SINGLE AND COMBINED EFFECTS OF CANNABINOIDS ON NEUROPATHIC PAIN AND COGNITION

A Thesis
Submitted to
the Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE

---

by
Alyssa M. Myers
May 2016

Examining Committee Members:

Sara Jane Ward, PhD, Thesis Advisor, Center for Substance Abuse Research
Dianne Langford, PhD, Department of Neuroscience
Ronald Tuma, PhD, Center for Substance Abuse Research
Lynn Kirby, PhD, Center for Substance Abuse Research

ABSTRACT

Rationale.

For centuries, medications derived from the marijuana plant have been used for therapeutic purposes across numerous cultures. In 1964, the primary psychoactive ingredient in cannabis, delta-9-tetrahydrocannabinol (Δ-9-THC) was defined. This, followed by the discovery of the endocannabinoid system, marked the beginning of comprehensive research into the beneficial exploitation of this system.

The cannabis plant contains various other cannabinoids besides Δ-9-THC. Most of the effects of cannabinoid-based therapies are based on the agonistic action of Δ-9-THC through cannabinoid receptors. Alternatively, some of these effects are caused by the actions of other cannabinoids, like cannabidiol. Cannabidiol (CBD), the non-psychoactive phytocannabinoid in *Cannabis sativa*, has been hypothesized to ameliorate adverse effects of Δ-9-THC. Cannabidiol possesses neuroprotective, antiemetic, and anti-inflammatory properties. Sativex, a 1:1 ratio of CBD and Δ-9-THC, is currently an approved medication in Europe for the treatment of conditions such as neuropathic pain, and has been fast tracked by the USFDA for late stage clinical trials for a host of disorders, ranging from epilepsy to irritable bowel disease. Additionally, increasing preclinical evidence demonstrates that treatment with Cannabidiol alone produces efficacy on a variety of nervous system injuries, including neuropathic pain, schizophrenia and anxiety disorders.

Furthermore, there is mounting evidence of an "entourage effect" in cannabinoid-based pharmacotherapies. This effect occurs when treatment with a combination of cannabinoids derived from the plant produce more efficacy than treatment with a single cannabinoid (1). As cannabinoid-based treatments continue to develop and clinical data increases, further investigation of the entourage effect is necessary to facilitate the appropriate future treatment regimens for nervous system disorders.

Hypotheses.

We **hypothesized** that treatment with the non-psychoactive cannabis compound cannabidiol would be as effective as the psychoactive cannabis compound Δ-9-THC, or a combination of the two, in mitigating neuropathic pain in a mouse model of chemotherapy-induced peripheral neuropathy. We additionally **hypothesized** that cannabidiol would not affect classic cannabinoid-agonist induced cognitive impairment in rodent models of learning and memory.

Methodology.

Neuropathic pain was induced by repeated injections of the chemotherapeutic agent Paclitaxel. Mechanical hypersensitivity to Paclitaxel was assessed using the Von Frey assay. Cognition was assessed using three rodent models of learning and memory: 1) Conditional Discrimination, 2) Conditional Discrimination with a reversal component, and 3) Barnes Maze.

Results.

Cannabidiol was found to be more potent and more effective than Δ-9-THC in attenuating neuropathic pain in a dose dependent manner. Combinations of CBD+Δ-9-THC revealed that lower, ineffective doses of CBD and Δ-9-THC display supra-additive effects when given in combination while higher, individually effective doses exhibit sub-additive effects in combination.

Cognitively, no deficits were observed over a range of doses of any cannabinoid tested in the conditional discrimination tasks, although a slight trend was observed in animals administered the synthetic mixed CB1/CB2 agonist WIN55,212-2. In the Barnes Maze task, treatment with Δ-9-THC alone and in combination with cannabidiol dose-dependently decreased number of entries and total time spent in the target zone. Cannabidiol did not produce any effects in the Barnes Maze alone, nor did it attenuate the effects seen in animals treated with Δ-9-THC alone. Lastly, Δ-9-THC did not affect total distance traveled or average speed, whereas combination treatment increased both locomotor measurements at all but the highest combination dose.

Conclusions.

The results of these studies indicate that cannabidiol is more potent than Δ-9-THC in attenuating neuropathic pain. Results of cognitive testing indicate subtle impairment in animals treated with Δ-9-THC and WIN55,212-2 that were not reversed by CBD.

This thesis is dedicated to my family for their

unconditional love and support throughout this academic journey

ACKNOWLEDGMENTS

"What you *get* by achieving your goals is not as important as what you *become* by achieving your goals" –Henry David Thoreau

I cannot express enough gratitude and thanks to my committee for their continued support and encouragement: Dr. Sara Jane Ward, my mentor, Dr. Dianne Langford and Dr. Ron Tuma. My sincerest appreciation for the opportunities and experiences each of you have provided me.

The completion of this thesis could not have been accomplished without the support of my parents, friends, and lab mates. To my parents, Donna and Edward Myers, thank you for always pushing me to achieve more than I thought I was capable of achieving. Your continued support throughout this journey means more to me than you could ever know.

I would also like to thank Ellen Walker: finding the words to express how much of an impact you've had on the development of my scientific career is not easy. The support, guidance, and experiences that you've given to me are not only unforgettable, but are what made me love becoming a scientist.

To Sara Jane Ward, my mentor: my most sincere thanks go to you. You've introduced me to the wonders and frustrations of cannabinoid research, and I want to thank you for your continuous guidance, friendship, support and encouragement over the last two years.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
cAMP	Cyclic adenosine monophosphate
CB1r	Cannabinoid 1 receptor
CB2r	Cannabinoid 2 receptor
CB	Cannabinoid
CBD	Cannabidiol
CBD+Δ-9-THC	Cannabidiol + Δ-9-THC
CIPN	Chemotherapy induced peripheral neuropathy
DAGL	Diacylglycerol lipase
Δ-9-THC	Delta-9-Tetrahydrocannabinol
Eadd	Predicted additive effect levels
ECS	Endocannabinoid system
ED50	Effective Dose which achieves 50% effect level
Eobs	Observed combination effect levels
FAAH	Fatty acid amide hydrolase
FR1	Fixed-ratio 1

GABA	Gamma-aminobutyric acid
GLU	Glutamate
GPCR	G-protein coupled receptor
%MPE	Percent maximal possible effect
MAGL	Monoacylglycerol lipase
PAC	Paclitaxel
TRPV	Transient receptor potential vanilloid
TRPV1	Transient receptor potential vanilloid-1

CHAPTER 1

INTRODUCTION

History of Cannabis

Marijuana is the most widely used illicit substance in western societies and additionally, the one with the longest recorded history of human use (2). Coveted by many cultures for its numerous therapeutic effects, such as reducing nausea and vomiting during chemotherapeutic treatment, appetite stimulation in HIV/AIDS patients, and treatment of chronic pain and spasticity associated with neurodegenerative diseases such as multiple sclerosis, marijuana seems to contain a natural healing ability.

The Cannabis sativa plant contains many bioactive compounds referred to as phytocannabinoids. The most commonly studied phytocannabinoids are delta-9-tetrahydrocannabinol (Δ-9-THC), the primary psychoactive compound in cannabis, and cannabidiol, a non-psychoactive compound hypothesized to ameliorate the adverse effects of Δ-9-THC.

In 1964, Dr. Raphael Mechoulam and colleagues isolated the psychoactive constituent of cannabis, delta-9-tetrahydrocannabinol. Δ-9-THC is the most prevalent cannabinoid in the marijuana plant. This phytocannabinoid is a partial agonist at cannabinoid 1 and 2 receptors, producing both therapeutic and psychoactive effects. The therapeutic potential of cannabinoids active at the CB1 receptor, however, is limited due to psychoactive effects mediated by the CB1 receptor. Alternatively, the

expression of CB2 receptors on immune cells offers an opportunity to regulate the function of the immune system via CB2 receptor ligands (3).

