between increased freezing prior to the tone with increased freezing during the tone (Jacobs et al., 2010). As discussed in Chapter 2, the “baseline” freezing measure used throughout the experiments is rather imperfect. As such future studies should record the freezing of their mice prior to conditioning. Moreover, the increased freezing prior to the

tone, in the novel context, might be suggestive of contextual generalization. Increased contextual generalization would not indicate that the mice did not form the context/shock or tone/shock pairing, rather it would suggest that they did not retain a distinct representation of the conditioning context (Jacobs et al., 2010; Wiltgen & Silva, 2007). While some may argue that the Tau P301S mice are less mobile than the noncarrier littermates, this is unlikely as the total exploration of the transgenic mice did not differ from the noncarrier littermates during NOR. Nevertheless, CBD did not impact the fear associative memory of the mice in the present experiment. Like the present experiment, Cheng et al. (2014(B)) treated AβPP/PS1 with 20mg/kg and found that the baseline freezing response of AβPP/PS1 mice was increased, but the freezing of the transgenic mice during context and tone recall did not differ from noncarrier littermates. Similarly, CBD did not impact the fear associative behaviour of their mice.

At large, when observing the age of cognitive decline amongst the Tau P301S mice there are clearly conflicting results between laboratories. The conflicting results might be due to experimental differences (i.e., training duration, apparatuses, housing). It may also be a result of the background strain of the mice. The Tau P301S mice in the current experiment were kept on a C57BL/6N background, previous papers have used the Tau P301S mice on a B6C3H/F1 background (Hashem et al., 2021; Yoshiyama et al., 2007). A direct comparison of the transgenic mice depending on the background has not yet been characterized, however previous research suggests that the background of Aβ mouse models of AD has implications on the severity and onset of cognitive decline and pathology (Neuner, Heuer, Huentelman, O'Connell, & Kaczorowski, 2019; Ryman, Gao, & Lamb, 2008). IN my view, it is important to consider the background of the mice when comparing

experimental results as it might have implications on the onset of symptoms and development of pathologies. In support of this, the ethnicity of AD cases impacts the risk factors associated with the onset and severity of the disease in humans (Alzheimer's Disease Facts & Figures, 2022). Future studies should directly compare Tau P301S-Line PS19 mice on a C57BL/6N background to the mice on B6C3H/F1 background to further characterize the differences in their behaviour and brain pathology at different ages. Based off the findings in the present experiment there is reason to believe that the Tau P301S mice decline at a slower rate when bred on a C57BL/6N background. Despite the potential delay in the onset of cognitive decline, the pathology of the mice was in-line with previous experiments.

Additionally, while there were no clear effects of CBD on the behaviour of the Tau P301S it is important to note that the male and female mice were grouped together for the behavioural analysis. Grouping the behaviour of the mice regardless of sex may have resulted in increased variability rendering it difficult to view statistically significant results. In support of this, Sun et al. (2020) have reported that the onset and severity of learning and memory, as well as motor impairments amongst Tau P301S mice differs significantly between males and females. The same observation was made in the present experiment. Furthermore, the pathology of the male and female mice differed significantly in the present experiment and in previous experiments (Sun et al., 2020). Future studies should include sex as a factor when assessing the behaviour of Tau P301S mice to limit variability. Unfortunately, this was not possible in the present experiment as there would not have been enough animals to include sex as a factor when analysing the behavioural results.

The Effects of CBD on AD Related Pathologies in Tau P301S Mice

Overall, the 10-month-old Tau P301S mice had visible degeneration within the HPC formation, particularly the male Tau P301S mice. The observed HPC volume loss and sex difference was statistically supported. Consistent with our findings, previous experiments have reported significant HPC cell loss in Tau P301S mice at 8 (Fan et al., 2020) and 9 months of age (Yoshiyama et al., 2007). CBD was unable to restore the HPC volumes of the Tau P301S mice around 10 months of age. In contradiction to these reports CBD has been shown to prevent cell death, promote cell survival and promote neurogenesis *in vitro* (Chen et al., 2016; di Giacomo et al., 2020; Esposito et al., 2006 (B); Esposito et al., 2011; Iuvone et al., 2004) and *in vivo* (Esposito et al., 2011; Fogaça et al., 2018). However, to our knowledge, the effects of CBD on cell loss in a tauopathy mouse model has not been reported. Neurodegeneration in Tau P301S mice is related to the toxic effects of tau, as such CBD might be ineffective in delaying this cascade of events. Future studies should assess neurogenesis to observe if CBD is able to promote neurogenesis in the presence of tau abnormalities.

In addition to the reduced HPC volumes, the Tau P301S mice demonstrated increased positively stained GFAP cells and Iba1 cells in the HPC and PFC around 10 months of age. Specifically, the morphology of the GFAP and Iba1 stained cells appeared reactive. Reactive astrocytes are classified to have enlarged cell bodies and long, thick processes, whereas reactive microglial have enlarged cell bodies and short processes. In support of this, previous papers have also found that astrogliosis and microgliosis to be increased in the brains of Tau P301S mice beginning at 3-6 months of age (Hashem et al., 2021; Yoshiyama et al., 2007), increasing from 9-10 months of age (López-González et al.,

2015; Sun et al., 2020). Furthermore, the HPC of the male Tau P301S mice demonstrated increased levels of GFAP relative to the female Tau P301S mice. The affect of sex was not observed on the quantity of Iba1 in the HPC or PFC of the Tau P301S mice. This result is supported by a previous experiment that also found GFAP, but not microglial to be increased in the HPC of the male Tau P301S mice relative to females (Sun et al., 2020).

While the findings that the pathology of the male Tau P301S mice progresses faster relative to the female Tau P301S mice is in line with previous reports on this strain (Sun et al., 2020), it contradicts AD as seen in humans. AD related pathologies are more severe in females than males (Braak et al., 2011). Female AD patients have more severe atrophy of regions within the MTL (Juottonen et al., 1998). Furthermore, the prevalence of AD amongst females is higher than the prevalence of AD amongst males (Alzheimer's Disease Facts & Figures, 2022). Despite the discrepancy between the sex differences observed within this mouse model and AD as seen in humans, there was no consistent trends on CBD impacting the pathology of the male and female mice differently. To our knowledge there have been no previous papers that report the effects of CBD on the progression of AD depending on sex. Future studies should further assess if the cannabinoid compound impacts male and female AD mouse models differently, particularly given the differences in the prevalence and presentation of AD in humans.

Finally, CBD did not significantly affect on the percentage of GFAP in the HPC or PFC of the mice. However, there was a marginal trend towards an effect of CBD on reducing the quantity of Iba1 in the HPC, but not PFC of the Tau P301S mice. Perhaps if a more sensitive measure of assessing reactive astrocytes and microglial was used more clear effects from CBD would have been observed. Hovens et al. (2014) suggest that cell body to

cell size and dendrite size to cell size are accurate measurements of reactive microglial, as such future experiments should use a stereological microscope or a confocal microscope to observe if CBD significantly alters the conformation of the inflammatory markers.

Previous experiments have reported anti-inflammatory effects of CBD *in vitro* and *in vivo* in the presence of AD toxicities (Aso et al., 2015; Cheng et al., 2014 (B); Esposito et al., 2007; Esposito et al., 2011; Martin-Moreno et al., 2011; Watt et al., 2020(A)). To our knowledge this is the first experiment to assess the effects of pure CBD, *in vivo,* on the pathology of a tauopathy mouse model. As such there is a need for more experiments to further understand the impact of CBD on inflammation related to the hyperphosphorylation of tau. Researchers have reported that microglial, but not astrocytes precede the accumulation of hyperphosphorylated tau amongst Tau P301S mice (Sun et al., 2020; Yoshiyama et al., 2007). Therefore, the marginal trend towards a decrease in microglial, but not astrocytes might suggest that CBD could also delay the hyperphosphorylation of tau within this strain of animals, which would further support its therapeutic usage for AD. In support of this, in the presence of inflammation the phosphorylation of tau is increased (Yang et al., 2020). Specifically, within this strain the distribution of inflammation is believed to correlate with the distribution of tau pathology (Sun et al., 2020). Interestingly, when Tau P301S mice were treated with immunosuppressants at 2 months of age their survival rate increased and the mice demonstrated reduced inflammation, atrophy, and ultimately a reduction in phosphorylated tau and insoluble tau (Yoshiyama et al., 2007). Similarly, Asai et al. (2015) found that depleting microglial in Tau P301S mice reduced phosphorylated tau by 47% and 70%. Therefore, there is reason to believe that reducing inflammation would reduce the phosphorylation of tau. Our laboratory will be quantifying

the tau pathology of these mice, which will help shed light on the relationship between microglial and tau, as well as the effects of CBD on the progression and accumulation of hyperphosphorylated tau. If CBD can reduce the hyperphosphorylation of tau this would support its use to directly target one of the well-known neural hallmarks of AD, NFTs.

Concluding Remarks

Ultimately, the results suggest that at 6 months of age the Tau P301S mice were only impaired during spatial recall and then around 9 months of age their object recognition, spatial navigation, and hindlimb strength began to decline. The motor coordination and fear associative memory was unaffected at either age tested. Overall, CBD did not improve the observed learning and memory impairments. However, CBD might be promoting the motor coordination of the mice independent of genotype. Finally, only the CBD treated Tau P301S mice did not explore the novel object significantly above chance during NOR at 9 months of age. As such CBD might be in part impairing the object recognition of the Tau P301S mice as they age.

In addition to the learning and memory impairments, the Tau P301S mice did demonstrate severe neurodegeneration in the HPC and significant accumulation of microglial and astrocytes in the HPC and PFC. The neurodegeneration and the accumulation of astrocytes in the HPC was more severe in the male Tau P301S mice relative to the female Tau P301S mice. CBD did not restore the severe HPC volume reduction amongst the Tau P301S mice. Finally, while CBD was unable to reduce the quantity of GFAP in the HPC and PFC, there was a marginal trend towards a reduction of Iba1 in the HPC, but not PFC of the Tau P301S mice. Importantly, the chronic

administration of CBD over the period of 7 months was well tolerated amongst all the mice.

CHAPTER FOUR - General Discussion

In these experiments, I assessed the effects of various dosages of CBD and THC on APP^{NL-G-F} mice, a mouse model of AD that develops Aβ. Additionally, I assessed the effects of 20mg/kg of CBD relative to a vehicle, on a tauopathy mouse model, Tau P301S-Line PS19 and their noncarrier littermates. The learning and memory of the mice was assessed on tasks thought to be dependent on brain regions centered around the MTL (i.e., the HPC, perirhinal cortex, and amygdala) (Bucci et al., 2000; Ferbinteanu et al., 2003 Goosens & Maren, 2001; Kochli et al., 2015; Morris et al., 1982; Mumby et al., 2007; Sutherland et al., 1982). The motor coordination of the Tau P301S, but not APP^{NL-G-F} mice was analyzed as this strain has been shown to develop paralysis as they age (López-González et al., 2015; Yoshiyama et al., 2007). Following behavioural testing the brain pathology of the mice was assessed to observe the effects of the cannabinoid compounds on the volume of the HPC and inflammation in the HPC and PFC. Additionally, the effects of the cannabinoid compounds on the quantity of Aβ was assessed in the HPC and PFC of the APP^{NL-G-F} mice, but not in the Tau P301S mice.

The Effects of Cannabinoid Compounds on the Behaviour of AD Mouse Models

Marginal trends towards positive drug effects were observed when various dosages of CBD and THC were administered to APP^{NL-G-F} mice, as well as when 20mg/kg of CBD was administered to Tau P301S mice and their noncarrier littermates. Indeed, higher dosages of CBD and THC appear to promote object recognition and spatial recall amongst the APP^{NL-G-F} mice. Human AD cases are known to have impaired object recognition (Laatu et al., 2003) and impaired spatial recall (de Toledo-Morrell et al., 2000), as such CBD and THC might provide therapeutic potential with regards to these learning and

memory networks in the presence of Aβ. Moreover, 20mg/kg of CBD improved the motor coordination and balance of Tau P301S mice and their noncarrier littermates at 6 months of age. Motor impairments have also been reported amongst AD cases (Suttanon et al., 2012), further supporting a potential therapeutic benefit of CBD. However, it is worth mentioning that the effect of treatment on the motor coordination of the mice was not observed at 9 months of age. As such more studies are needed to further understand if CBD does in fact promote motor coordination.

In contrast to the observed trends towards benefits of the compounds, CBD might be impairing the contextual recall of the $APP^{NL\text{-}G\text{-}F}$, but not Tau P301S mice. Moreover, as the Tau P301S mice develop cognitive impairments, CBD might be impairing their object recognition. These results are not supported by previous papers, which found no impairments following CBD administration on context recall (Cheng et al., 2014(B); Watt et al., 2020(A)), or on object recognition (Aso et al., 2015; Cheng et al., 2014(A); Coles et al., 2020; Khodadadi et al., 2021). The differing results might be due to experimental differences but do warrant further investigation to confirm whether CBD impairs select learning and memory functions in the presence of AD pathologies. Importantly, there were no impairments associated with the administration of THC on the behaviour of the $APP^{NL\text{-}G\text{-}F}$ mice, or from the chronic administration of CBD on noncarrier littermates. From this we can conclude that the dosages chosen were non-psychoactive, safe, and well tolerated.

Overall, there were more observable trends towards the benefits of CBD on the behaviour of the $APP^{NL\text{-}G\text{-}F}$ mice relative to the Tau P301S mice. The $APP^{NL\text{-}G\text{-}F}$ mice only demonstrated slight impairments in comparison to the Tau P301S mice, which were significantly impaired on various tasks. As such cannabinoid compounds might be

therapeutic up until a certain point in the disease course. Though, previous experiments have found benefits from the administration of CBD on Aβ mouse models that were behaviourally tested during late disease stages (Coles et al., 2020; Watt et al., 2020(A)). Alternatively, as discussed in Chapter 3, higher dosages of CBD might be needed in the presence of hyperphosphorylated tau (Kreilaus et al., 2022; Watt et al., (2020 (A)(B)). Finally, we did not assess the effects of THC on the Tau P301S mice, perhaps future studies will find more therapeutic potential from the chronic administration of THC in the presence of hyperphosphorylated tau. In support of this Hashem et al. (2021) found that inhibiting the hydrolysis of 2-AG improved cognitive impairments in a tauopathy mouse model. The benefits of inhibiting the degradation of 2-AG were believed to be in part due to the activation of CB1 and CB2 receptors. As such, THC might be a better treatment option in the presence of NFTs since it is a well-known partial agonist for CB1 and CB2 receptors (Petitet et al., 1998; Pertwee, 2008).

Ultimately, these observations and conclusions are speculative as there were no significant effects of the cannabinoid compounds with the exception that CBD might be promoting motor coordination in the Tau P301S mice and noncarrier littermates at 6, but not 9 months of age. Limitations and future directions will be discussed in detail later in the discussion.

The Effects of Cannabinoid Compounds on the Pathology of AD Mouse Models

Like the behavioural data, there were some marginal trends and observable effects of CBD on the brain pathology of $APP^{NL\text{-}G\text{-}F}$ mice and Tau P301S mice. Surprisingly, THC did not demonstrate any potential therapeutic effects on the pathology of the $APP^{NL\text{-}G\text{-}F}$ mice. Suggesting that CBD might be more effective than THC at delaying the progression

of AD in the brain. Moreover, the marginal trends observed on the effects of CBD on AD related pathologies differed between APP^{NL-G-F} mice and Tau P301S mice suggesting that cannabinoid compounds might impact the neural hallmarks of AD in a differing manner.

We found a marginal trend towards a dose dependent increase in the HPC volume of APP^{NL-G-F} mice treated with CBD, but not THC. As such higher dosages of CBD might provide protective effects on the HPC in the presence of Aβ, possibly by increasing neurogenesis (Esposito et al., 2010; Fogaça et al., 2018). In contrast to this observation, 20mg/kg of CBD was ineffective in delaying or preventing the severe neurodegeneration in the HPC of the Tau P301S mice. Perhaps if the brains of the Tau P301S mice were observed at a younger age a protective effect of CBD would have been observed. However, this is unlikely as the neurodegeneration in the HPC of the female Tau P301S mice was less severe than the male Tau P301S mice and there were no benefits of the compound on the HPC volume of either sex. As such CBD might be ineffective in preventing neurodegeneration related to the toxic effects of hyperphosphorylated tau. Tau abnormalities are more closely related to neurodegeneration amongst AD cases relative to Aβ (Giannakopoulos et al., 2003; Petersen et al., 2019), as such this observation needs further investigation. Importantly, there were no observable trends towards either cannabinoid compound decreasing the HPC volume of any group of mice, further supporting the safety of the chosen dosages.

Moreover, various dosages of CBD and THC were ineffective at delaying or preventing the percentage of Aβ and microglial in the HPC or PFC of the APP^{NL-G-F} mice, however CBD appears to dose dependently decrease the percentage of astrocytes in the HPC and PFC of the APP^{NL-G-F} mice. The same trend was not observed from the

administration of various dosages of THC. As such CBD but not THC might provide protective effects on the quantity of astrocytes in the HPC in the presence of Aβ. In contrast to this, when observing the brains of the Tau P301S mice there was a marginal trend towards CBD decreasing the quantity of microglial, but not astrocytes in the HPC. Therefore, CBD might impact inflammation in the brains of the mice differently depending on if the disease is driven by Aβ or hyperphosphorylated tau. Future studies should observe the effects of CBD on the inflammatory markers in the presence of Aβ and hyperphosphorylated tau to further understand the effects of the cannabinoid compounds in the presence of both pathologies.