More specifically, the unfavorable effects associated with CB1 receptor activation include deficits in learning and memory, psychosis, alterations to brain morphology and connectivity, and poor psychosocial outcomes (4). Particularly, functional changes in areas of the brain with a high density of cannabinoid receptors, such as the hippocampus and striatum, are thought to mediate observed cognitive impairment in cannabis users (5, 6, 7).

Cannabidiol, the second most widely studied phytocannabinoid, is devoid of psychotropic effects and is not thought to act predominantly through either CB1 or CB2 receptors. There is significant interest in the exploitation of this compound due to the discovery of its anti-inflammatory, anti-oxidative, and neuroprotective effects that typically occur independently of CB1 and CB2 receptors (8). The actions of cannabidiol on immune cells have been reported to be concentration-dependent, some of which include inhibiting cell proliferation and maturation, immune cell migration, antigen presentation and humoral response (9,10).

Overall, cannabidiol seems to be a promising starting point for drug development given its antioxidant and anti-inflammatory actions on immune cells (8). Preclinical models of human disease support the notion that cannabidiol attenuates inflammation beyond its antioxidant properties (8), however, the details of this mechanism are not yet fully understood.

The neuroprotective effects of cannabidiol are also speculated to work through other receptor targets (which may be associated with neuronal or immune cell populations), including the 5HT1A receptor and TRPV1 vanilloid receptor. Currently, six classes of G-protein coupled serotonin receptors have been identified (11). Specifically, the 5HT1A serotonin receptor is the main receptor associated with the neuroprotective effects of cannabidiol. Several studies have reported that cannabidiol is an agonist at this receptor to produce its neuroprotective effects (12, 13, 14, 15). Furthermore, in a study by Comelli et al (16), it was found that the neuroprotective effects of cannabidiol were also mediated by TRPV1 vanilloid receptors in a rodent model of neuropathic pain. These, amongst numerous other studies, suggest that the neuroprotective effects of cannabidiol may be mediated through specific pharmacological targets, such as 5HT1A and TRPV1 receptors, while other actions are mediated through other targets, including cannabinoid receptors.

The Endocannabinoid System

The endocannabinoid system (ECS) is comprised of 1) endogenous compounds which are ligands, enzymes for endocannabinoid ligand synthesis and degradation, and at least two cannabinoid receptor proteins located throughout the nervous system and beyond. This system is involved in a variety of physiological processes including appetite, learning and memory, stress response, pain sensation, and the modulation of pain and inflammation.

Within the endocannabinoid system, there are two primary arachidonate-based lipids. These endogenous substances, known as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are synthesized on demand from membrane phospholipid precursors and are released to modulate the activity of cannabinoid receptor targets. Biosynthesis of 2-AG is initiated by diacylglycerol lipase (DAGL) alpha or beta. Currently, the mechanism by which anandamide is synthesized is not fully understood, although it has been suggested that AEA has several potential pathways for synthesis. Eventually, both 2-AG and AEA are metabolized and degraded by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively, back into arachidonic acid.

Cannabinoid Receptors

In mammals, there are two well characterized cannabinoid receptors containing distinct physiological features that contribute to their differing pharmacological properties. While structurally similar, their distribution within the body differs.

The cannabinoid CB1 receptor is primarily found in the central nervous system. As the most abundant G-protein coupled receptor in the brain, its overall widespread distribution corresponds well with the known pharmacodynamic effects of cannabinoids, and activation of this receptor leads to inhibition of adenylate cyclase activity (17) and inhibition of neurotransmitter release (18). Specifically, it is found in high densities in regions such as the hippocampus, basal ganglia and limbic system. In contrast, CB1 receptor expression is limited in regions such as the thalamus, brain stem

and medulla. Outside of the central nervous system CB1 receptors are also found on peripheral nerves, as well as in the digestive tract and adipose tissue.

The CB2 receptor is primarily found in non-neuronal areas. It is largely involved in anti-inflammatory and immunosuppressive actions (19), and its expression is markesly upregulated on immune cells such as T cells and microglia. It is largely believed to be devoid of psychoactivity (20). Evidence suggests that activation of this receptor results in the regulation of inflammation, and immune function.

Signaling

Unlike classical neuronal signaling, the CB1 receptor utilizes retrograde signaling, a negative feedback mechanism. In anterograde signaling, when action potentials reach an axon terminal, depolarization of the neuron triggers the release of neurotransmitter. When released, the neurotransmitters bind to postsynaptic receptors. Under certain conditons, activation of these post synaptic receptors leads to opening calcium channels and the on-demand synthesis and release of endocannabinoid lipids, which retrogradedly to the presynaptic terminal to activate CB1 receptors there.

Activation of cannabinoid receptors blocks the activation of adenylate cyclase, preventing signaling through cyclic AMP (cAMP). By activating the CB1 receptor, signaling in the presynaptic neuron is modulated to produce a prolonged reduction in the release of neurotransmitter. These presynaptic CB1-containing terminals are typically GABAergic or glutamatergic. Thus, CB1 receptor activation can either decrease glutamate or GABA release from the presynaptic terminal. Within the

hippocampus, for example, there is an abundance of CB1 receptors on both presynaptic glutamatergic pyramidal neurons and on GABAergic inhibitory neurons. When endocannabinoids bind to the CB1 receptor on the presynaptic terminal, activation of that receptor decreases the release of inhibitory GABA and reduces the release of excitatory glutamate. The net effect is to decrease glutamate release from the presynaptic side while depolarizing the postsynaptic side (21).

Current Treatment

Due to the increasing legalization and use of medicinal marijuana, there has been great interest in utilizing the endocannabinoid system to provide effective therapeutic endpoints across a variety of health issues. A recent advance in novel therapeutic strategies is the cannabinoid-based buccal spray Sativex. Sativex is a 1:1 preparation of Δ-9-THC and cannabidiol, and is prescribed for the treatment of spasticity and pain in multiple sclerosis. Since this cannabinoid-based pharmacotherapy has had great success in Canada and Europe, it has been recently fast-tracked by the USFDA into late-stage clinical trials.

Regrettably, concerns regarding dependence, tolerance, and cognitive deficits produced by cannabinoid-based pharmacotherapies remain significant issues, despite the recent increase in the use of medicinal cannabis, as well as the development of licensed cannabinoid-based pharmacotherapies like Sativex (22). Therefore, it is imperative to consider exploring non-psychoactive treatment strategies, beginning with cannabidiol as treatment alone for neuropathic pain states.

Chemotherapy Induced Neuropathic Pain and Cannabinoids

> ***"Neuropathic Pain** is a complex, chronic pain state that usually is accompanied by tissue injury. With neuropathic pain, the nerve fibers themselves might be damaged, dysfunctional, or injured. These damaged nerve fibers send incorrect signals to other pain centers. The impact of a nerve fiber injury includes a change in nerve function both at the site of injury and areas around the injury."*
>
> -The American Chronic Pain Association (23)

Neuropathic pain is a significant and disabling clinical problem with very few treatment options available (24). Specifically, chemotherapy induced peripheral neuropathy (CIPN) is an extremely disabling side effect of chemotherapy treatment associated with several commonly used chemotherapeutic agents, including vinca alkaloids, taxanes, and platinum agents. Unfortunately, neuropathic pain is refractory to conventional pharmacotherapies, necessitating validation of novel analgesics (25).

Patients experiencing CIPN commonly present clinically with sensory neuropathies, such as pain and paresthesia, which can begin weeks to months after initial treatment and peak at, or after the end of treatment (26). Symptoms are variable depending on the type of nerve fibers involved.

The exact mechanism of CIPN has not been fully elucidated and can differ amongst chemotherapeutic agents. Generally, chemotherapeutic agents cause peripheral nerve damage by affecting microtubules, mitochondrial disruption or via cytotoxic effects on DNA. This damage often leads to alteration of voltage-gated

sodium and transient receptor potential vanilloid (TRPV) channel activity and expression (27, 28), spontaneous activity and sensitization of peripheral nerve fibers (29), and infiltration of activated microglia and release of pro-inflammatory cytokines (30). Taken together, these disruptions cause a central sensitization effect, or pain hypersensitivity due to the amplification of neural signaling in the central nervous system (31).

Paclitaxel is a chemotherapeutic agent used for the treatment of many forms of cancer, including breast and ovarian (32). Mechanistically, Paclitaxel induces neuropathic pain by disrupting sensory nerve fiber structure and function (25, 34). Specifically, Paclitaxel causes damage to peripheral nerves by impeding microtubule-based axonal transport and disrupting mitochondrial function (35).

Within the last decade, several studies have reported that cannabinoids possess an ability to suppress neuropathic pain induced by toxic insults and traumatic nerve injury (15, 36, 37). Furthermore, preclinical evidence has demonstrated that treatment with cannabidiol alone, is an effective therapy for the treatment of neuropathic pain. For example, Toth et al (38) found that CBD prevented mechanical and thermal hypersensitivity in a rodent model of diabetic neuropathy. Two studies by Ward et al (39, 15) reported that treatment with cannabidiol prevents chemotherapy-induced mechanical allodynia in mice.

We hypothesized that treatment with the non-psychoactive cannabis compound cannabidiol would be as effective as the psychoactive cannabis compound Δ-9-THC, or combinations of the two, in mitigating neuropathic pain in a mouse model of

chemotherapy-induced peripheral neuropathy. To investigate this hypothesis, we employed a mouse model of chemotherapy-induced neuropathic pain to determine whether treatment with cannabidiol alone is as effective as Δ-9-THC or combinations of CBD+Δ-9-THC for the treatment of neuropathic pain.

Cognition and Cannabinoids

Acute effects of exposure to cannabis include euphoria, relaxation, intoxication, cognitive impairment, poor executive functions, distorted conscious perception, and analgesia (18). In humans, cannabinoids have been shown to impair encoding and recall of verbal and non-verbal information dose-dependently (40). Animal studies have also reported cognitive impairment in a variety of experimental assays such as discrimination tasks and maze tasks (41). Taken together, it is reasonable to suggest that cognitive impairment is a side effect of major concern although data in the literature are variable.

Given the high density of CB1 receptors in the hippocampus, and the important role of the hippocampus in learning and memory, it is very likely that the adverse effects of cannabinoids shown in experimental models of learning and memory are accredited to their actions within this brain region (42). Spatial learning and memory in particular are hippocampal dependent. Because potentiation of glutamatergic synaptes within the hippocampus is thought to be the cellular mechanism underlying memory formation, blocking this neurotransmission can result in abnormal cognitive behaviors such as impaired ability to remember a precise location and difficulty in goal-

directed navigation (43). Similarly, the striatum, which is important in operant learning, is densely populated with CB1 receptors.

To date, no studies have investigated the effects of cannabidiol alone or in combination with Δ-9-THC in rodent models of cognition. Therefore, we further hypothesized that cannabidiol would not affect classic-cannabinoid agonist induced cognitive impairment in rodent models of learning and memory. To test this hypothesis, we employed three models of cognition that assessed spatial and operant learning to determine whether cannabidiol ameliorates cognitive deficits commonly observed with Δ-9-THC.

In summary, cannabis use is associated with psychoactive and neuroprotective effects. The use of cannabis, both recreationally and medicinally, can be associated with psychosis and cognitive impairment. It is thought that these effects are generated by the activation of CB1 receptors and alteration of glutamatergic (and GABAergic) neurotransmission in brain regions densely populated by CB1 receptors. Although human research is limited, it still supports the evidence that cannabis use is promising in a variety of conditions, especially neuropathic pain (39, 15, 44). Taken together, the identification of cannabis-derived therapies which possess efficacy but are devoid of CB1 associated adverse effects, such as learning and memory impairment, is warranted. Overall, we hypothesize that CBD+Δ-9-THC combinations will be effective in the treatment of neuropathic pain but may still cause learning and memory deficits, while CBD alone will be equally efficacious without the unwanted side effects.

CHAPTER 2

METHODOLOGY

Subjects.

Male C57Bl6 mice (Taconic, 6-8 weeks upon delivery) were used for all experiments. The Temple University Institutional Animal Care and Use Committee approved all experiments. Animals were group housed, four to a cage, in a temperature controlled animal facility. Mice were habituated to the animal facility for at least one week prior to experiments with a light-dark cycle of 12:12h and had access to food and water ad-libitum.

Drugs.

For the mechanical sensitivity studies, CBD was provided by INSYS Therapeutics, Inc. (Austin, TX). For the cognition studies, CBD was provided by the NIDA drug supply program. Δ-9-THC and the synthetic CB1/CB2 mixed agonist WIN-55,212 were also provided through the NIDA drug supply program (Rockville MD). Paclitaxel was purchased through the Temple University Pharmacy (Philadelphia PA). All compounds were dissolved in a vehicle of 1:1:18 parts ethyl alcohol, cremophor, and saline. All agents were injected intraperitoneally (IP) based on body weight.

Paclitaxel-induced mechanical sensitivity.

Prior to drug administration, baseline sensitivity to mechanical stimuli was assessed in each mouse using standard Von Frey filaments and the up down method, as described in Ward et al 2011, 2014. Drugs and/or vehicle were then administered on experimental days 1, 3, 5, and 7. Paclitaxel was given at a dose of 8.0 mg/kg/injection. Fifteen minutes prior to paclitaxel injection, mice were also pretreated with vehicle or a range of CBD (0.625 – 20 mg/kg), Δ-9-THC (1.25 – 20 mg/kg), or CBD+Δ-9-THC combination (0.04+0.04 – 5.0+5.0 mg/kg) doses. Mechanical sensitivity was reassessed in all treatment groups on days 9, 14, and 21. N=8/group.

Conditional Discrimination.

Instrumental discrimination learning was assessed in mice using an appetitively-motivated operant model auditory conditional discrimination. Mice were trained using a two-component conditional discrimination task that requires animals to differentiate between two tones (2s vs. 8s) by making nose-pokes in one of two nose-poke holes, each being paired with a specific tone duration. This procedure involves a series of 8 phases, each with a specific criterion that must be met before a subject can proceed to the next phase. Phases 1-5, and 7 are considered training phases, and phases 6 and 8 are tests. The number of sessions within a phase varies depending on the criterion. Subjects are tested in one session per day, which lasts for 60 minutes. Additionally, subjects are weighed and food restricted 24 hours prior to testing and are given ~2.5 grams of rodent chow for each day of the conditional discrimination

procedure to maintain 90% of their free-feeding bodyweight. After mice were trained to criteria, they were pre-treated with WIN-55212 (2.5 – 10 mg/kg), Δ-9-THC (2.5 – 10 mg/kg), CBD (2.5 – 10 mg/kg), or combination of CBD+WIN (2.5+2.5 – 10+10 mg/kg) or CBD+Δ-9-THC (2.5+2.5 – 10+10 mg/kg) 15 minutes prior to their last conditioning session. N=8/group. Phases are outlined as follows:

Phase 1: Left Poke Training

Poking in the left hole will be reinforced on a fixed-ratio schedule (FR1). If at any time during the session a poke occurs in the left hole, a dipper containing a solution of Ensure will be presented for 6 seconds. The left nose-poke hole will remain illuminated for the entire duration of the session. Responses made in the right and center holes will be counted but have no programmed consequence. *Criterion:* 20 Ensure deliveries; 75% of responding in the left (active) nose-poke hole.