To our knowledge these were the first experiments to report the effects of *various dosages* of CBD and THC on the behaviour and brain pathology of an Aβ mouse model of AD. Additionally, to our knowledge, this is the first study to report the effects of pure CBD on the behaviour *and* brain pathology of a tauopathy mouse model. In addition, to contributing to the literature with regards to the effects of cannabinoid compounds in relation to AD, we were able to demonstrate that the daily, oral administration of 20mg/kg of CBD to healthy mice was safe and well tolerated over the period of 7 months. For translational purposes understanding the safety and tolerability of cannabinoid compounds over prolonged periods of time is crucial. Despite the novelty of the present experiment there were a few limitations, which will be discussed below.

Limitations and Future Directions

Firstly, it is important to highlight that $APP^{NL\text{-}G\text{-}F}$ mice are a mouse model of FAD (Saito et al., 2014) which only accounts for 1% of AD cases (Cruchaga et al., 2018). Furthermore, the Tau P301S-Line PS19 mouse model is technically a model of FTLD, with

pathologies that overlap with AD (Yoshiyama et al., 2007). As such neither model is a pure representation of the most common form of AD, SAD. This limitation is not specific to the present experiment, most, if not all, animal models used for AD research do not model pure SAD (Chin, 2011). As such there is a clear need for the development of better mouse models of the disease. Perhaps if the mouse models better represented the disease, stronger effects from the cannabinoid compounds would have been observed. Despite this discrepancy the observable trends should not be discounted as the results can still speak to the effects of cannabinoid compounds on AD related cognitive impairments and AD like pathologies.

Moreover, it is possible that cannabinoid compounds might be more effective at delaying the progression and onset of SAD rather than FAD. SAD is associated with aging and several risk factors, including, but not limited to, stroke, stress, and seizures (McDonald, 2002). Whereas FAD is largely associated with mutations on the following genes: APP, PSEN-1, or PSEN-2 (Weggen & Beher, 2012). Interestingly research suggests that cannabinoid compounds provide therapeutic potential to aged rodents (Nidadavolu et al., 2021) and in the presence of strokes (Meyer, de Mattos, Kirchhoff, & de Oliveira, 2021), stress (Fogaça et al., 2018), and seizures (Devinsky et al., 2018). Perhaps if future studies model SAD by utilizing the co-factor theory proposed by McDonald (2002) more clear benefits from the administration of the cannabinoid compounds might be observed. If cannabinoid compounds demonstrate more benefits in SAD models rather than FAD models, this will provide hope for 99% of AD cases (Cruchaga et al., 2018).

Secondly, the concentrations of CBD and THC were not assessed in the brains of any animals in the present experiments. As such, we can only speculate that the observable

trends towards drug effects were a result of the cannabinoid compounds acting on the CNS. While we have no reason to believe that the drugs were not entering the brain, particularly because CBD and THC are known to easily penetrate the blood brain barrier (Calapai et al., 2020) it is still important for future studies to directly confirm this.

Additionally, assessing the concentrations of the cannabinoid compounds in the brain will shed light on whether the mice are developing a tolerance to the compounds. If the mice are developing a tolerance to CBD and/or THC higher concentrations of the drugs might be necessary as the treatments progress. Future studies should have a subset of animals that are sacrificed for fresh tissue collection at various time points throughout the duration of treatments. Previous papers have successfully detected cannabinoids in the brains of rodents through high-performance liquid chromatography analysis when the tissue was collected 2 to 6 hours following treatment administration (Deiana et al., 2012). If the concentrations of the cannabinoid compounds decrease in the brains of the mice throughout the duration of treatments this might be suggestive of tolerance. Future studies should also assess the effects of the long-term administration of cannabinoid compounds on the endogenous cannabinoid system. Research suggests that a down regulation in CB1 receptors might be associated with a developed tolerance following the long-term consumption of *Cannabi*s products (Hirvonen et al., 2012). Importantly, these findings are associated with the recreational use of the drug, particularly THC. As such more studies are needed to further our understanding on the effects of low doses of THC and CBD on the endogenous cannabinoid system.

Of note, Sativex, which is a 1:1 ratio of THC and CBD is currently being prescribed to multiple sclerosis patients and longitudinal clinical trials have *not* reported that the

patients require higher dosages of the drug as treatments progress (See Giacoppo, Bramanti, & Mazzon, (2017) for a review). Similarly, a long-term study on epileptic patients reported benefits of 20mg/kg per day of CBD for up to 48 weeks (Devinsky et al., 2018). Finally, Cheng et al. (2014(B)) found social recognition improvements in an Aβ mouse model administered 20mg/kg of CBD, orally, daily, for 8 months. Therefore, at non-psychoactive concentrations we have no reason the believe that the animals are developing drug tolerances, but it is still important that this concern be addressed moving forward. It is also important to highlight that, like the present experiments, these studies further support that the long-term administration of cannabinoid compounds is safe and well tolerated.

Despite the clear benefits of cannabinoids compounds, traditionally, it is believed that the recreational use of cannabinoid compounds, specifically THC, is associated with addictive tendencies, reduced gray matter volume in select brain regions, and impaired cognitive functions (Battistella et al., 2014; Cousijn et al., 2012; Calabrese & Rubio-Casillas, 2018; Hirvonen et al., 2012). As such select individuals may argue that the long-term consumption of cannabinoid compounds may be detrimental rather than therapeutic. Interestingly, reduced gray matter volume correlated with *Cannabis* use is more closely associated with the quantity of the product consumed (g) (Cousijn et al., 2012) or the age at which the use first began (adolescents) rather than the length of consumption (Battistella et al., 2014). Additionally, these beliefs are based off the recreational use of the drug and are correlational rather than causative. As such I do not believe that at low concentrations this long-term format of cannabinoid consumption would lead to negative effects in the brain or on behaviour. In support of this we did not find that the cannabinoid compounds altered the behaviour of the mice negatively, nor did it reduce the volume of their HPC. Nevertheless,

more long-term studies are needed to further understand the effects of cannabinoid compounds on the volume of the brain and on cognitive abilities, particularly when consumed at low dosages.

Ultimately, the present experiments contribute to our understand of the effects of CBD and THC in the presence of Aβ, as well as the effects of CBD in the presence of tau abnormalities and when chronically administered to healthy mice. While the findings are novel and exciting, there is still a large gap in the literature on the effects of cannabinoid compounds particularly regarding routes of administration and rates of absorption. As such it is difficult to directly compare the results on the effects of cannabinoid compounds on AD mouse models and there is still a need for more studies to further our understanding on the effects of the drugs.

Much of the cited literature found benefits from the administration of cannabinoid compounds on AD mouse models when the drugs were administered via an i.p injection (Aso et al., 2015; Cheng et al., 2014(A); Martin-Moreno et al., 2011; Suliman et al., 2017). The present experiment orally administered the drugs, as such the dosages might not be translational when the routes of administration differ. Interestingly, Deiana et al. (2012) found that the concentration of CBD in the plasma and brain of rodents was increased following an i.p injection relative to following oral administration. Perhaps higher dosages are necessary when the compounds are being ingested. In support of this Malfait et al. (2000) assessed the effects of CBD on a mouse model of arthritis. Their results suggest that when CBD was administered via an i.p injection 5mg/kg was the most optimal dosage relative to 2.5mg/kg, 10mg/kg, and 20mg/kg. However, following oral administration 25mg/kg was the most optimal dosage relative to 10mg/kg and 50mg/kg. Future studies

should increase the dosage of pure CBD and pure THC to assess whether more clear effects from the cannabinoid compounds on the behaviour and brain pathology of APP^{NL-G-F} and Tau P301S mice can be observed.

Furthermore, cannabinoid compounds are lipid metabolites and when ingested through the digestive tract the rate of absorption varies between individuals (Fasinu, Phillips, ElSohly, & Walker, 2016). Therefore, it is possible that the absorption of CBD, THC and their concentrations were not consistent among the mice rendering it difficult to view statistically significant results. Variability differs less when drugs are administered mucosal or sublingual, therefore these routes of administration might be alternatives to look at (Fasinu et al, 2016). Nevertheless, the oral consumption of the cannabinoid compounds would be optimal for translational purposes. Interestingly, in humans the bioavailability of cannabis-based products is increased when the compounds are consumed during a fed state, relative to a fasted state (Silmore et al., 2021). In the present experiments the mice were treated near the end stage of their light cycle. As such it is likely that the mice were in a fasted state prior to treatments. Future studies should treat the mice during their dark cycle as this is when the mice are the most active, as such they would be more inclined to be in a fed state prior to treatments.

Nevertheless, while most studies administered cannabinoid compounds via an i.p injection, select studies did administer the drugs through oral consumption and found significant effects on their AD mouse models (Cheng et al., 2014 (B); Martin-Moreno et al., 2012). However, Cheng et al. (2014 (B)) and Martin-Moreno et al. (2012) tested the effects of the oral consumption cannabinoid compounds on transgenic Aβ mouse models. Therefore, their mice might have expressed artificial phenotypes. As such it is difficult to

conclude whether their results are due to the cannabinoid compounds impacting pathologies and behavioural abnormalities directly related to AD. Furthermore, the gut microbiota of their mice might have differed from the mice in the present experiments. Alterations to gut microbiota is believed to have implications in AD (Sochocka et al., 2019; Vogt et al., 2017). Moreover, this might affect the digestion and absorption of the cannabinoid compounds.

Finally, it is plausible that to observe benefits of the compounds that they should be administered as a combination therapy. It has been proven that CBD can mitigate the psychoactive components of THC, which would allow patients to receive the benefits of CBD and THC in the absence of the aversive side effects exerted from THC (Calabrese & Rubio-Casillas, 2018; Giacoppo et al., 2017; Hudson, Renard, Norris, Rushlow, & Laviolette, 2019; McPartland et al., 2015). Additionally, as previously mentioned Sativex is currently being prescribed to help alleviate the burden of multiple sclerosis in humans (Giacoppo et al., 2017). Like AD, multiple sclerosis is associated with increased neuroinflammation, therefore it is likely that Sativex will also help alleviate symptoms among AD patients. In support of this, Casarejos et al. (2013) gave a tauopathy mouse model Sativex, daily for one month and found the compound was able to improve behavioural abnormalities, reduce oxidative stress, astrogliosis, microgliosis, NPs, and NFTs. Furthermore, Aso et al. (2015) found more benefits from CBD combined with THC, relative to either compound alone. Overall, while there is supporting research for the compounds alone, a combination therapy might be the best option to alleviate the burden of AD in the brain and on behaviour.

Concluding Remarks

Ultimately there is a dire need for new treatment options that will delay symptom onset amongst AD cases while delaying the development of multiple pathologies in the brain. There is reason to believe that cannabinoid compounds could improve multiple pathologies and cognitive impairments related to AD, thereby improving the quality of life of patients and caregivers while improving the overall economy.

The present experiments found marginal trends towards benefits from the chronic administration of various dosages of CBD on the behaviour and brain pathology of APP^{NL-G-F} mice. Various dosages of THC appear to promote select learning and memory functions, but not pathologies in the APP^{NL-G-F} mice. Additionally, 20mg/kg of CBD might be promoting motor coordination in Tau P301S mice and their noncarrier littermates, as well as decreasing microglial in the HPC of Tau P301S mice. While the experiments are novel and exciting more research is required to further our understanding on the effects of cannabinoid compounds in the presence of AD related cognitive impairments and pathologies. Particularly, future experiments should assess mouse models that develop Aβ in conjunction with NFTs, observe higher dosages of the compounds when orally ingested, assess the concentrations of the cannabinoid compounds in the brain, and observe the effects of a combination treatment option. If cannabinoid compounds are proven to significantly improve the learning and memory and AD related pathologies in AD mouse models this will provide hope for AD cases.

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Figures

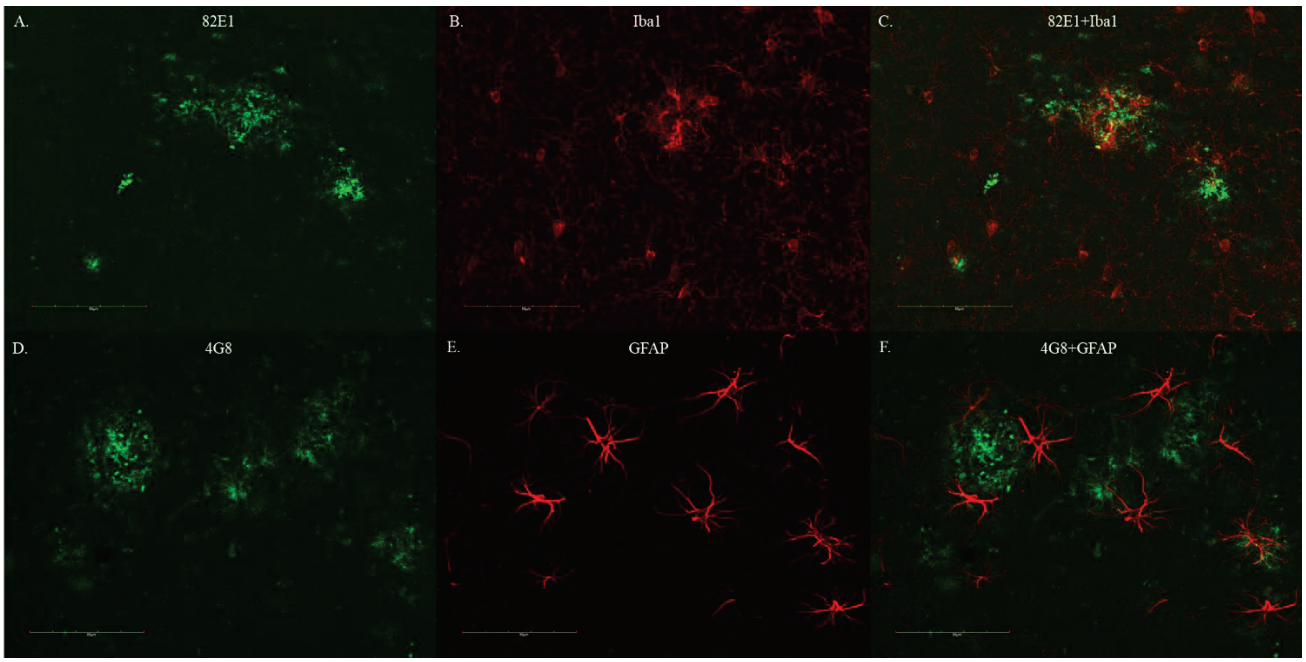


Figure 1. A representation of the immunohistochemical staining in the HPC of an APP^{NL-G-F} mouse. Image A-C represents the double staining of $A\beta_{1-16}$, stained with 82E1 and microglial, stained with Iba1. Image A is a demonstration with only the $A\beta_{1-16}$ channel. Image B is a demonstration with only the microglial channel. Image C demonstrates both channels, combined. Image D-F is a demonstration of the double staining of $A\beta_{7-24}$, stained with 4G8 and astrocytes, stained with GFAP. Image D represents only the $A\beta_{7-24}$ channel. Image E represents only the astrocyte channel. Image F represents both channels, combined. The images were taken using a confocal microscope at 40x magnification. The scale bar is 80µm.

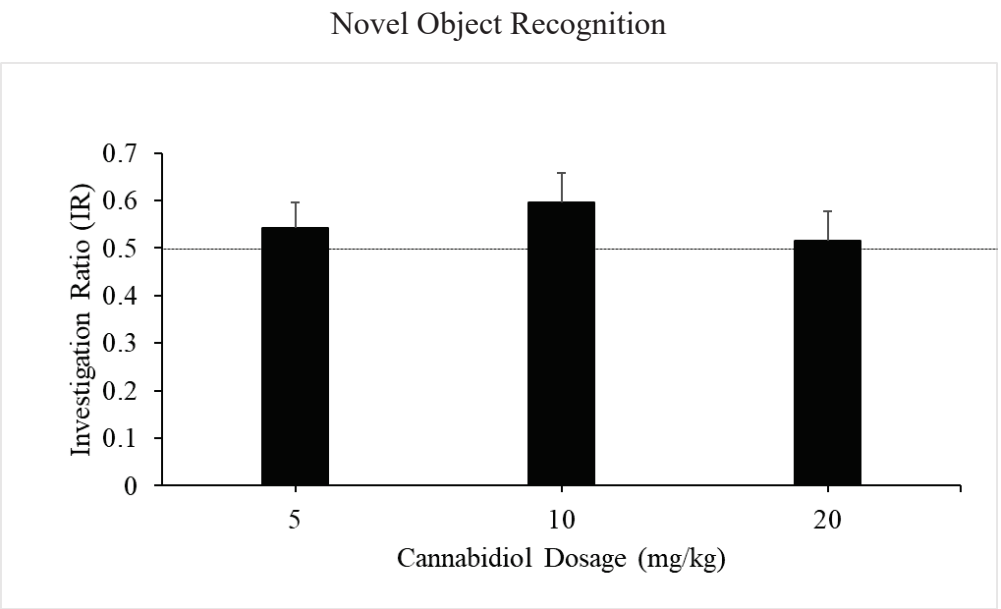


Figure 2. The average investigation ratio (IR) for the novel object during novel object recognition (NOR) for $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. Overall, each group did not have an IR value significantly above 0.5, indicating that they did not retain object recognition. There was no statistically significant effect of drug dosage on the novel object exploration time, suggesting that various dosages of CBD did not impact the object recognition of the mice.