Phase 2: Right Poke Training

Poking in the right hole will be reinforced on a fixed-ratio schedule (FR1). If at any time during the session a poke occurs in the right hole, a dipper containing a solution of Ensure will be presented for 6 seconds. The right nose-poke hole will remain illuminated for the entire duration of the session. Responses made in the left and center holes will be counted but have no programmed consequence. *Criterion:* 20 Ensure deliveries; 75% of responding in the right (active) nose-poke hole.

Phase 3: Short Tone/Light Duration Training

An audible tone of 2 seconds will be presented along with an illuminated stimulus light, which is located above the left nose-poke hole. The tone/light combination will be presented on a fixed-time schedule (30 seconds). Once the tone/light ends after 2 seconds, the left poke will illuminate, and if a response is made in the left nose-poke hole within 6 seconds, a dipper containing an Ensure solution will be presented for 6 seconds. Responses made in the right and center holes will be counted, but have no programmed consequence. *Criterion:* 20 Ensure deliveries; 75% of responding in the left (active) nose-poke hole.

Phase 4: Long Tone/Light Duration Training

An audible tone of 8 seconds will be presented along with an illuminated stimulus light, which is located above the right nose-poke hole. The tone/light combination will be presented on a fixed-time schedule (30 seconds). Once the tone/light ends after 8 seconds, the right poke will illuminate, and if a response is made in the right nose-poke hole within 6 seconds, a dipper containing an Ensure solution will be presented for 6 seconds. Responses made in the right and center holes will be counted, but have no programmed consequence. *Criterion:* 20 Ensure deliveries; 75% of responding in the right (active) nose-poke hole.

Phase 5: Conditional Discrimination Training

An audible tone and paired stimulus light will be presented on a fixed-time schedule (30 seconds). For each trial, the duration of the tone/light cue will either be short (2 seconds) or long (8 seconds). In the presence of a short tone/light, a response in the left nose-poke hole will be reinforced with the presentation of an Ensure solution. In the presence of a long tone/light, a response in the right hole will be reinforced. Responses made during each trial will be recorded, but only a response in the active hole will be reinforced. *Criterion*: 10 Ensure deliveries for each cue duration; 70% correct discrimination for two consecutive sessions. Correct discrimination is measured using the discrimination ratio, which is defined as: Discrimination ratio = [correct responding/ (correct + incorrect responding)]*100 (Dunn et al 2005).

Phase 6: Extensive Training ***TEST***

Following the extensive training phases defined above, a test will occur. The procedure during this phase is defined the same as Phase 5. Correct discrimination is measured.

Phase 7: Rapid Training

During this phase, the previously trained contingencies are reversed. An audible tone and paired stimulus light will be presented on a fixed-time schedule (30 seconds). An audible tone of 2 seconds will be presented along with an illuminated stimulus light, which is located above the right nose-poke hole. An audible tone of 8 seconds will be

presented along with an illuminated stimulus light, which is located above the left nose-poke hole. For each trial, the duration of the tone/light cue will either be short (2 seconds) or long (8 seconds). In the presence of a short tone/light, a response in the right hole will be reinforced with the presentation of an Ensure solution. In the presence of a long tone/light, a response in the left hole will be reinforced. Responses made during each trial will be recorded, but only a response in the active hole will be reinforced. *Criterion:* This phase will only last one session.

Phase 8: Rapid Training ***TEST***

Following the rapid training session defined above, a test will occur. The procedure during this phase is defined the same as Phase 7. Correct discrimination is measured.

After mice were trained to criteria, they were pre-treated with WIN-55212 (2.5 – 10 mg/kg), Δ-9-THC (2.5 – 10 mg/kg), CBD (2.5 – 10 mg/kg), or combination of CBD+WIN (2.5+2.5 – 10+10 mg/kg) or CBD+Δ-9-THC (2.5+2.5 – 10+10 mg/kg) 15 minutes prior to their last conditioning session. N=8/group.

Barnes Maze.

Spatial learning was assessed in mice using an aversively-motivated standard, automated Barnes Maze. The Barnes maze is a round maze with a center platform and a series of mock escape holes and one true escape hole along its perimeter. At the beginning of each trial an aversive tone is played directly above the mouse placed in

the center of the maze, and the animal's escape behavior is recorded with an automated tracking system. A five-day procedure was used, wherein on days 1-4 mice received 4 5-min acquisition trials per day to locate the true escape hole. On day five the escape hole is replaced with a mock escape hole and the mouse's exploratory behavior is measured in 1 5-min retention trial. Animals were pretreated with CBD (20 mg/kg), Δ-9-THC (2.5—20 mg/kg), or combination of CBD+Δ-9-THC (2.5—20 mg/kg) for 30 minutes prior to the retention trial.

Data and Statistical Analysis.

Data are represented as mean +/- SEM. Results were analyzed with one- and two- way ANOVA (GraphPad Prism 6); $p<0.05$ was considered significant. Mechanical sensitivity data are expressed as percent mechanical sensitivity to normalize baseline differences between groups. Potential synergistic effects of cannabinoid combinations on mechanical sensitivity were alternately analyzed in collaboration with Dr. Ronald Tallarida using the principle of dose equivalence. This technique is used to compare actual drug combination data with mathematically predicted results if the drugs were to act together additively.

CHAPTER 3

RESULTS

Paclitaxel-induced mechanical sensitivity.

Pretreatment with CBD or Δ-9-THC significantly attenuated paclitaxel-induced mechanical sensitivity. Representative doses along the ascending limb of the dose response curve are shown in Figure 1. For CBD, two-way ANOVA revealed a significant effect of treatment ($F(3, 112) = 11.18$, $p<0.0001$), of time ($F(3, 112) = 11.09$, $p<0.0001$), but no significant interaction ($F(9, 112) = 1.473$, n.s.). For Δ-9-THC, two-way ANOVA revealed a significant effect of treatment ($F(4, 140) = 7.738$, $p<0.0001$), of time ($F(3, 140) = 29.33$, $p<0.0001$), but no significant interaction ($F(12, 112) = 1.290$, n.s.) CBD produced this effect with higher potency, in that the minimal effective dose for CBD was 1.25 mg/kg IP, while the minimal effective dose for Δ-9-THC was 2.5 mg/kg IP. Two-way ANOVA also revealed a significant difference between the CBD and Δ-9-THC dose response curves, with the 1.25 mg/kg dose of CBD producing a significantly higher % baseline score as compared to 1.25 mg/kg dose of Δ-9-THC. Both drugs appeared to be equally efficacious against mecahincal sensitivity.

Pretreatment with CBD+Δ-9-THC combinations in a 1:1 ratio based on dose produced a robust leftward and upward shift in the dose-response curve, suggesting a synergistic interaction. Representative doses along the ascending and descending limb are shown in Figure 2.

Full dose response data for the single and combined agents were transformed to percent maximal possible effect (%MPE), which was necessary in order to determine effect levels for specific single agent doses and predicted effect levels for combination doses (Figure 3).

Dose equivalence analysis was applied using the ascending limbs of the single agent dose response curves shown to determine the predicted additive effect levels (Eadd) for the combination doses tested. These were then statistically compared to the observed combination effect levels (Eobs) using modified a t-test, revealing a statistically significant synergistic effect of the CBD+Δ-9-THC combinations (Table 1).