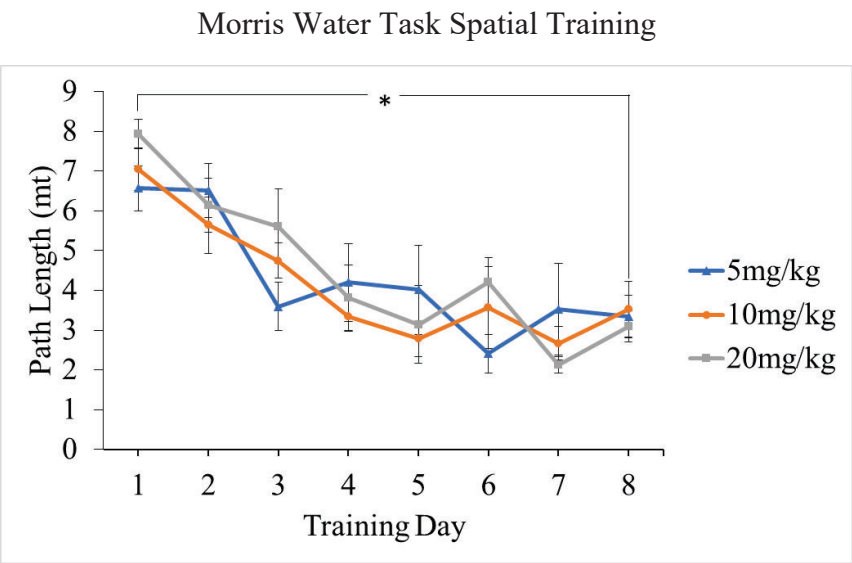
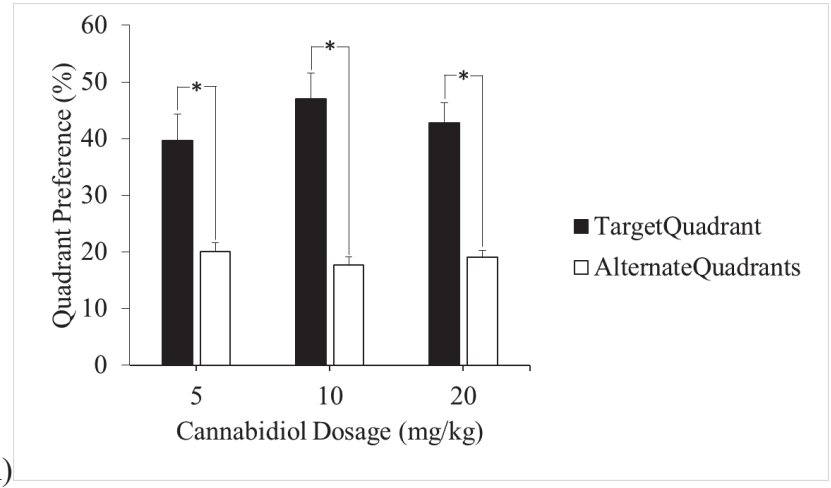
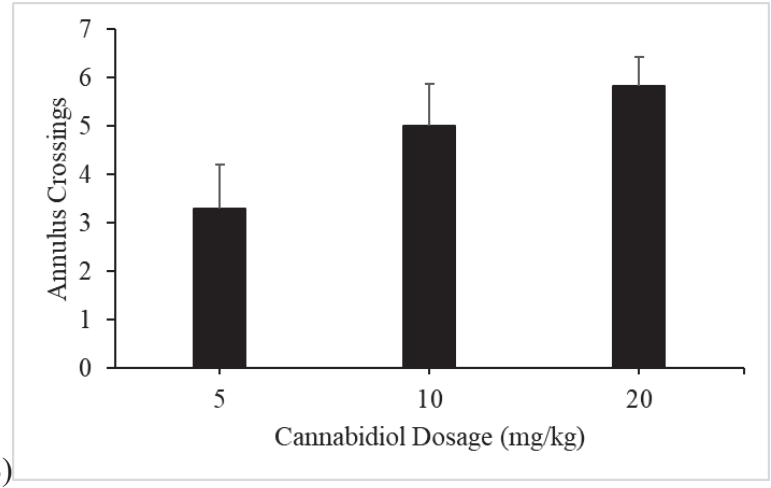


Figure 3. The average path length of the $APP^{NL\text{-}G\text{-}F}$ mice that received 5mg/kg, 10mg/kg, or 20mg/kg of CBD during MWT spatial training. The mice exhibited spatial learning, evidenced by a significant reduction in path length from training day 1 to training day 8 ($p < 0.001$). Various dosages of CBD did not significantly impact the spatial learning of the mice. A "*" indicates significance.

Probe



(A)



(B)

Figure 4. The data collected from the probe trial for $APP^{NL\text{-}G\text{-}F}$ mice treated with various dosages of CBD. (A) The average time the mice spent in the target quadrant relative to the alternate quadrants during the probe trial. The results suggest that all the mice recalled the platform location regardless of the dosage of CBD they received. There was a significant effect of quadrant ($p < 0.001$). There was no significant effect of CBD dosage. (B) The average number of annulus crossings during the probe trial. The number of annulus crossings appear to increase in a dose dependent manner. However, the results suggest that there was no significant effect of CBD dosage. A "*" indicates significance.

Visible Platform

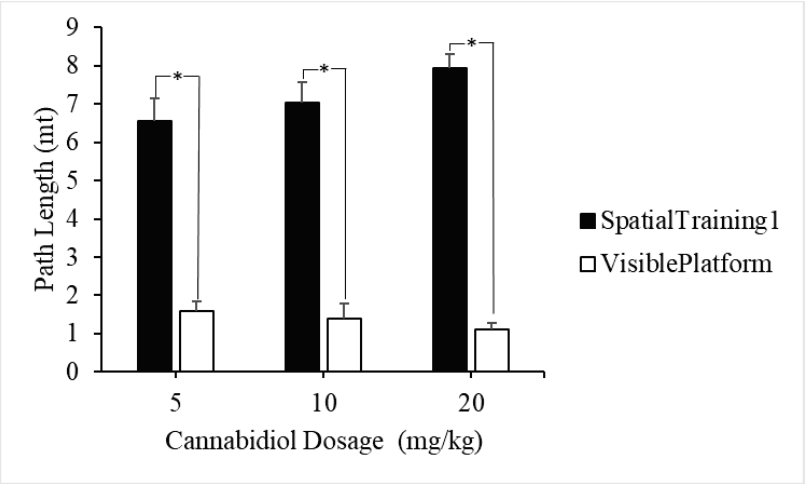
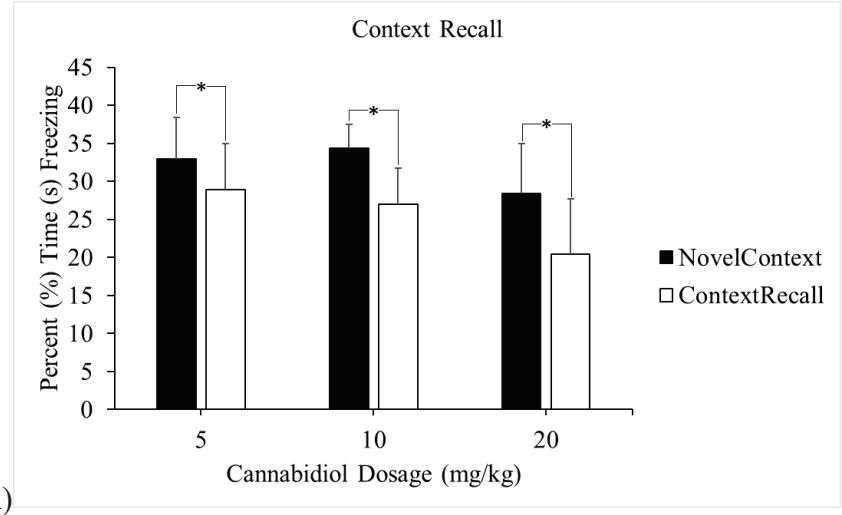
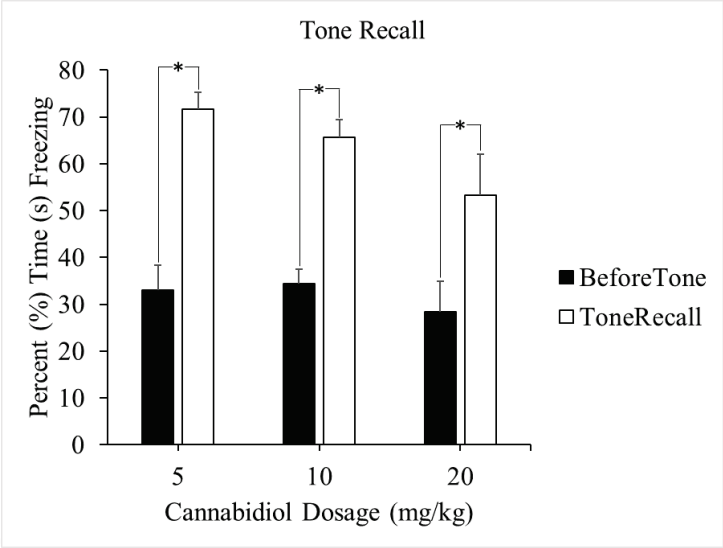


Figure 5. A comparison of the path length of the $APP^{NL\text{-}G\text{-}F}$ mice treated with various dosages of CBD on the first day of spatial training, when the platform was hidden, relative to visible platform test, when the platform was visible. The results suggest that the mice do not have sensory, motor, or motivational deficits. Evidenced by a significant reduction in path length when the platform was visible compared to when the platform was hidden ($p < 0.001$). There was no significant effect of CBD dosage. A "*" indicates significance.

Fear Conditioning



(A)



(B)

Figure 6. The data collected during context recall and tone recall following fear conditioning for the $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. (A) A comparison of the time the mice spent freezing during the novel context relative to during context recall. Overall, the mice had a higher freezing percentage in the novel context, suggesting that they did not recall the context/shock pairing. There was a significant effect of freezing ($p < 0.005$). There was no significant effect of CBD dosage. (B) The percent time spent freezing before the tone relative to during tone recall. Overall, the mice spent more time freezing during the tone suggesting that they recalled the tone/shock pairing. There was a significant effect of freezing ($p < 0.001$). There was no significant effect of CBD dosage. A "*" indicates significance.

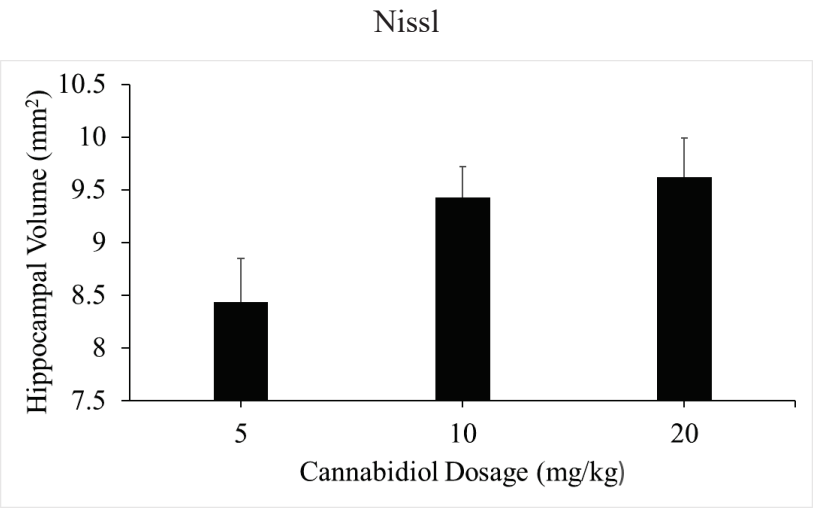
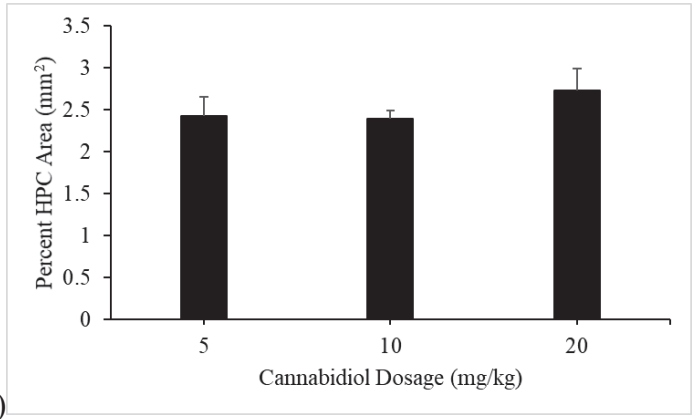
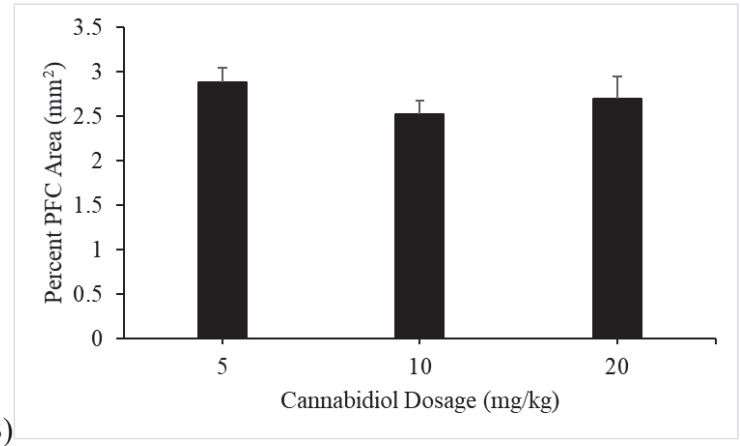


Figure 7. The average HPC volume of the $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. There was a marginal trend towards a dose dependent effect of CBD dosage on the HPC volume ($p < 0.072$).

Aβ1-16



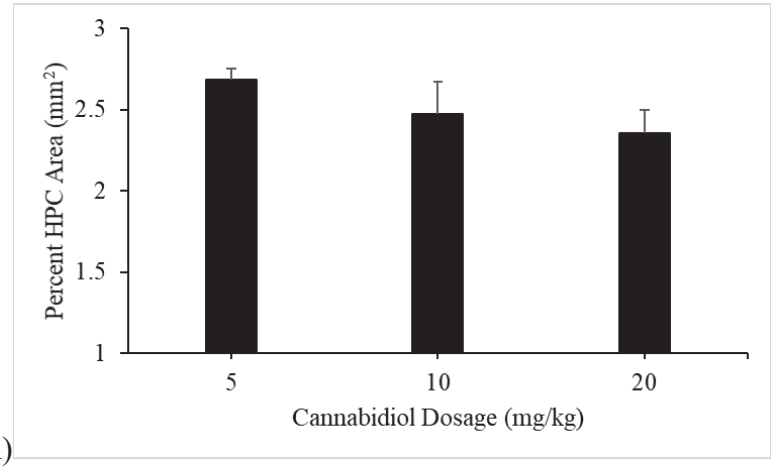
(A)



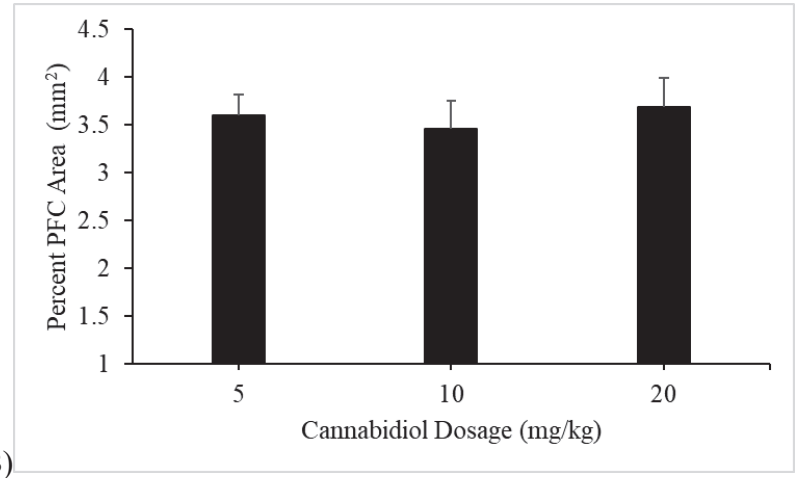
(B)

Figure 8. The percentage of Aβ1-16 (stained with 82E1) in the HPC and PFC of APP^{NL-G-F} mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. (A) The percentage of 82E1 in the HPC. There was no significant effect of CBD dosage on the percentage of Aβ1-16 in the HPC. (B) The percentage of 82E1 in the PFC depending on the given dosage of CBD. There was no significant effect of CBD dosage on the percentage of Aβ1-16 in the PFC. Overall, various dosages of CBD did not significantly affect the quantity of Aβ1-16 in the HPC and PFC of the APP^{NL-G-F} mice around 7 months of age.

Aβ7-24



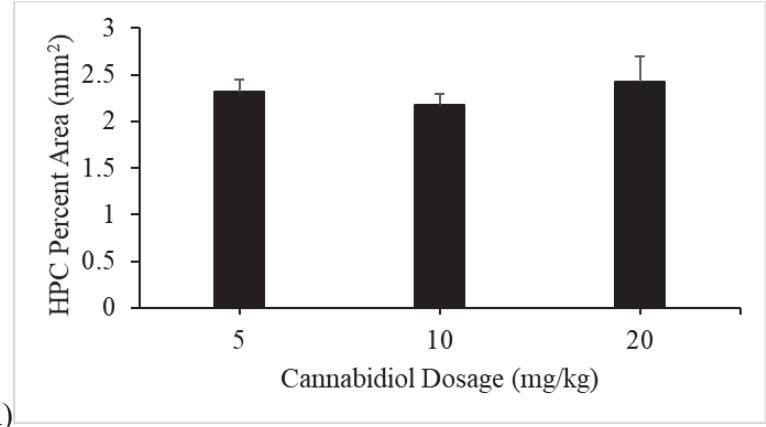
(A)



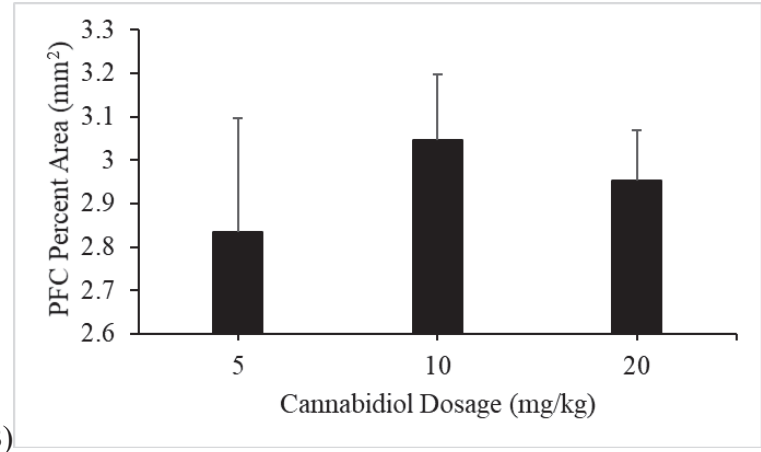
(B)

Figure 9. The percentage of Aβ7-24 (stained with 4G8) in the HPC and PFC of APP^{NL-G-F} mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. (A) The percentage of Aβ7-24 in the HPC depending on the given dosage of CBD. There was no significant effect of CBD dosage on the percentage of Aβ7-24 in the HPC. (B) The percentage of Aβ7-24 in the PFC depending on the given dosage of CBD. There was no significant effect of CBD dosage on the percentage of Aβ7-24 in the PFC. Overall, various dosages of CBD did not significantly affect the quantity of Aβ7-24 in the HPC and PFC of the mice around 7 months of age.

Iba1



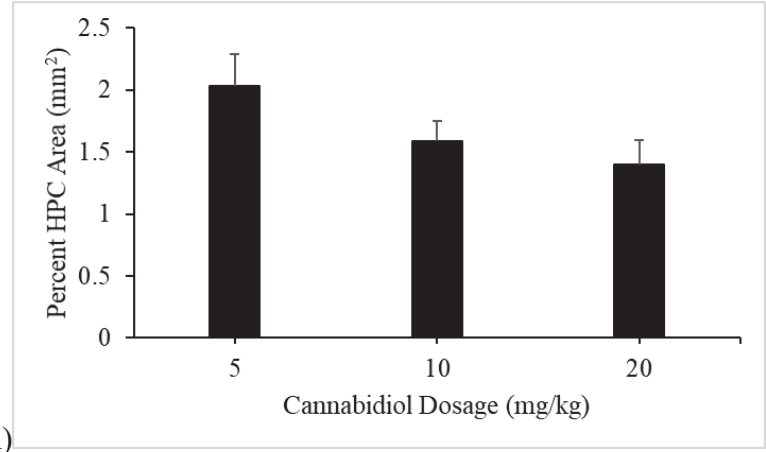
(A)



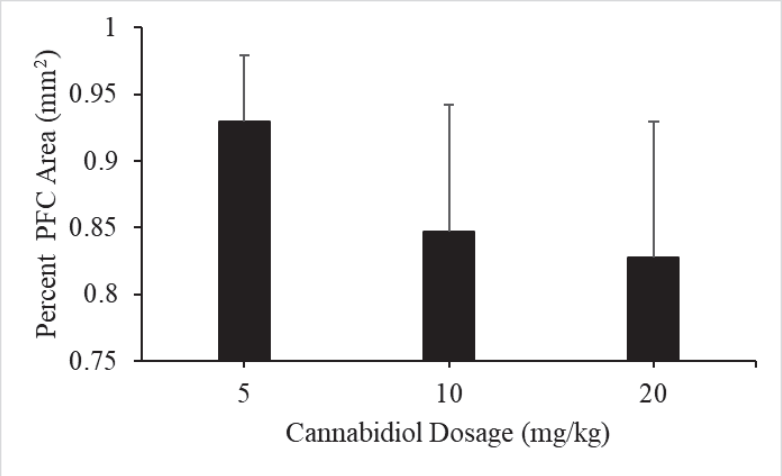
(B)

Figure 10. The percentage of Iba1 in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. (A) The percentage of Iba1 in the HPC between groups. There was no significant effect of CBD dosage on the percentage of Iba1 in the HPC. (B) The percentage of Iba1 in the PFC. There was no significant effect of CBD dosage on the percentage of Iba1 in the PFC. Overall, various dosages of CBD did not significantly affect the quantity of Iba1 in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

GFAP



(A)



(B)

Figure 11. The percentage of GFAP in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice treated with 5mg/kg, 10mg/kg, or 20mg/kg of CBD. (A) The percentage of GFAP in the HPC between groups. There was no significant effect of CBD dosage on the percentage of GFAP in the HPC. (B) The percentage of GFAP in the PFC. There was no significant effect of CBD dosage on the percentage of GFAP in the PFC. Overall, various dosages of CBD did not significantly affect the quantity of GFAP in the HPC and PFC of the mice around 7 months of age.

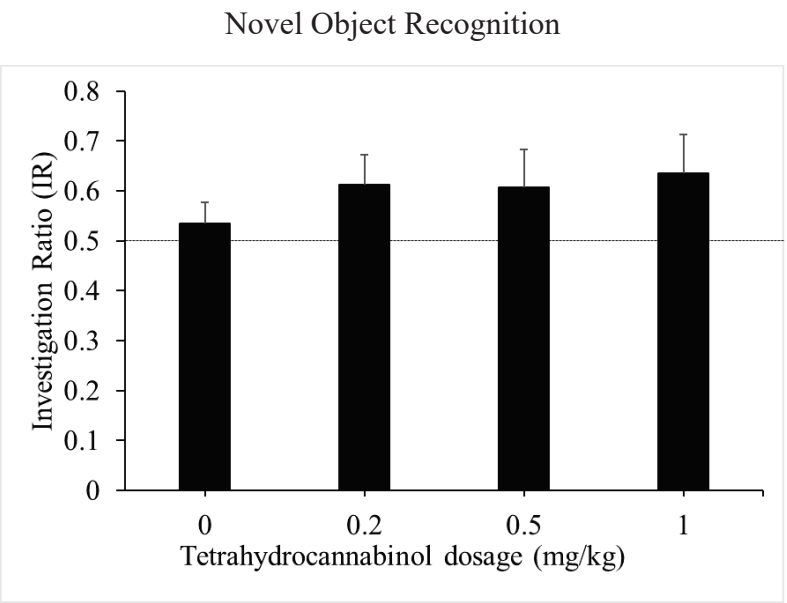


Figure 12. The average IR for the novel object during NOR for the APP^{NL-G-F} mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg of THC. The APP^{NL-G-F} mice did not retain object recognition as evidenced by an IR value non significantly above chance (0.5). There was no significant effect of THC dosage on object recognition.

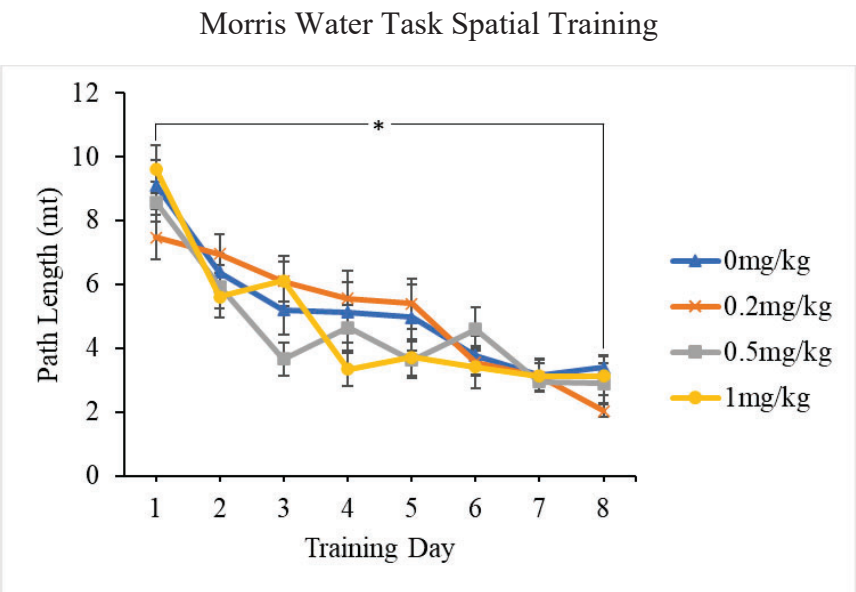
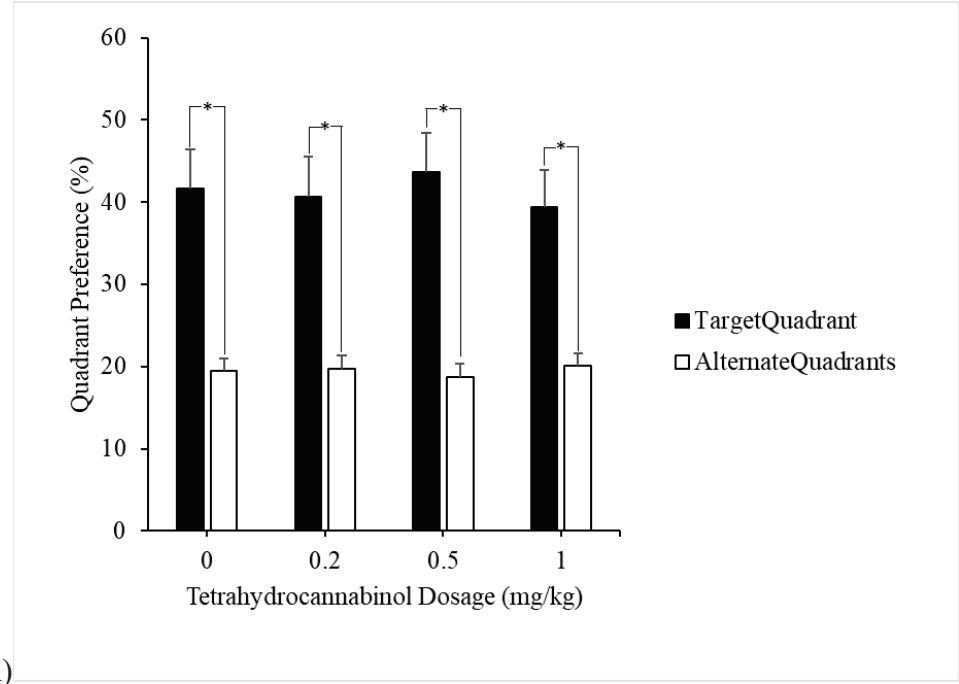
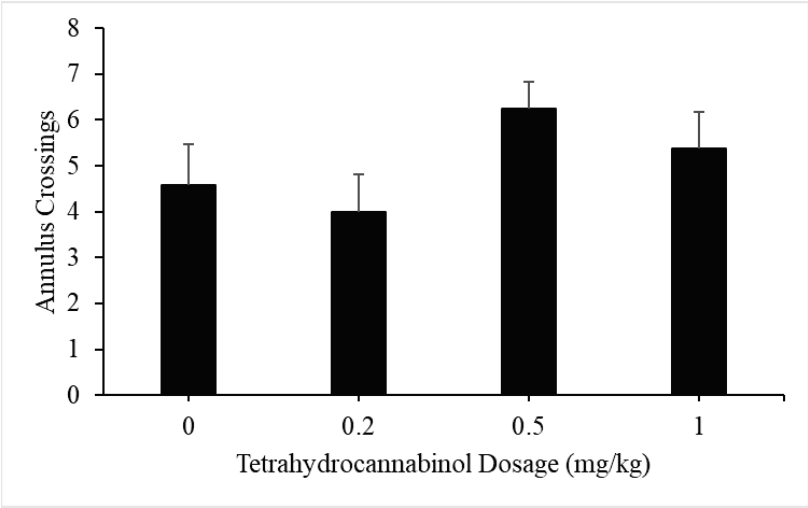


Figure 13. The average path length during MWT spatial training for the $APP^{NL\text{-}G\text{-}F}$ mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. The training data suggests that the mice were able to learn the location of the hidden platform, evidenced by a significant reduction in path length dependent on training day ($p < 0.001$). There was no significant effect of THC dosage. A "*" indicates significance.

Probe



(A)



(B)

Figure 14. The data collected during the probe trial for $APP^{NL\text{-}G\text{-}F}$ mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) The average time that the mice spent in the target quadrant relative to the average time spent in the alternate quadrants during the probe trial. The quadrant preference results suggest that all the mice recalled the previous platform location. There was a significant difference in the time spent in the target quadrant relative to the alternate quadrants ($p < 0.001$). There was no significant effect of drug dosage. (B) The average number of annulus crossings during the probe trial. The performance of the vehicle mice and mice that received various dosages of THC was similar. Therefore, THC did not impact the spatial recall of the mice. There was no significant effect of THC dosage on the number of annulus crossings. A "*" indicates significance.

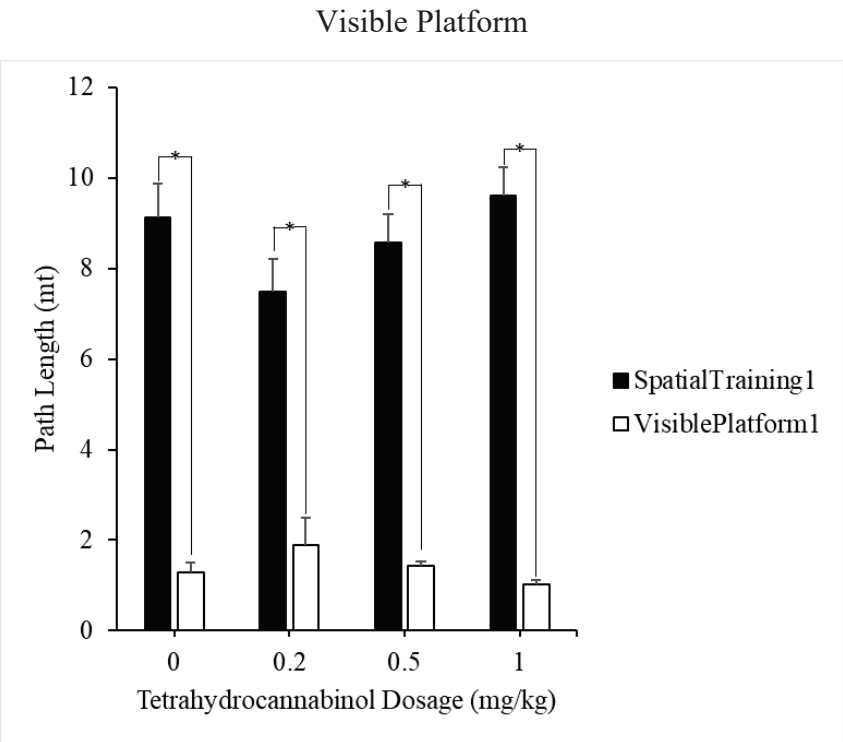
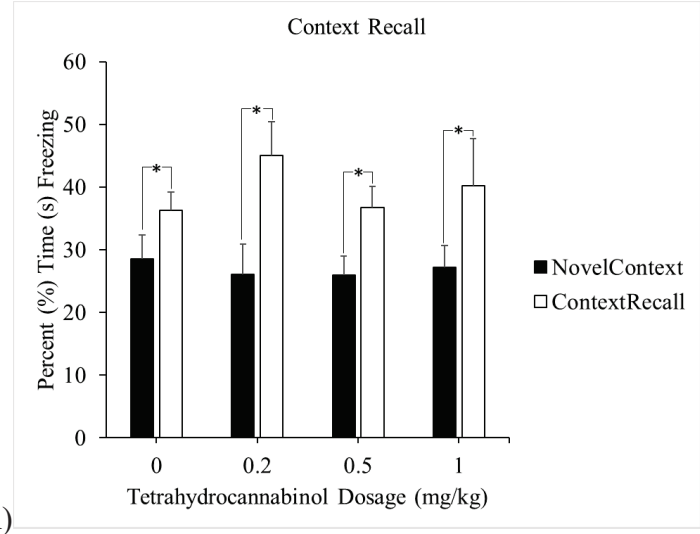
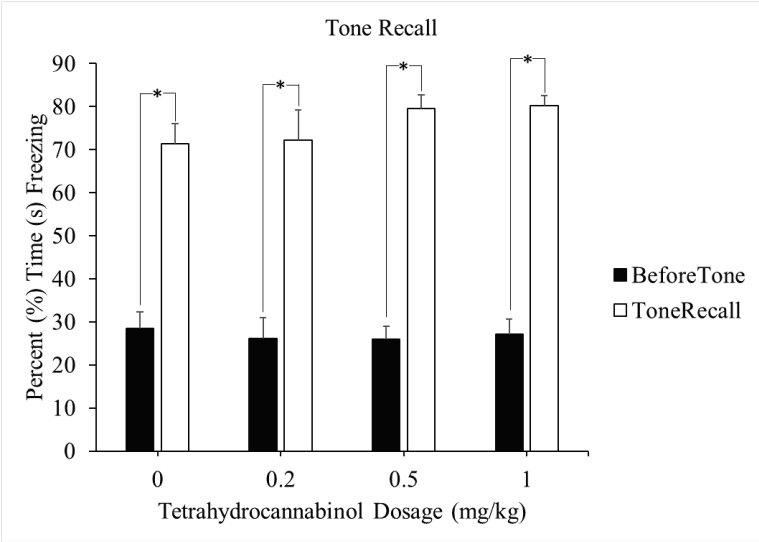


Figure 15. The path length of the APP^{NL-G-F} mice treated with a 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC, during spatial training day one relative to their path length during the visible platform test. All the mice demonstrate a reduced path length when the platform was visible. There are no observable differences depending on the given dosage of THC. Therefore, the mice do not demonstrate sensory, motor, or motivational impairments. There was a significant difference in path length when the platform was hidden relative to when the platform was visible ($p < 0.001$). There was no significant effect of THC dosage. A "*" indicates significance.