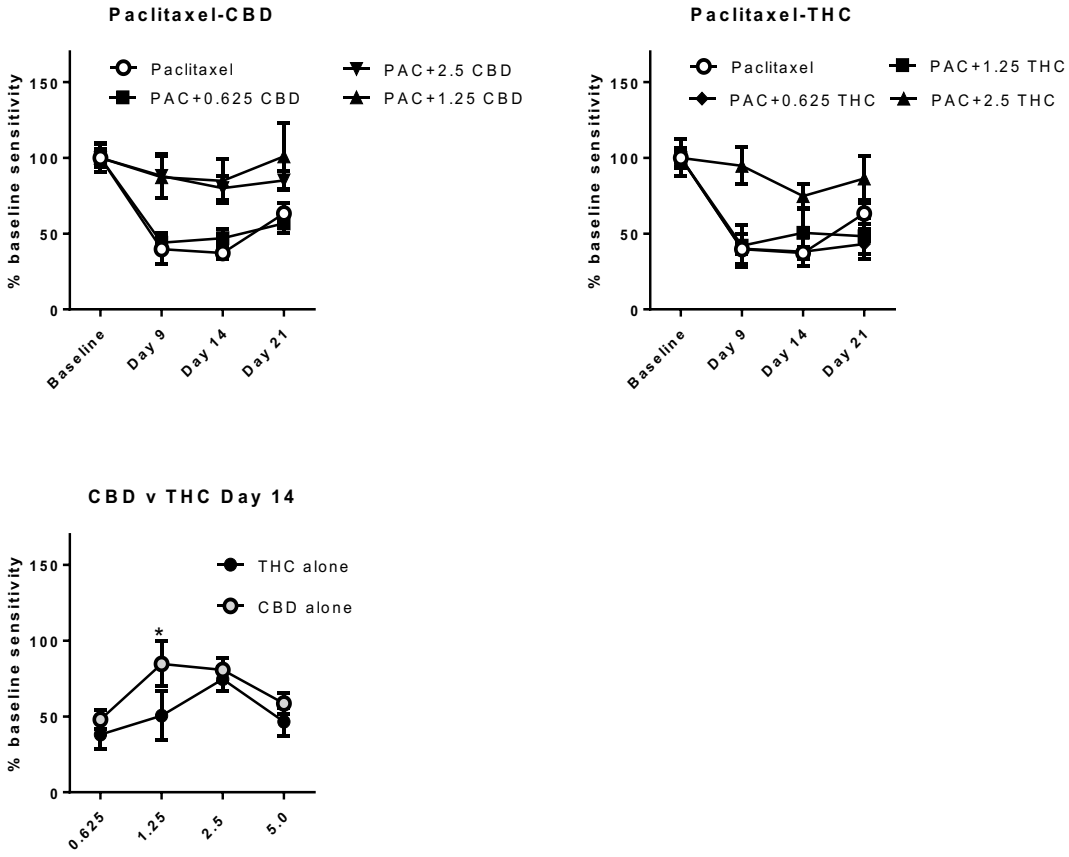

Figure 1. Effects of CBD or Δ-9-THC on Paclitaxel-induced mechanical sensitivity. Data are expressed as percent baseline sensitivity to Von Frey filament stimulation. Top left: CBD dose dependently prevents Paclitaxel-induced mechanical sensitivity. Top right: Δ-9-THC dose dependently prevents Paclitaxel-induced mechanical sensitivity. Bottom left: CBD is more potent and slightly more effective than Δ-9-THC. Ns=8/group.

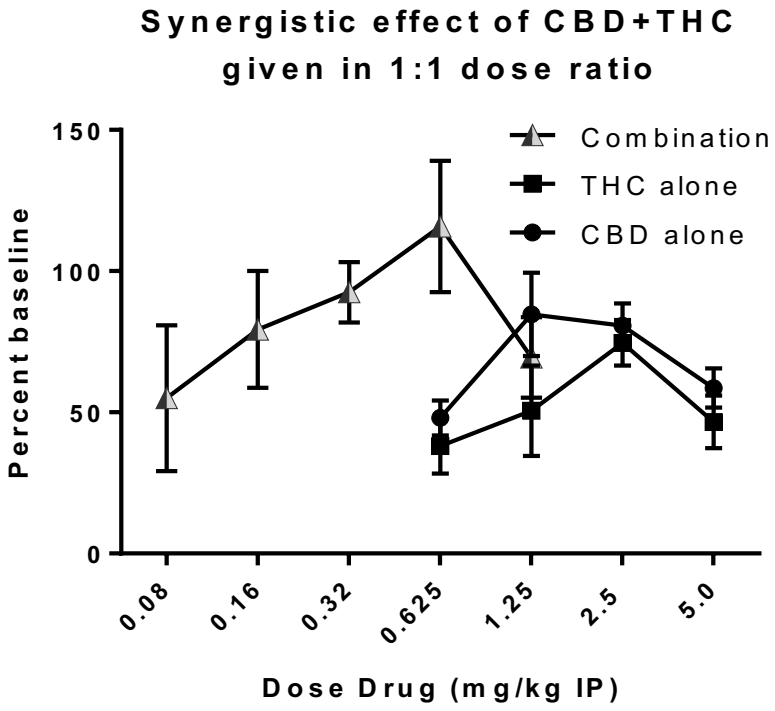

Figure 2. Effects of CBD+Δ-9-THC administered in 1:1 ratio based on dose. CBD+Δ-9-THC dose dependently prevents paclitaxel induced mechanical sensitivity, displaying both increased efficacy and potency compared with either agent alone. Ns=8/group.

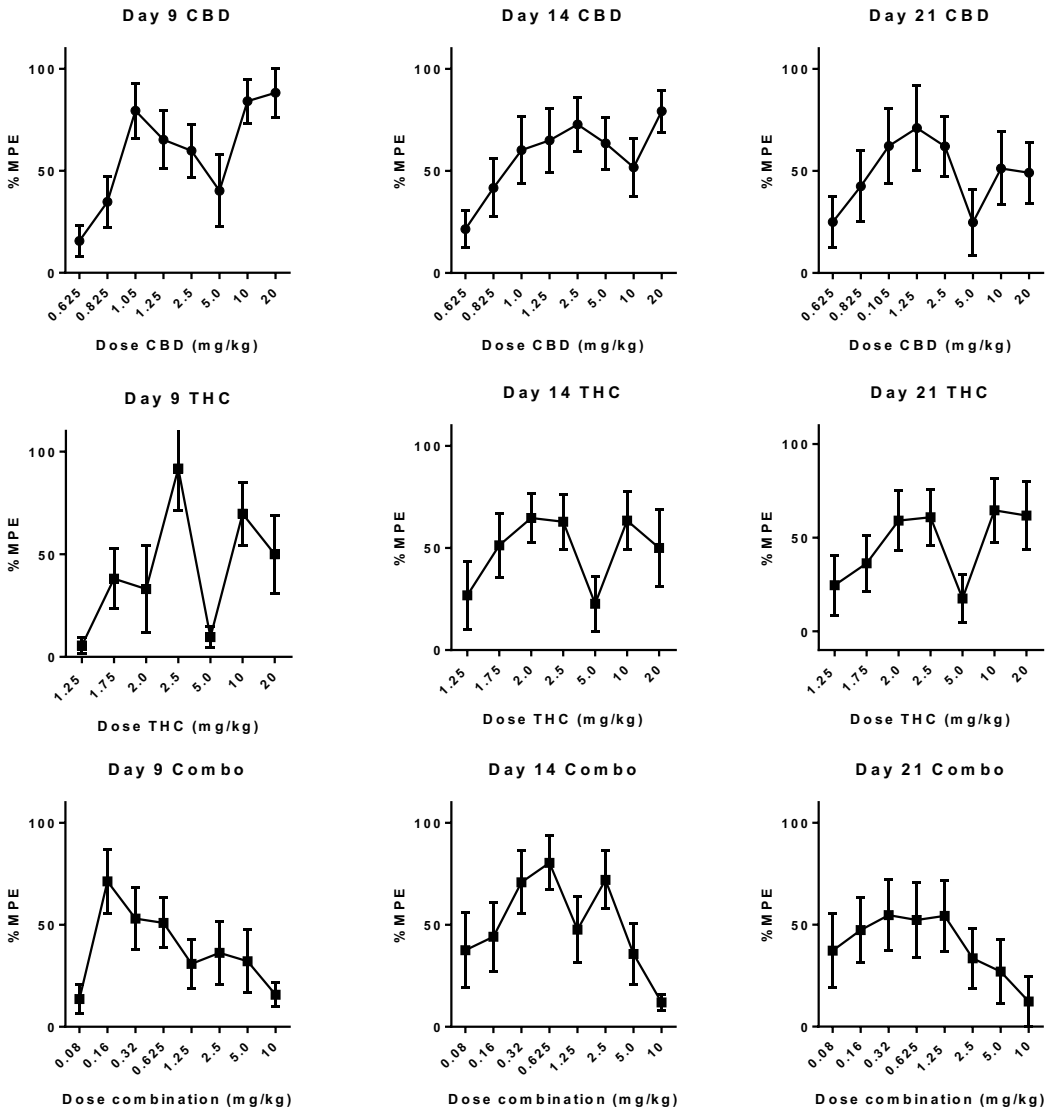

Figure 3. Full dose response curves expressed as percent maximal possible effect for CBD, Δ-9-THC, and CBD+Δ-9-THC. The ascending limbs of these dose response curves were used to derive predicted additive effects and quantitatively compare them to observed combination effects to determine synergy. Ns=8/group.