Fear Conditioning



(A)



(B)

Figure 16. The context recall and tone recall data following fear conditioning for the $APP^{NL\text{-}G\text{-}F}$ mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) A comparison of the percentage of time that the mice spent freezing in the novel context relative to during context recall. Overall, all the mice spent more time freezing in the conditioning context, suggesting that they recalled the context/shock pairing independent of THC dosage. There was a significant difference in percent time freezing ($p < 0.001$). There was no significant effect of THC dosage. (B) The percentage of time the mice spent freezing prior to the tone relative to during tone recall. Overall, the mice spent more time freezing during the tone suggesting that they recalled the tone/shock pairing independent of THC dosage. There was a significant difference in the percent time freezing ($p < 0.001$). There was no significant effect of THC dosage. A "*" indicates significance.

Nissl

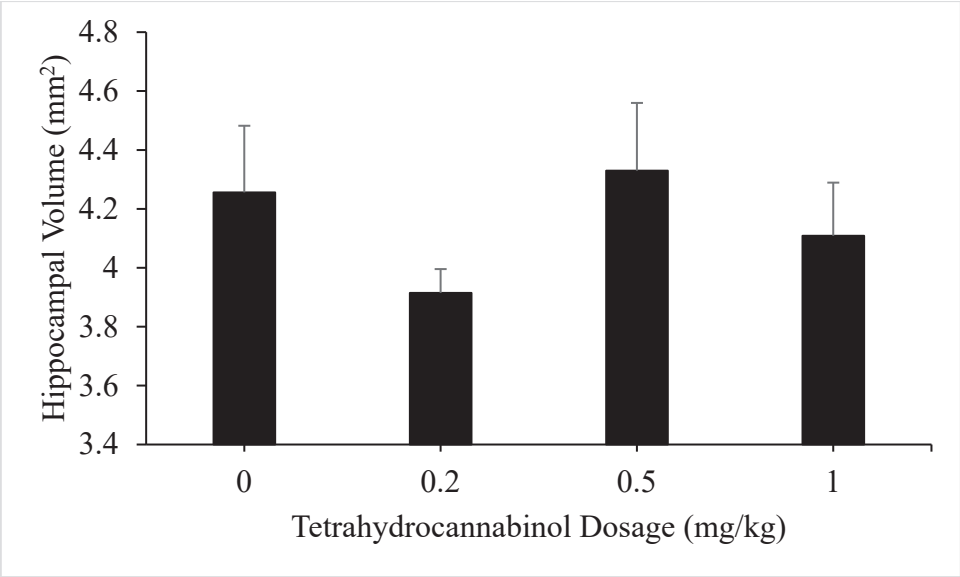
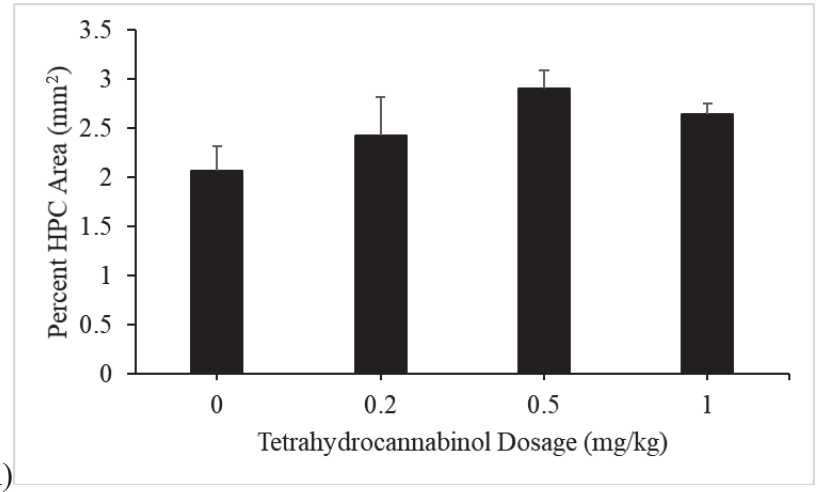
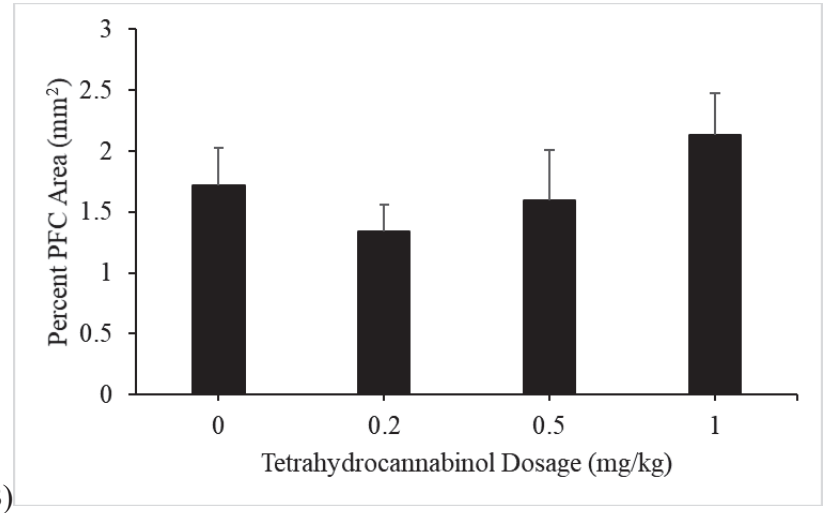


Figure 17. The HPC volume of the 0mg/kg, 0.2mg/kg, 0.5mg/kg, and 1.0mg/kg THC treated $APP^{NL\text{-}G\text{-}F}$ mice. The results suggest that there was no significant difference in the HPC volumes of the mice depending on the given dosage of THC around 7 months of age.

Aβ1-16



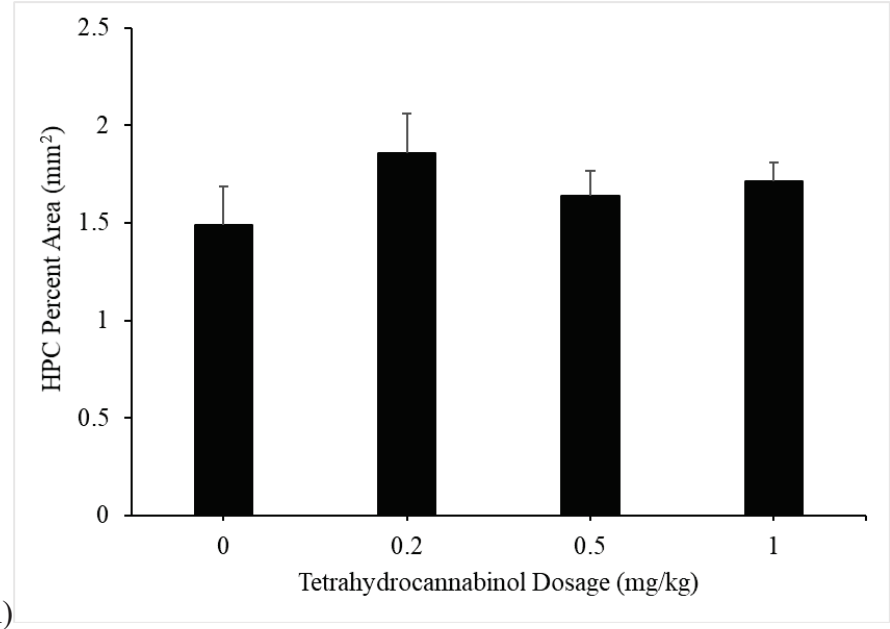
(A)



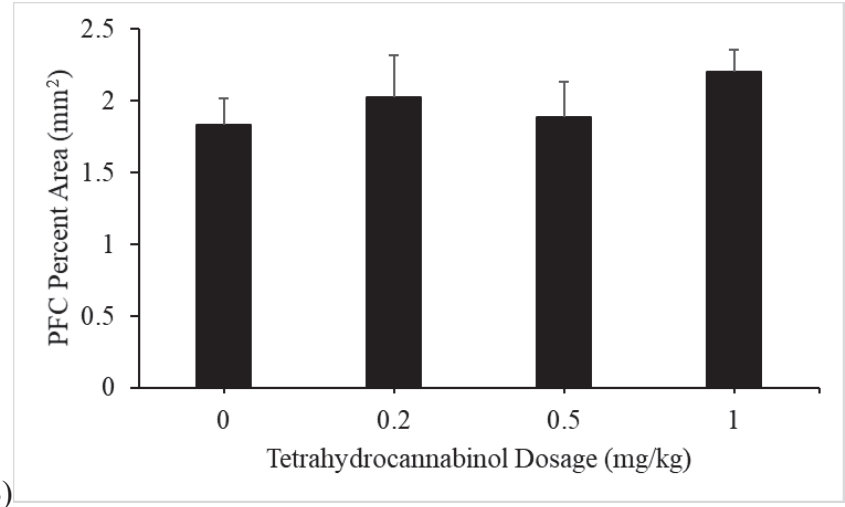
(B)

Figure 18. The percentage of Aβ1-16 (stained with 82E1) in the HPC and PFC of the APP^{NL-G-F} mice treated with a 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) The percentage of Aβ1-16 in the HPC depending on the dosage of THC. There was no significant effect of THC dosage on the percentage of Aβ1-16 in the HPC. (B) The percentage of Aβ1-16 in the PFC between groups. There was no significant effect of THC dosage on the percentage of Aβ1-16 in the PFC. Overall, various dosages of THC did not significantly affect the quantity of Aβ1-16 in the HPC and PFC of APP^{NL-G-F} mice around 7 months of age.

Aβ7-24



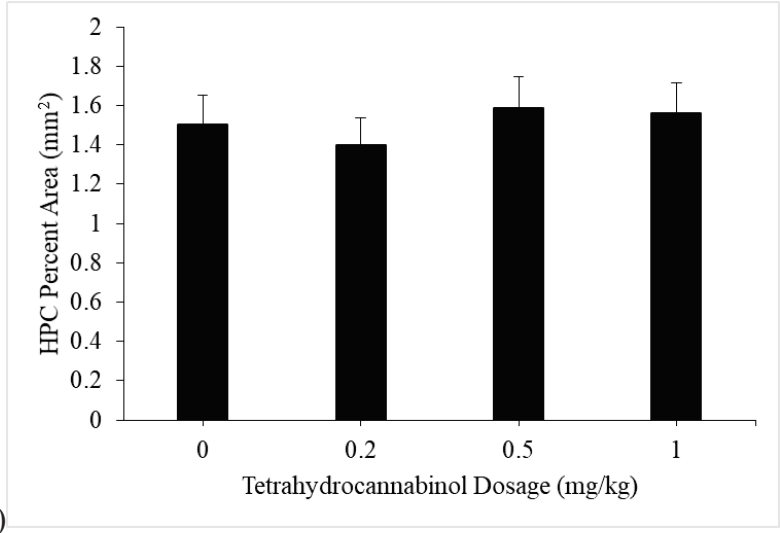
(A)



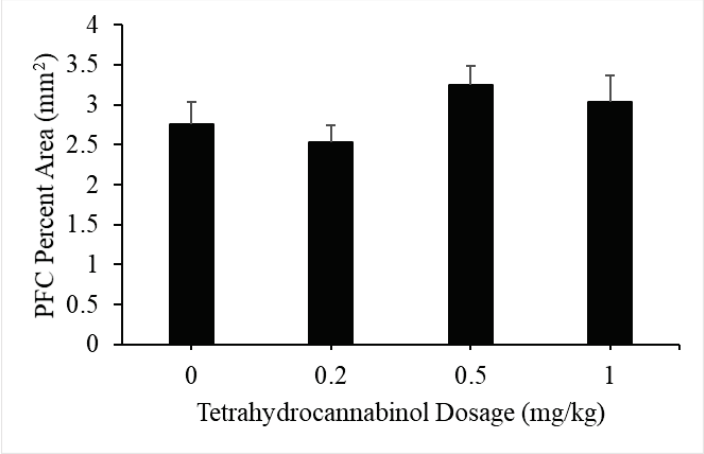
(B)

Figure 19. The percentage of Aβ7-24 (stained with 4G8) in the HPC and PFC of APP^{NL-G-F} mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) The percentage of Aβ7-24 in the HPC between groups. There was no significant effect of THC dosage on the percentage of Aβ7-24 in the HPC. (B) The percentage of Aβ7-24 in the PFC. There was no significant effect of THC dosage on the percentage of Aβ7-24 in the PFC. Overall, various dosages of THC did not significantly affect the quantity of Aβ7-24 in the HPC and PFC of APP^{NL-G-F} mice around 7 months of age.

Iba1



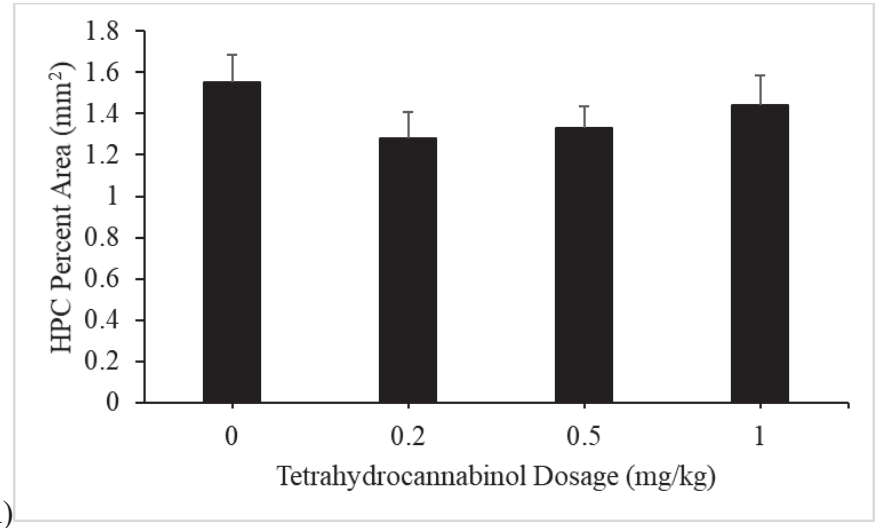
(A)



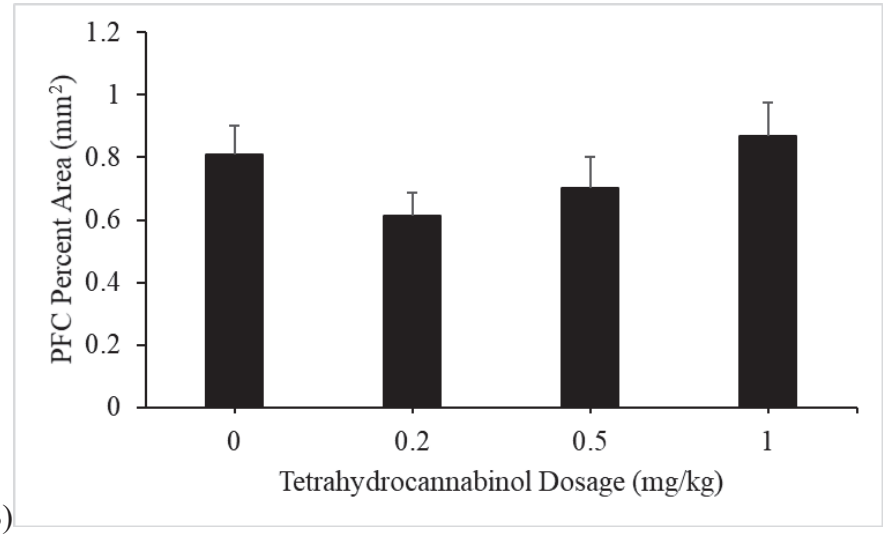
(B)

Figure 20. The percentage of Iba1 in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) The percentage of Iba1 in the HPC depending on the given dosage of THC. There was no significant effect of THC dosage on the percentage of Iba1 in the HPC. (B) The percentage of Iba1 in the PFC depending on the given dosage of THC. There was no significant effect of THC dosage on the percentage of Iba1 in the PFC. Overall, various dosages of THC did not significantly affect the quantity of Iba1 in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

GFAP



(A)



(B)

Figure 21. The percentage of GFAP in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice treated with 0mg/kg, 0.2mg/kg, 0.5mg/kg, or 1.0mg/kg of THC. (A) The percentage of GFAP in the HPC between groups. There was no significant effect of THC dosage on the percentage of GFAP in the HPC. (B) The percentage of GFAP in the PFC depending on the given dosage of THC. There was no significant effect of THC dosage on the percentage of GFAP in the PFC. Overall, various dosages of THC did not significantly affect the quantity of GFAP in the HPC and PFC of $APP^{NL\text{-}G\text{-}F}$ mice around 7 months of age.

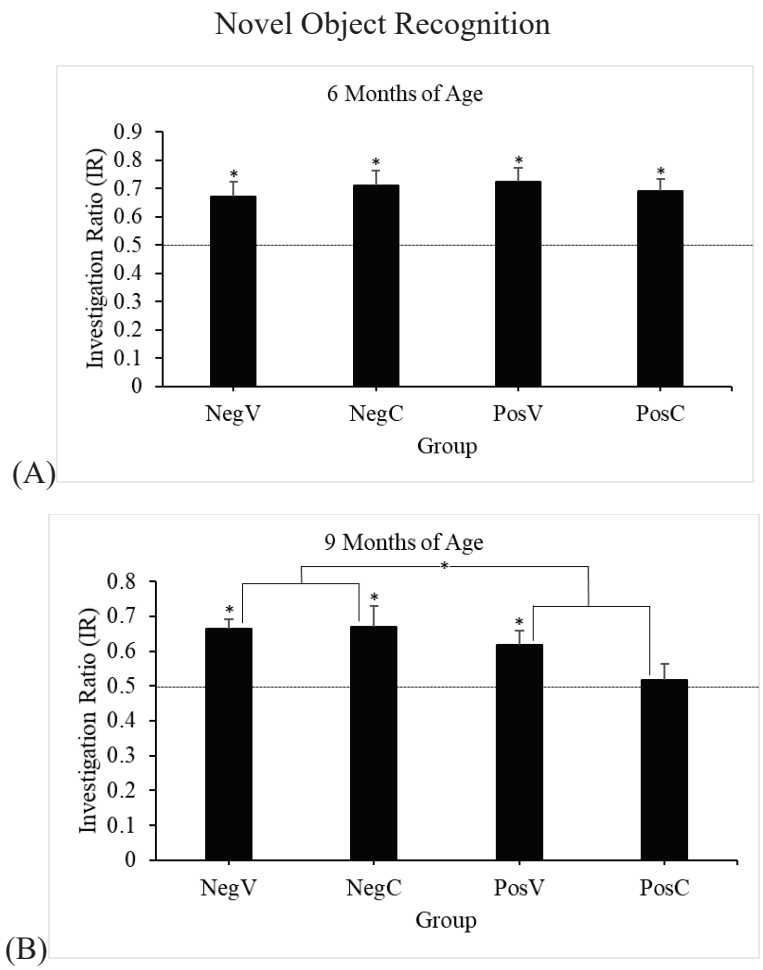


Figure 22. The average IR for the novel object during NOR for noncarrier littermates and Tau P301S mice treated with a vehicle or 20mg/kg of CBD. (A) The NOR data when the mice were 6 months of age. Overall, each group had an IR value significantly above 0.5, indicating that they explored the novel object above chance relative to the familiar object (noncarrier-vehicles ($p < 0.006$), noncarrier-CBD ($p < 0.003$), Tau P301S-vehicles ($p < 0.001$), and Tau P301S-CBD ($p < 0.001$)). There was no statistically significant effect of genotype or treatment on the object recognition of the mice. (B) The NOR data when the mice were 9 months of age. The noncarrier-vehicles ($p < 0.001$), noncarrier-CBD ($p < 0.031$), and Tau P301S-vehicles ($p < 0.014$) explored the novel object significantly above chance, but the Tau P301S-CBD mice did not. Additionally, between subjects there was a significant effect of genotype ($p < 0.034$), but there was no significant effect of treatment. Overall, at 9 months of age the object recognition of the Tau P301S mice was impaired relative to the noncarrier littermates. The Tau P301S mice treated with CBD were the only group that did not explore the novel object above chance. A "*" indicates significance.

Balance Beam

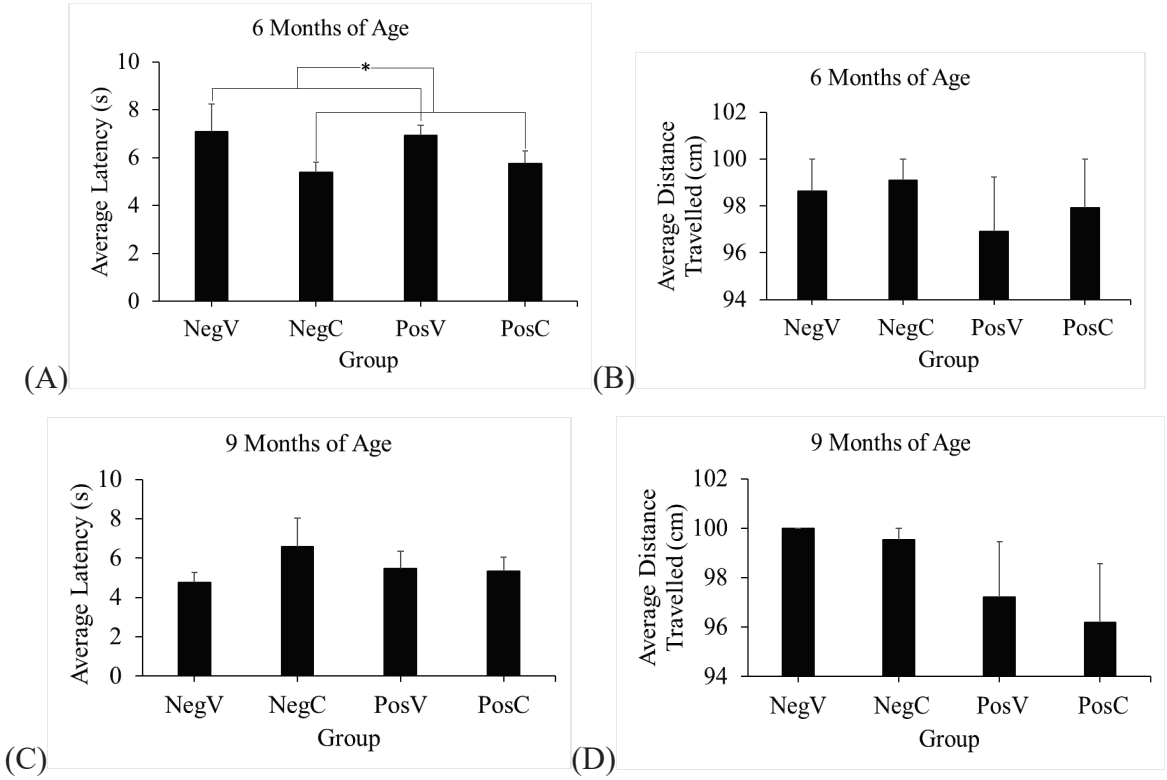
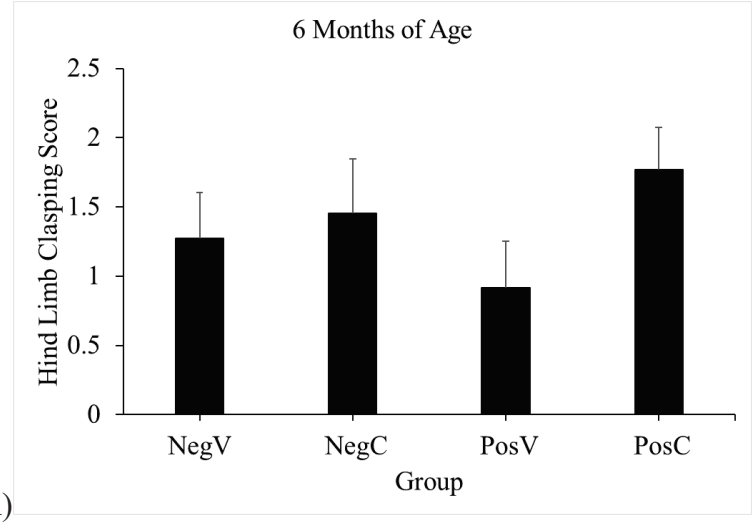
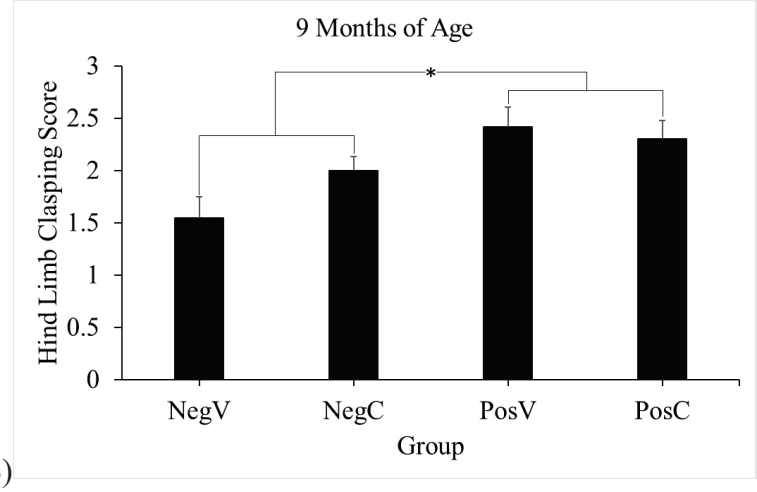


Figure 23. The balance beam data collected from the Tau P301S mice and noncarrier littermates treated with a vehicle or 20mg/kg of CBD. (A) The average latency of the mice to traverse the beam during successful trials at 6 months of age. The are no clear differences depending on genotype. The CBD mice appear to have a shorter latency relative to the vehicle mice. There was no significant effect of genotype. There was a significant effect of treatment ($p < 0.047$). (B) The average distance travelled across all three trials when the mice were 6 months of age. The average distance travelled was similar, regardless of genotype and treatment. There was no significant effect of genotype or treatment. (C) The average latency of the mice to traverse the beam during successful trials at 9 months of age. All the mice performed similarly, there was no significant effect of genotype or treatment. (D) The average distance travelled across the beam during all three trials at 9 months of age. The Tau P301S mice appear to have a shorter distance travelled relative to the noncarrier littermates. However, there was no significant effect of genotype or treatment. Overall, the motor coordination of the Tau P301S mice was not impaired relative to the noncarrier littermates at 6 or 9 months of age. CBD might be promoting the motor coordination of the Tau P301S mice and noncarrier littermates at 6, but not 9 months of age. A "*" indicates significance.

Tail Suspension



(A)



(B)

Figure 24. The hind limb clasping score of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle. (A) The hind limb clasping score of the mice at 6 months of age. At 6 months of age the hindlimb clasping of the mice was similar regardless of genotype and treatment. The observed results were supported by a nonsignificant effect of genotype and treatment. (B) The hind limb clasping score of the mice at 9 months of age. The clasping score of the Tau P301S mice was higher relative to the noncarrier littermates, suggestive of hind limb weakness. There was a significant effect of genotype ($p < 0.002$). CBD did not impact the clasping of the mice, evidenced by a nonsignificant effect of treatment. Overall, at 9, but not 6 months of age the Tau P301S mice began to demonstrate hindlimb weakness. A "*" indicates significance.

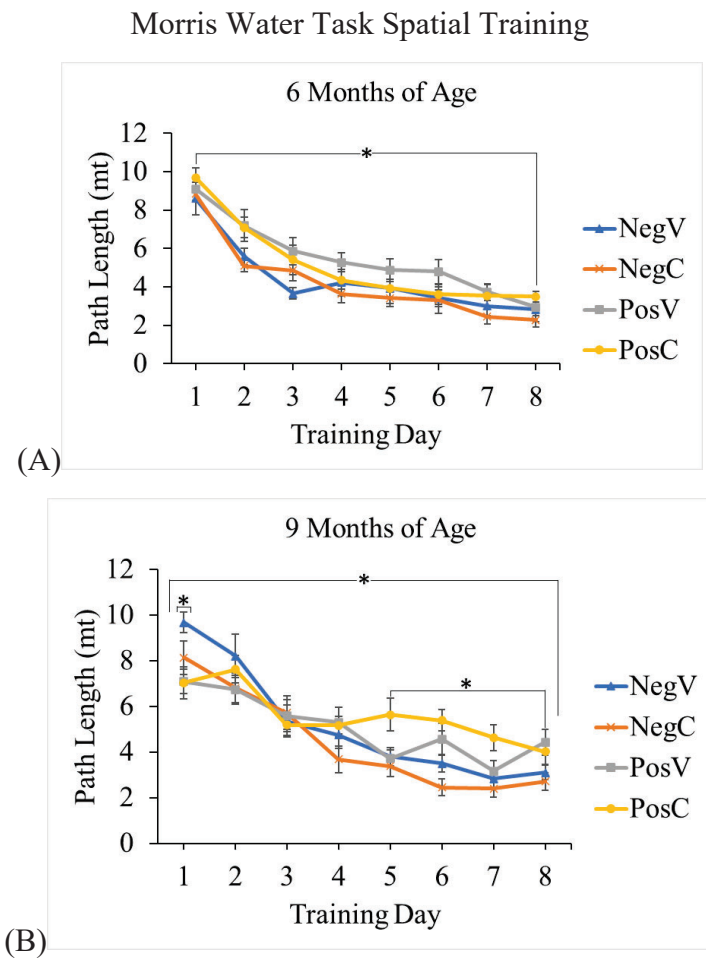


Figure 25. The path length of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle during MWT spatial training. (A) The path length of the mice from training day 1 to training day 8, at 6 months of age. The mice were able to learn the location of the platform, evidenced by a significant effect of training day ($p < 0.001$). There was no significant effect of genotype or treatment on the path length of the mice. Therefore, the Tau P301S mice retained spatial learning at 6 months of age and CBD did not impact the learning of the mice. (B) The path length of the mice from spatial training day 1 to spatial training day 8. All the mice were able to learn the location of the hidden platform as evidenced by a significant effect of day ($p < 0.001$). The Tau P301S mice were impaired compared to the noncarrier littermates as evidenced by a significant effect of genotype on the path length ($p < 0.001$). A pairwise comparison with Bonferroni corrections revealed that the path length of the Tau P301S mice and noncarrier littermates significantly differed on day 1 ($p < 0.004$), day 5 ($p < 0.034$), day 6 ($p < 0.000$), day 7 ($p < 0.011$), and day 8 ($p < 0.006$). CBD did not restore the spatial learning of the mice as there was no significant effect of treatment. Overall, the spatial learning of the Tau P301S mice began to decline at 9 months of age. A "*" indicates significance.