Combination	Eadd	Eobs
0.04/0.04	0.114	40
0.08/0.08	0.691	45
0.16/0.16	4.08	71
0.31/0.31	13.68	78
	*p=0.004	

Table 1. Predicted additive versus observed effect levels of CBD+Δ-9-THC combinations.

Conditional Discrimination.

Δ-9-THC alone or in combination with CBD did not affect tone discrimination in trained mice, with no effect of treatment ($F(2, 63) < 1.0$, n.s., Figure 4 left panel). Although pretreatment with WIN-55,212-2 produced a trend toward decreased discrimination performance, one-way ANOVA of the WIN dose response curve revealed no significant effect ($F(3, 28) = 1.279$, n.s., Figure 4 right panel). Two-way ANOVA revealed a significant effect of cannabinoid treatment ($F(2, 63) = 3.658$, $p<0.05$), but multiple comparisons post hoc analysis revealed no significant effect of any cannabinoid treatment dose versus another. There was also no effect of any cannabinoid treatment on the reversal phase of the conditional discrimination task (data not shown).

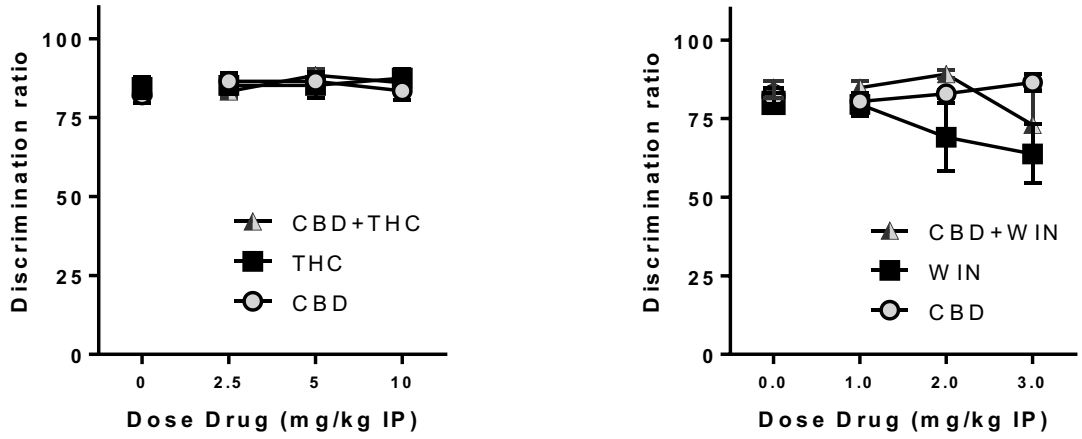

Figure 4. Effects of Δ-9-THC or WIN55,212-2 alone or in combination with CBD on retention of a tone discrimination task. Treatment with WIN55,212-2 produced a non-significant trend to decrease discrimination and this effect was attenuated by CBD at the moderate dose of WIN55,212-2. Ns=8/group.

Barnes Maze.

Four behavioral outcomes were analyzed following Barnes Maze performance on retention day (day 5; Figure 5). Two outcome measures are indicative of memory retention, those being the number of entries the mouse made into the zone with the escape hole, and the total time spent in that zone (Figure 5 top panel). There was a nearly significant effect of drug dose on entries into the target zone ($F(4, 60) = 2.445$, $p=0.056$), but no significant effect of cannabinoid treatment ($F(4, 60) = <1.0$, n.s.) and no interaction ($F(4, 60) < 1.0$, n.s.). There was a significant effect of drug dose on time spent in the target zone ($F(4, 60) = 4.085$, $p<0.005$), but no significant effect of cannabinoid treatment ($F(4, 60) = <1.0$, n.s.) and no interaction ($F(4, 60) < 1.0$, n.s.). Two additional outcome measures were analyzed that are indicative of motor performance, those being total distance traveled and average speed (Figure 5 bottom panel). There was no significant effect of drug dose on total distance traveled ($F(4, 60) = 1.675$, n.s.), but a significant effect of cannabinoid treatment ($F(4, 60) = 19.63$, $p<0.0001$) and a interaction ($F(4, 60) < 2.759$, $p<0.05$). There was a significant effect of drug dose on average speed ($F(4, 60) = 7.796$, $p<0.0001$), a significant effect of cannabinoid treatment ($F(4, 60) = 6.054$, $p<0.01$) and a significant interaction ($F(4, 60) = 6.556$, $p<0.001$).

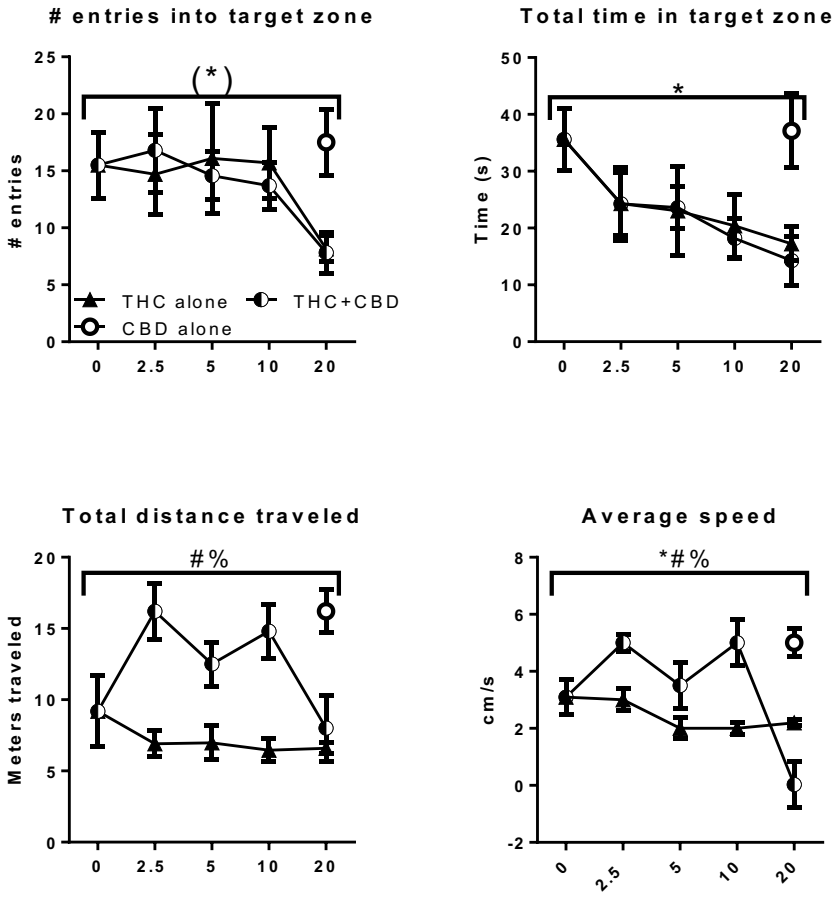

Figure 5. Effects of Δ-9-THC or CBD+Δ-9-THC administered in 1:1 ratio based on dose on retention of spatial memory. Top panels: Δ-9-THC and CBD+Δ-9-THC dose dependently decrease number of entries and total time in the target zone. CBD did not attenuate the effects of Δ-9-THC alone. Bottom panels: Δ-9-THC did not effect total distance traveled or average speed, while the CBD+Δ-9-THC treatment increased both locomotor measurements at all but the highest combination dose. Ns=8/group. * denotes main effect of dose, # denotes main effect of treatment, and % denotes interaction.