Probe

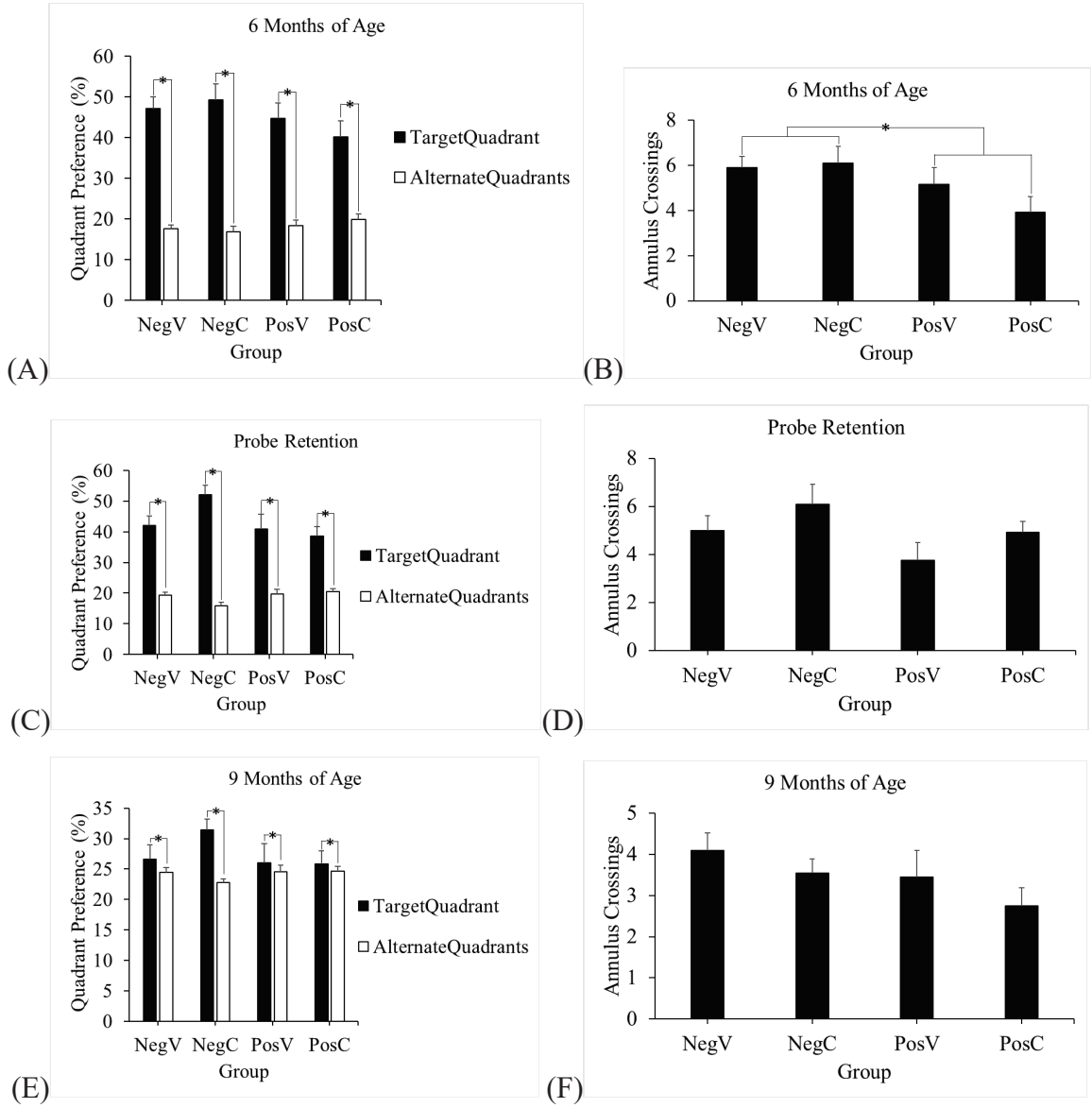
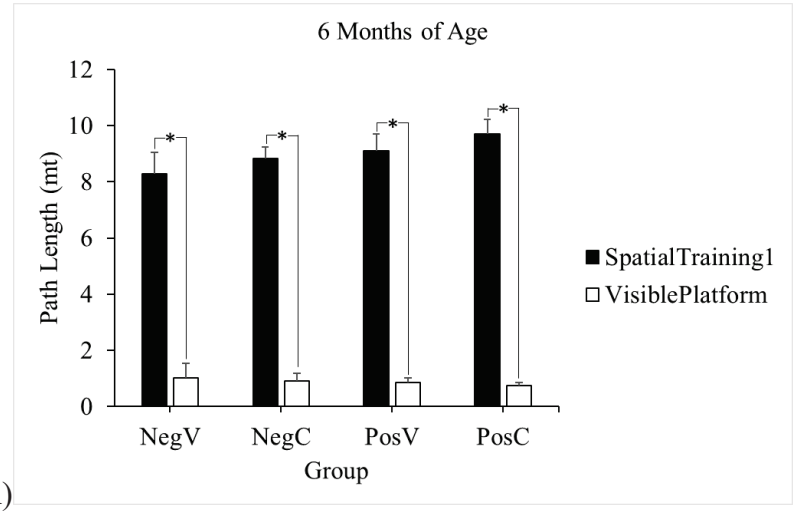


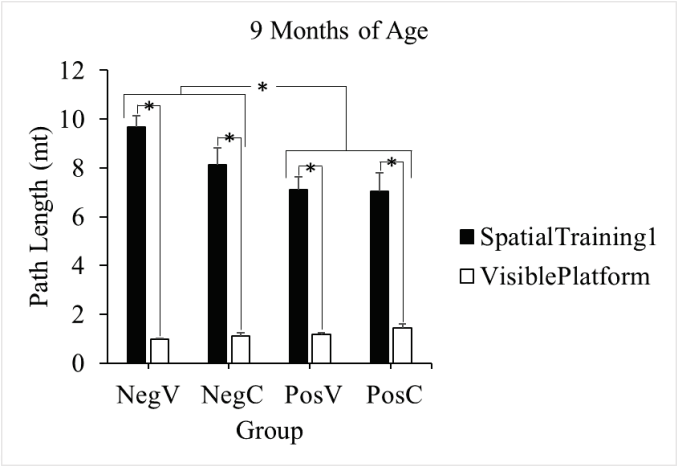
Figure 26. The probe data of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or vehicle. (A) The quadrant preference of the mice at 6 months of age during the probe trial performed 24 hours after training. There are no observable differences on the quadrant preference of the mice depending on genotype and treatment. The percentage of time spent in the target quadrant relative to the alternate quadrants was significantly increased ($p < 0.001$). There was no significant effect of genotype or treatment on quadrant preference. (B) The number of annulus crossings during the probe trial performed 24 hours after spatial training at 6 months of age. The number of annulus crossings for the Tau P301S mice was lower relative to the noncarrier littermates. CBD did not restore the spatial recall of the mice. There was a significant effect of genotype ($p < 0.039$). There was no significant effect of treatment. (C) The data collected during the probe retention, which was performed 1 week after training at 6 months of age. All the mice recalled the previous platform location, as evidenced by a significant effect of

quadrant ($p < 0.001$). There was a strong trend towards an effect of genotype on the quadrant preference of the mice ($p < 0.051$). There was no significant effect of treatment. (D) The number of annulus crossings during the probe retention at 6 months of age, performed one week after spatial training. Overall, the performance of the mice was similar. There was no significant effect of genotype or treatment. (E) The quadrant preference during the probe trial when the mice were 9 months. The noncarrier littermates treated with CBD demonstrate the strongest quadrant preference. The performance of the noncarrier littermates-vehicle, Tau P301S-vehicle, and Tau P301S-CBD mice was similar. There was a significant effect of quadrant preference ($p < 0.043$). There was no significant effects of genotype or treatment on quadrant preference. (F) The number of annulus crossings during the probe trial at 9 months of age depending on genotype and treatment. There was no significant effect of genotype or treatment. Ultimately the probe data suggests that the spatial recall of the mice began to decline at 6 months of age. While there were no effects of genotype at 9 months, the overall performance of each group of mice appears worse than their performance at 6 months of age. A "*" indicates significance.

Visible Platform



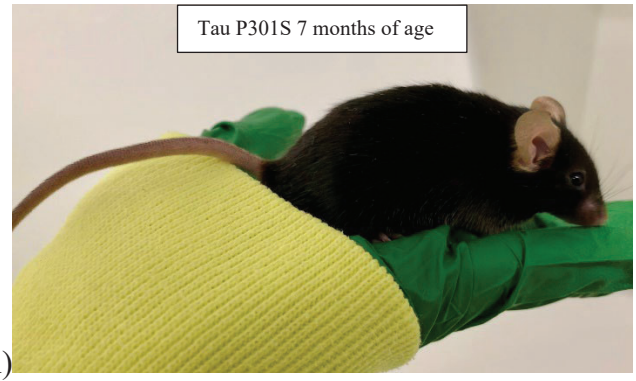
(A)



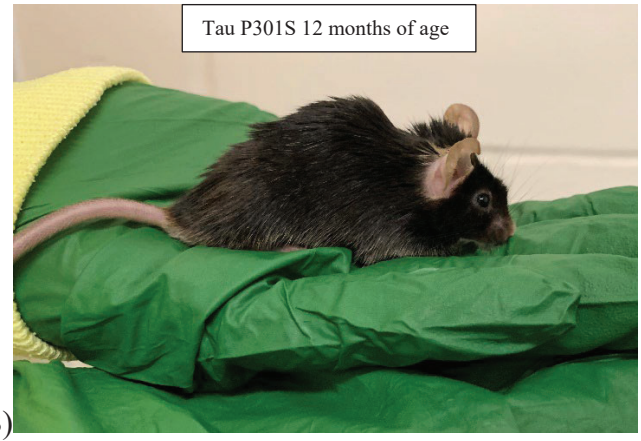
(B)

Figure 27. A comparison of the path length of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or vehicle during spatial training day 1 relative to the visible platform test. (A) The path length of the mice during spatial training day 1 and the visible platform test at 6 months of age. All the mice demonstrate a decreased path length when the platform was visible, ($p < 0.001$). There was no significant effects of genotype or treatment. (B) The path length of the mice during training day 1 and during the visible platform test at 9 months of age. All the mice demonstrate a decreased path length during the visible platform test ($p < 0.001$). However, there was a significant effect of genotype on the performance of the mice ($p < 0.003$). There was no significant effect of treatment on the performance of the mice. Overall, the motor, motivational and/or sensory behaviour of the Tau P301S mice might be declining at 9, but not 6 months of age. A "*" indicates significance.

Posture of Tau P301S mice



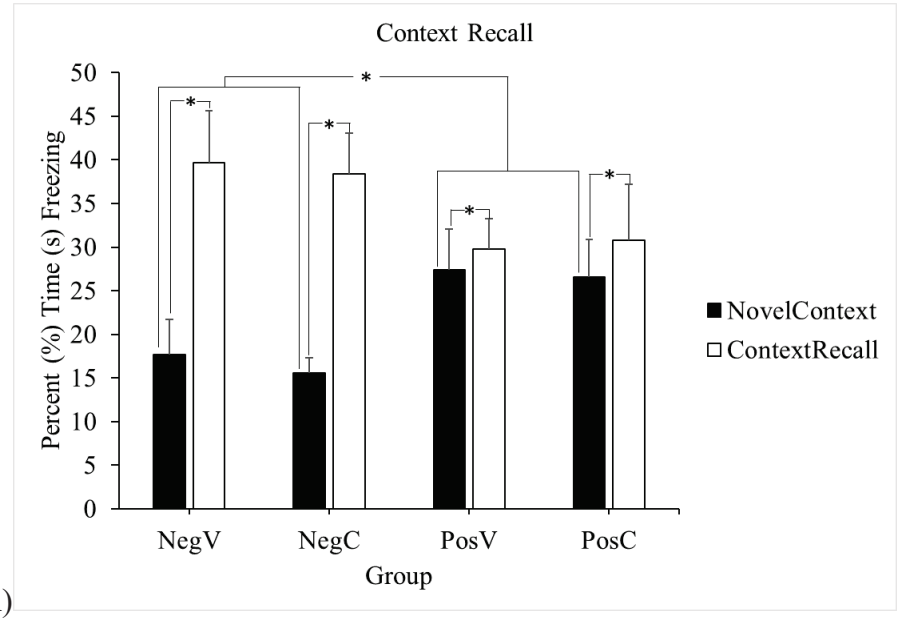
(A)



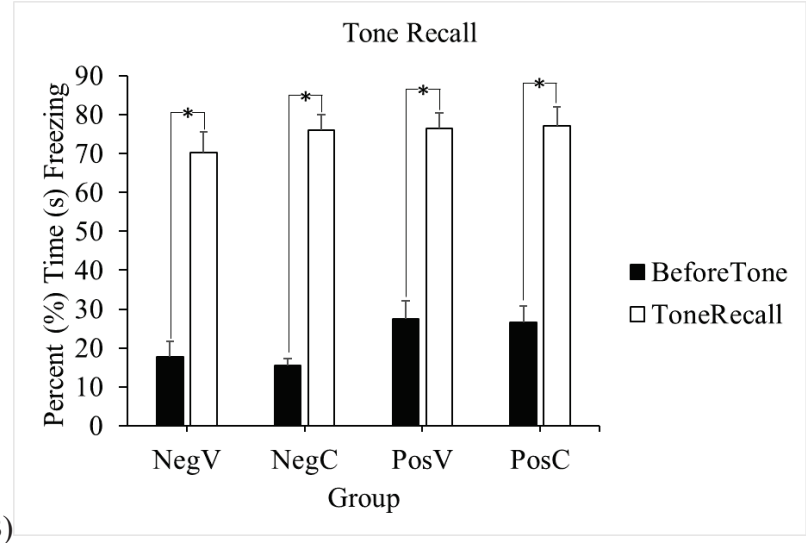
(B)

Figure 28. A demonstration of the posture of the Tau P301S mice as they age. (A) A 7-month-old male Tau P301S mouse that demonstrates no alterations in their posture. There is no observable atrophy or hunch at this age. (B) A 12-month-old male Tau P301S mouse that demonstrates atrophy and a hunched back. This posture was observed in the male Tau P301S mice in the present experiment around 10 months of age. The hunched posture is associated with motor impairments and hind limb weakness, followed by paralysis.

Fear Conditioning



(A)

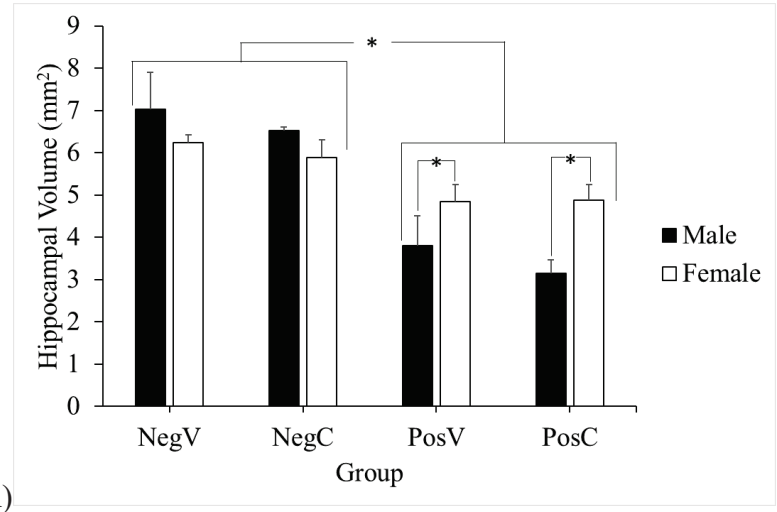


(B)

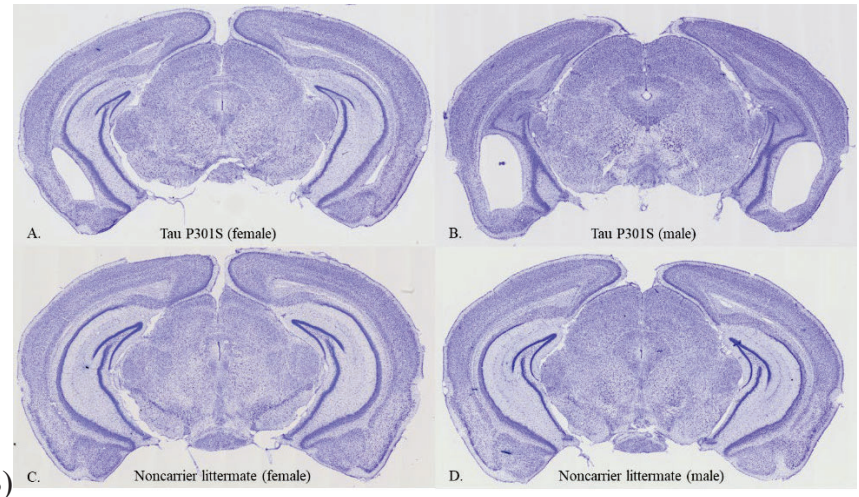
Figure 29. The fear conditioning data of the Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle. (A) A comparison of the freezing response of the mice during the novel context relative to during context recall. While the context recall of the mice appears similar, the Tau P301S mice have an increased freezing response in the novel context. As such there is a less pronounced difference in the freezing of the Tau P301S mice in the conditioning context relative to in the novel context. CBD does not appear to impact the context recall of the mice. There was a significant increase in the freezing behaviour of all the mice during context recall compared to in the novel context ($p < 0.001$). There was a significant effect of genotype on the freezing response of the mice ($p < 0.002$). A pairwise comparison with Bonferroni corrections suggests that the freezing response of the Tau P301S and noncarrier littermates only significantly differed in the novel context ($p < 0.012$) but not during context recall. There was no significant effect of

treatment on the freezing behaviour of the mice. Overall, the Tau P301S were able to recall the conditioning context. However, the transgenic mice did demonstrate increased freezing in the novel context relative to the noncarrier littermates. CBD did not impact the context recall of the mice. (B) A comparison of the freezing response of the mice before the tone compared to during the tone. All the groups of mice demonstrate a higher percentage of freezing during the tone compared to before the tone. There was a significant difference in the freezing behaviour of the mice during the tone relative to before the tone ($p < 0.001$). There was no significant effect of genotype or treatment on the freezing response. Overall, the Tau P301S mice retained tone recall. CBD did not impact the tone recall of the mice. A "*" indicates significance.