CHAPTER 4

DISCUSSION

We found that treatment with cannabidiol and Δ-9-THC are effective in a rodent model of chemotherapy-induced peripheral neuropathy. Additionally, we observed a synergistic effect when testing cannabinoids in combination. In the conditional discrimination assay, we did not observe a cognitive deficit in animals administered Δ-9-THC. However, we did observe a trend towards impairment in animals treated with WIN55,212-2. Results of the Barnes Maze procedure revealed that THC alone and in combination with CBD produced a dose-dependent effect on retention. CBD alone produced no cognitive deficits in the learning and memory assays.

The success of efficacious cannabinoid treatment in attenuating neuropathic pain in rodents has been previously examined using mixed affinity CB1/CB2 receptor ligands such as Δ-9-THC and WIN55,212-2 (3). Cannabidiol and its derivatives, reported to be devoid of psychoactive effects, may offer a beneficial approach. Among these properties, the neuroprotective, alteration of inflammation, and immunomodulatory properties of cannabidiol are most promising for treatment of neuropathic pain.

Previously, our lab has demonstrated that treatment with cannabinoids produces a synergistic effect in an established model of chemotherapy induced neuropathic pain. In the present study, we compared cannabidiol and Δ-9-THC first as monotherapies. We observed that treatment with either cannabidiol or Δ-9-THC dose-dependently

prevents Paclitaxel-induced mechanical sensitivity. Additionally, we found that treatment with cannabidiol alone is more potent and slightly more efficacious than treatment with Δ-9-THC in a dose response curve. Data suggest that the minimal effective dose of cannabidiol to produce attenuation of neuropathic pain was 1.25 mg/kg. This effect was produced with greater potency than Δ-9-THC. Comparably, the minimal effective dose for Δ-9-THC to produce significant attenuation of neuropathic pain was 2.5 mg/kg. Subsequently, we evaluated cannabidiol and Δ-9-THC in combination, in equivalent doses modeled after Sativex. We observed that pretreatment with combinations of CBD+Δ-9-THC dose-dependently produce a leftward and upward shift in the dose response curve, suggesting synergistic action. Conclusively, these data report an important, significant synergistic effect between cannabidiol and Δ-9-THC on neuropathic pain, leading us to further hypothesize about the role of possible interactions of cannabidiol and Δ-9-THC on learning and memory.

The phytocannabinoids Δ-9-THC and cannabidiol have efficacy in treating various pain conditions such as chemotherapy-induced peripheral neuropathy. Successful cannabinoid-based pharmacotherapies, such as Sativex, and the rapid legalization of medical marijuana, provide a gateway for the development of novel therapeutic strategies. However, the psychoactivity of Δ-9-THC and additional concerns regarding abuse and cognitive deficits continue to pose significant barriers for the acceptance of medicinal Cannabis use amongst patients and health care professionals.

The objective of our cognitive studies was to assess whether cannabidiol ameliorates cognitive deficits induced by classical cannabinoid agonists in an appetitively motivated operant model of learning and memory, and an aversively motivated, hippocampal-dependent model of spatial learning and memory. These two models of cognition allowed us to examine whether treatment with cannabidiol alters the adverse effects of Δ-9-THC, and whether treatment with cannabidiol itself produces cognitive impairment. Currently, we are the first to investigate the effects of cannabidiol alone and in combination with Δ-9-THC in rodent models of learning and memory.

We began assessing cognition with the conditional discrimination task. Data in this assay show that treatment with Δ-9-THC alone or in combination with cannabidiol did not impair cognition. However, a slight trend towards impairment in animals pretreated with WIN55,212-2 was observed. Because we did not see a deficit in animals treated with Δ-9-THC alone, we could not test our hypothesis regarding combination treatment. It is possible that we did not see any effects on cognition due to animals being too well trained, our dose of Δ-9-THC was not high enough to aversively impact cognition, or that acute dosing of Δ-9-THC does not produce an effect on learning and memory in this particular assay. Furthermore, treatment with cannabidiol did not impair cognitive performance nor did it reverse the slight impairment induced by WIN55,212-2. Based on these results, we concluded that cannabinoids in combination do not produce significant cognitive impairments in an appetitively motivated mouse model of learning and memory. These findings are not in agreement with the findings of other

established models of operant and spatial learning (44, 45). Possible reasons for observed differences are species and dose associated.

Next we chose to use a spatially motivated learning and memory assay. In the Barnes Maze task, we measured four behavioral outcomes. Two measures are indicative of memory retention, and two are indicative of motor activity. Results of the Barnes Maze task suggest that administration of Δ-9-THC alone produces retention deficits, and that cannabidiol does not block or ameliorate these effects in this model. We additionally report a marginally significant effect on dose for *entries into target zone* based on the data being ineffective at lower doses, and effective at the 20 mg/kg dose of CBD+Δ-9-THC. Also, we saw marginal significance on dose for *total time in target zone*. These results suggest that higher doses of cannabinoids decrease performance in this model. Overall, data from the spatial memory task are comparable to findings in similar spatial memory tasks in other species (46).

Interestingly, data reported for *total distance traveled* and *average speed* show an increase in motor activity in animals administered combinations of CBD+Δ-9-THC when compared to THC alone. The observed motor effects may be possible to the anxiolytic properties of cannabidiol. These effects are no longer present at the 20 mg/kg combination dose which is possibly due to the cataleptic properties of Δ-9-THC.

Overall, we report that THC alone and in combination with CBD produces an effect on retention without marked suppression of motor activity. This suggests that the results we found are truly retention deficits, and that we successfully examined an important side effect of cannabinoid treatment.

Taken together, results of the chemotherapy-induced peripheral neuropathy study demonstrate the ability of cannabidiol to significantly ameliorate Paclitaxel-induced neuropathic pain without inducing serious cognitive deficits.

Future Directions

Future studies aimed at developing novel cannabinoid-based pharmacotherapies should consider exploring a wider range of CBD: Δ-9-THC dose ratios, potential mechanisms underlying the observed interactions, and chronic dosing effects on cognition. Additionally, future studies should also examine lower doses of CBD and Δ-9-THC individually to determine ED50 values—the dose required to achieve 50% reduction in neuropathic pain. Determining ED50 values would allow researchers to further test these doses in combination in additional neuropathic pain and cognition studies. Lastly, future studies will test the hypothesis as to whether the combinations of Δ-9-THC and CBD are anxiolytic as the Barnes Maze locomotor data suggest.

Acknowledgements

This work was supported by grants R03DA034761 (SJW) and INSYS Rx (SJW). The authors acknowledge Kirsten King and Ariele Soroko-Monzo.

Conflict of Interest

The authors state no conflict of interest.

REFERENCES CITED

1. Catlow B, Sanchez-Ramos J. Cannabinoids for the Treatment of Movement Disorders. Curr Treat Options Neurol. 2015 Sep;17(9):370,015-0370-5.

2. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. 2006 Sep;58(3):389-462.

3. Kozela E, Lev N, Kaushansky N, Eilam R, Rimmerman N, Levy R, et al. Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. Br J Pharmacol. 2011 Aug;163(7):1507-19.

4. Broyd SJ, van Hell HH, Beale C, Yucel M, Solowij N. Acute and Chronic Effects of Cannabinoids on Human Cognition-A Systematic Review. Biol Psychiatry. 2016 Apr 1;79(7):557-67.

5. Bossong MG, Jager G, Bhattacharyya S, Allen P. Acute and non-acute effects of cannabis on human memory function: a critical review of neuroimaging studies. Curr Pharm Des. 2014;20(13):2114-25.

6. Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, et al. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology. 2010 Feb;35(3):764-74.

7. Martin-Santos R, Fagundo AB, Crippa JA, Atakan Z, Bhattacharyya S, Allen P, et al. Neuroimaging in cannabis use: a systematic review of the literature. Psychol Med. 2010 Mar;40(3):383-98.

8. Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med. 2011 Sep 1;51(5):105461.

9. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci. 2009 Oct;30(10):515-27.

10. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 2008 Jan;153(2):199-215.

11. Campos AC, Fogaca MV, Sonego AB, Guimaraes FS. Cannabidiol, neuroprotection and neuropsychiatric disorders. Pharmacol Res. 2016 Feb 1.
12. Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HTla receptors. Neurochem Res. 2005 Aug;30(8):1037-43.

13. Alves FH, Crestani CC, Gomes FV, Guimaraes FS, Correa FM, Resstel LB. Cannabidiol injected into the bed nucleus of the stria terminalis modulates baroreflex activity through 5-HT1A receptors. Pharmacol Res. 2010 Sep;62(3):228-36.

14. Gururajan A, Taylor DA, Malone DT. Effect of cannabidiol in a MK-801-rodent model of aspects of schizophrenia. Behav Brain Res. 2011 Sep 23;222(2):299-308.

15. Ward SJ, McAllister SD, Kawamura R, Murase R, Neelakantan H, Walker EA. Cannabidiol inhibits paclitaxel-induced neuropathic pain through 5-HT(1A) receptors without diminishing nervous system function or chemotherapy efficacy. Br J Pharmacol. 2014 Feb;171(3):636-45.

16. Comelli F, Giagnoni G, Bettoni I, Colleoni M, Costa B. Antihyperalgesic effect of a Cannabis sativa extract in a rat model of neuropathic pain: mechanisms involved. Phytother Res. 2008 Aug;22(8):1017-24.

17. Fine PG, Rosenfeld MJ. The endocannabinoid system, cannabinoids, and pain. Rambam Maimonides Med J. 2013 Oct 29;4(4):e0022.

18. Papathanasopoulos P, Messinis L, Lyros E, Kastellakis A, Panagis G. Multiple sclerosis, cannabinoids, and cognition. J Neuropsychiatry Clin Neurosci. 2008 Winter;20(1):36-51.

19. Ashton JC, Glass M. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol. 2007;5(2):73-80.

20. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philos Trans R Soc Lond B Biol Sci. 2012 Dec 5;367(1607):3353-63.

21. Soltesz I, Alger BE, Kano M, Lee SH, Lovinger DM, Ohno-Shosaku T, et al. Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. Nat Rev Neurosci. 2015 May;16(5):264-77.

22. Woodhams SG, Sagar DR, Burston JJ, Chapman V. The role of the endocannabinoid system in pain. Handb Exp Pharmacol. 2015;227:119-43.

23. American Chronic Pain Association - Neuropathic Pain. (n.d.). Retrieved April 12, 2016, from https://www.theacpa.org/condition/Neuropathic-Pain.

24. Schomberg D, Ahmed M, Miranpuri G, Olson J, Resnick DK. Neuropathic pain: role of inflammation, immune response, and ion channel activity in central injury mechanisms. Ann Neurosci. 2012 Jul;19(3):125-32.

25. Rahn EJ, Hohmann AG. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. Neurotherapeutics. 2009 Oct;6(4):713-37.

26. Pachman DR, Barton DL, Watson JC, Loprinzi CL. Chemotherapy-induced peripheral neuropathy: prevention and treatment. Clin Pharmacol Ther. 2011 Sep;90(3):377-87.

27. Adelsberger H, Quasthoff S, Grosskreutz J, Lepier A, Eckel F, Lersch C. The chemotherapeutic oxaliplatin alters voltage-gated Na(+) channel kinetics on rat sensory neurons. Eur J Pharmacol. 2000 Oct 6;406(1):25-32.

28. Gauchan P, Andoh T, Ikeda K, Fujita M, Sasaki A, Kato A, et al. Mechanical allodynia induced by paclitaxel, oxaliplatin and vincristine: different effectiveness of gabapentin and different expression of voltage-dependent calcium channel alpha(2)delta-1 subunit. Biol Pharm Bull. 2009 Apr;32(4):732-4.

29. Xiao WH, Bennett GJ. Chemotherapy-evoked neuropathic pain: Abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-L-carnitine. Pain. 2008 Apr;135(3):262-70.

30. Hu P, McLachlan EM. Macrophage and lymphocyte invasion of dorsal root ganglia after peripheral nerve lesions in the rat. Neuroscience. 2002;112(1):23-38.

31. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011 Mar;152(3 Suppl):S2-15.

32. Windebank AJ, Grisold W. Chemotherapy-induced neuropathy. J Peripher Nery Syst. 2008 Mar;13(1):27-46.

33. Dougherty PM, Cata JP, Cordella JV, Burton A, Weng HR. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated

fiber function in cancer patients. Pain. 2004 May;109(1-2):132-42.

34. Costa B, Giagnoni G, Franke C, Trovato AE, Colleoni M. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. Br J Pharmacol. 2004 Sep ;143 (2):247-50.

35. Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V. Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. Eur J Neurosci. 2004 Nov;20(9):2311-20.

36. Toth CC, Jedrzejewski NM, Ellis CL, Frey WH,2nd. Cannabinoid-mediated modulation of neuropathic pain and microglial accumulation in a model of murine type I diabetic peripheral neuropathic pain. Mol Pain. 2010 Mar 17;6:16,8069-616.

37. Ward SJ, Ramirez MD, Neelakantan H, Walker EA. Cannabidiol prevents the development of cold and mechanical allodynia in paclitaxel-treated female C57B16 mice. Anesth Analg. 2011 Oct;113(4):947-50.

38. Abush H, Akirav I. Short- and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. PLoS One. 2012;7(2):e31731.

39. Castellano C, Rossi-Arnaud C, Cestari V, Costanzi M. Cannabinoids and memory: animal studies. Curr Drug Targets CNS Neurol Disord. 2003 Dec;2(6):389-402.

40. Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, et al. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci. 2006 Mar 15 ;26(11):2991-3001.

41. Hebert AE, Dash PK. Nonredundant roles for hippocampal and entorhinal cortical plasticity in spatial memory storage. Pharmacol Biochem Behay. 2004 Sep;79(1):143-53.

42. Hohmann AG. Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. Chem Phys Lipids. 2002 Dec 31;121(1-2):173-90.

43. Dunn MJ, Futter D, Bonardi C, Killcross S. Attenuation of d-amphetamine-induced disruption of conditional discrimination performance by alpha-flupenthixol. Psychopharmacology (Berl). 2005 Jan;177(3):296-306.

44. Segev A, Akirav I. Differential effects of cannabinoid receptor agonist on social discrimination and contextual fear in amygdala and hippocampus. Learn Mem. 2011 Mar 29;18(4):254-9.

45. Sokolic L, Long LE, Hunt GE, Arnold JC, McGregor IS. Disruptive effects of the prototypical cannabinoid Delta(9)-tetrahydrocannabinol and the fatty acid amide inhibitor URB-597 on go/no-go auditory discrimination performance and olfactory reversal learning in rats. Behav Pharmacol. 2011 Jun;22(3):191-202.

46. Ruhl T, Prinz N, Oellers N, Seidel NI, Jonas A, Albayram 0, et al. Acute administration of THC impairs spatial but not associative memory function in zebrafish. Psychopharmacoldgy (Berl). 2014 Oct;231(19):3829-42.