Nissl

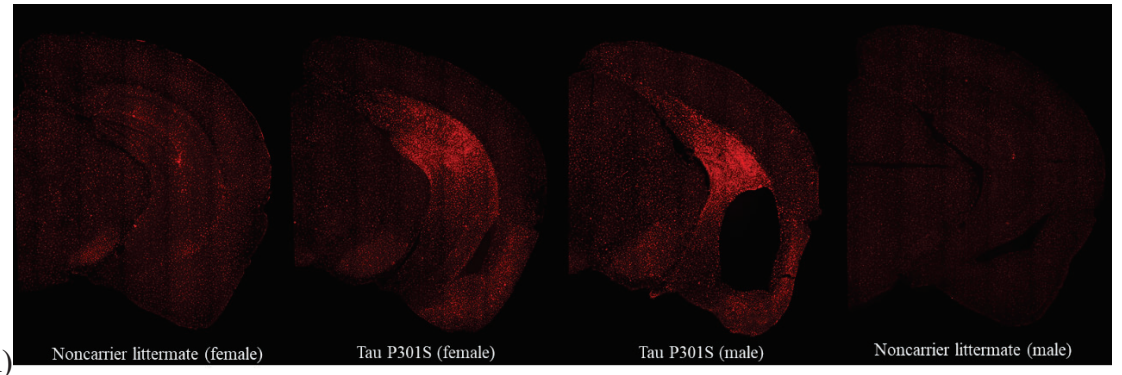


(A)



(B)

Figure 30. The HPC volume of Tau P301S and noncarrier littermates treated with 20mg/kg of CBD or vehicle. (A) The volumetric analysis of the HPC depending on genotype, treatment, and sex. Overall, the HPC of the noncarrier littermates was larger than the Tau P301S mice. The female Tau P301S mice have a larger HPC relative to the male Tau P301S mice. CBD does not appear to have any effect of the HPC volume of the mice. There was a significant effect of genotype ($p < 0.000$). There was no significant effect of treatment or sex. However, there was a significant interaction of genotype X sex ($p < 0.008$). A pairwise comparison with Bonferroni corrections revealed that the HPC volume of the female and male mice only significantly differed amongst Tau P301S mice ($p < 0.009$). Overall, the Tau P301S mice have a decreased HPC volume relative to the noncarrier littermates. The HPC volume loss was more severe amongst the male Tau P301S mice, compared to the female Tau P301S mice. (B) The Nissl-stained brains of the mice depending on genotype and sex. Image A demonstrates a Tau P301S female mouse, image B demonstrates a Tau P301S male mouse, image C demonstrates a noncarrier littermate female mouse, and image D demonstrates a male noncarrier littermate mouse. All the brains imaged were mice treated with CBD. A "*" indicates significance.



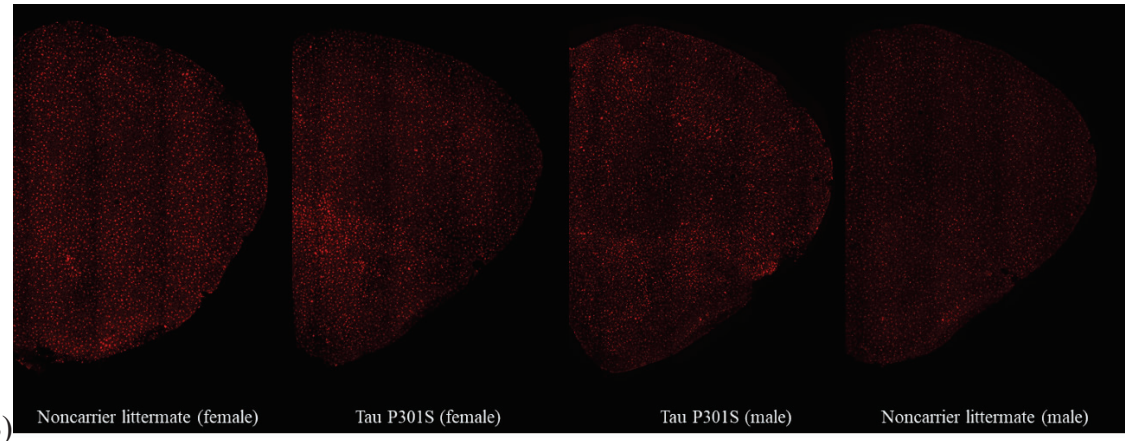
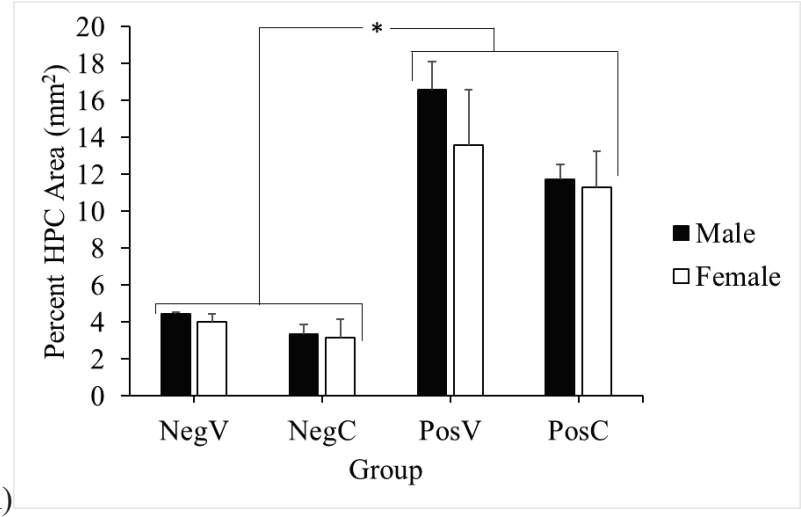
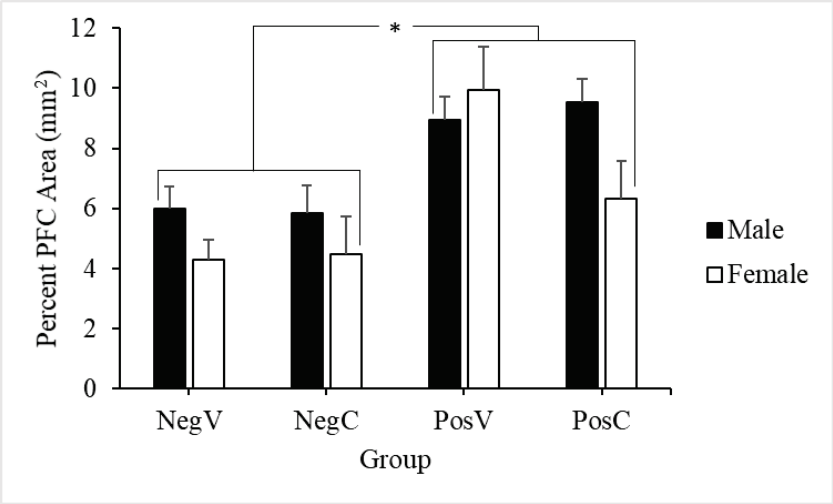


Figure 31. A representation of the staining and distribution of microglial, stained with Iba1, in the brains of the mice depending on genotype and sex. Each mouse in the given images was treated with CBD. All images were taken with a Nanozoomer microscope. (A) A representation of the staining of microglial in the HPC of the mice. From left to right demonstrates the staining in the HPC of a noncarrier littermate female, a Tau P301S female, a Tau P301S male, and a noncarrier littermate male. (B) A representation of the staining of microglial in the PFC of the mice. From left to right represents the staining in the PFC of a noncarrier littermate female, a Tau P301S female, a Tau P301S male, and a noncarrier littermate male.

Iba1



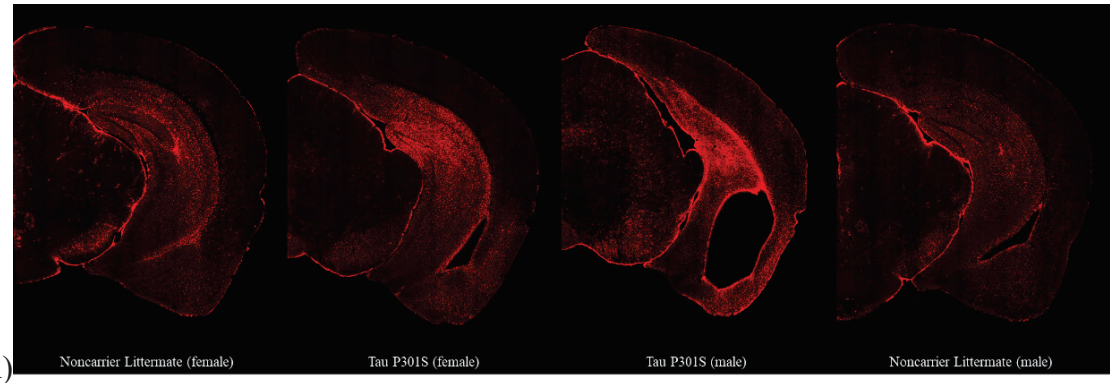
(A)



(B)

Figure 32. The percentage of Iba1 stained cells, a marker of microglial, in the HPC and PFC of the male and female Tau P301S mice and noncarrier littermates treated with 20mg/kg of CBD or a vehicle. (A) The percentage of Iba1 in the HPC of the mice. The Tau P301S mice have an overall higher percentage of Iba1 in the HPC. The CBD treated Tau P301S mice appear to have a slightly reduced quantity of Iba1 in the HPC than the vehicle treated Tau P301S mice. Additionally, it appears that the male mice of each group have more Iba1 relative to the females. There was a significant effect of genotype ($p < 0.001$). There was no significant effect of treatment or sex. (B) The percentage of Iba1 in the PFC of the mice. The Tau P301S mice have a higher percentage of Iba1 in the PFC relative to the noncarrier littermates. There is no consistent trend towards an effect of CBD or sex on the percentage of Iba1 in the PFC. There was an effect of genotype ($p < 0.001$). There was no significant effect of treatment. There was a marginally significant effect of sex ($p < 0.074$)). Overall, the Tau P301S mice have more Iba1 in the HPC and PFC. CBD and sex did not significantly impact the quantity of Iba1 in the HPC or PFC of the mice. A "*" indicates significance.

(A)



(B)

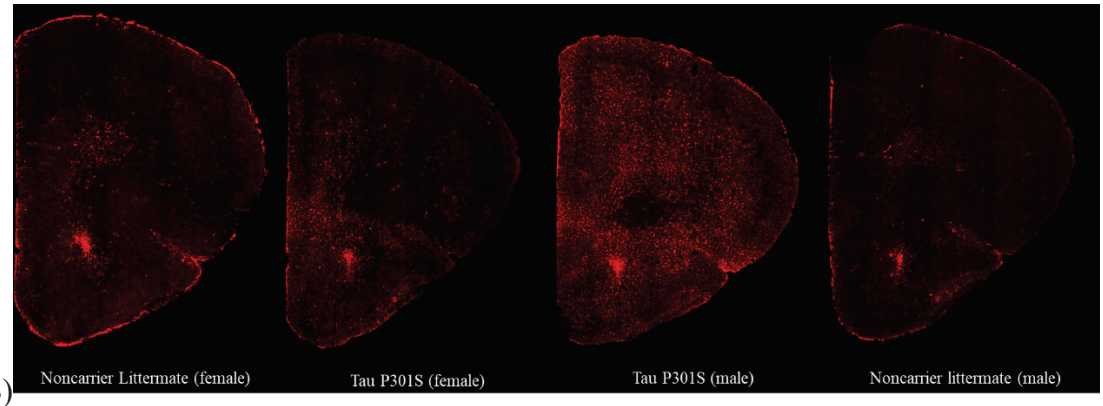
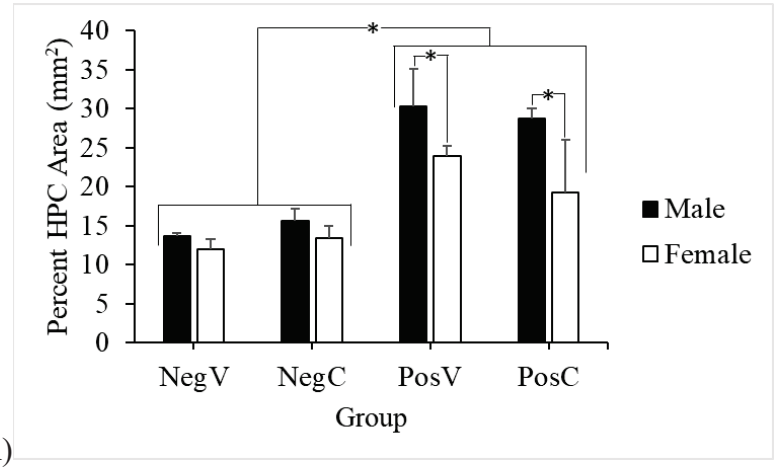
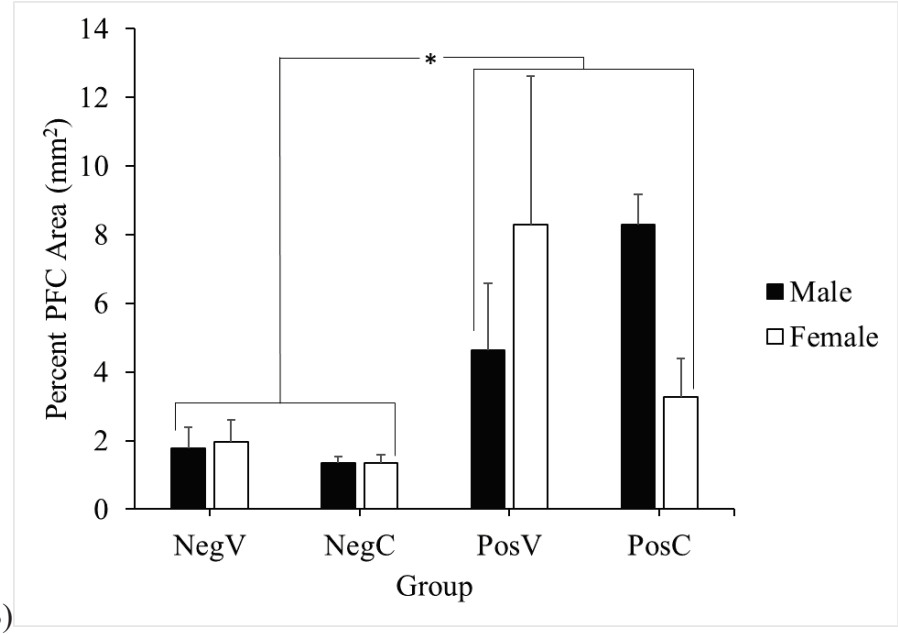


Figure 33. A representation of the staining and distribution of astrocytes, stained with GFAP, in the brains of the mice depending on genotype and sex. Each mouse in the given images was treated with CBD. All images were taken with a Nanozoomer microscope. (A) A representation of the staining of astrocytes in the HPC of the mice. From left to right demonstrates the staining in the HPC of a noncarrier littermate female, a Tau P301S female, a Tau P301S male, and a noncarrier littermate male. (B) A representation of the staining of astrocytes in the PFC of the mice. From left to right represents the staining in the PFC of a noncarrier littermate female, a Tau P301S female, a Tau P301S male, and a noncarrier littermate male.

GFAP



(A)



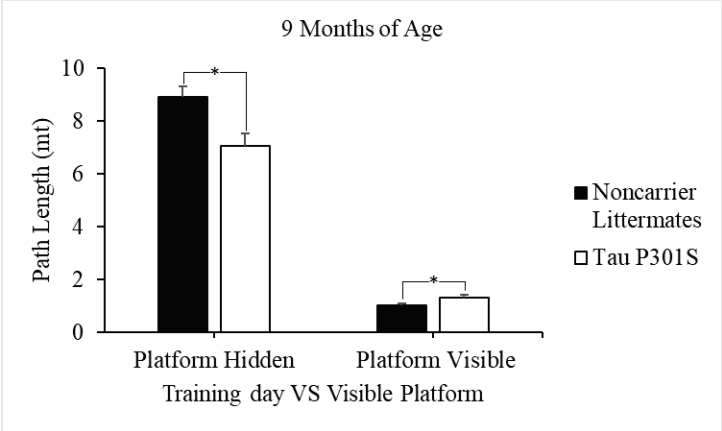
(B)

Figure 34. The percentage of GFAP in the HPC and PFC of the male and female Tau P301S mice and their noncarrier littermates treated with 20mg/kg of CBD or a vehicle. (A) The percentage of GFAP in the HPC of the mice. The Tau P301S mice have a higher percentage of GFAP in the HPC relative to the noncarrier littermates. There are no clear trends towards CBD impacting the percentage of GFAP in the HPC. Overall, the male mice in each group appear to have more GFAP in the HPC compared to the female mice. The results confirmed that there was a significant effect of genotype ($p < 0.001$)). There was no significant effect of treatment. There was a significant effect of sex ($p < 0.042$)). *A priori* assumptions were made that the effect of sex was only applicable to the Tau P301S mice. A pairwise comparison with Bonferroni corrections assessed this observation and revealed that there was only a significant effect of sex in the Tau P301S mice ($p < 0.018$) there was no significant effect of sex between the noncarrier littermates. (B) The percentage of GFAP in the PFC of the mice. The Tau P301S mice have more GFAP in the PFC compared to the noncarrier littermates. There are no consistent observations on a treatment or sex effect on

the percentage of GFAP in the PFC. The results confirmed that there was a significant effect of genotype ($p < 0.002$). There was no significant effect of treatment or sex. Ultimately the Tau P301S mice have a higher percentage of GFAP in the HPC and PFC relative to the noncarrier littermates. The male P301S mice have particularly more GFAP in the HPC, but not PFC compared to the female P301S mice. CBD did not delay the progression of GFAP in the HPC or PFC of the Tau P301S mice. A "*" indicates significance.

Supplemental Figures

Visible Platform



Supplemental Figure 1. A comparison of the path length of the 9-month-old noncarrier littermates and Tau P301S mice independent of treatment during training day 1, when the platform was hidden, and the during the visible platform test, when the platform was visible. There was a significant effect of genotype on the path length of the mice ($p < 0.003$). A pairwise comparison with Bonferroni corrections revealed a significant effect of genotype on training day 1 ($p < 0.005$) and during the visible platform test ($p < 0.010$). A "*" indicates significance.