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Cannabinoid-induced Behavioral Sensitization in Adolescent Sprague-Dawley Rats

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CANNABINOID-INDUCED BEHAVIORAL SENSITIZATION IN ADOLESCENT SPRAGUE-DAWLEY RATS

A Thesis

Presented to the

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California State University,

San Bernardino

In Partial Fulfillment

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in

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by

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ABSTRACT

Adolescent cannabis use has grown because of increased availability and higher societal acceptance. This increase in cannabis use is problematic as adolescents who experiment with cannabis are more likely to abuse cannabis and experiment with other illicit drugs such as cocaine. The reason for the greater susceptibility to drugs use is unclear and may be the result of altered drug sensitivity after cannabis exposure. Thus, the present investigation used the behavioral sensitization paradigm to examine the behavioral response of early adolescent rats to the cannabinoid agonist CP 55,940 (CP) or cocaine after repeated cannabinoid administration. It was hypothesized that: (1) CP would cause a sensitized response in both male and female adolescent rats, (2) female rats would have a greater behavioral response than male rats, (3) pretreatment with CP would induce cross-sensitization to cocaine, (4) pretreatment with cocaine would cause behavioral sensitization and conditioned activity in male and female adolescent rats. In the first experiment, 137 male and female Sprague-Dawley rats were given CP (4, 13.2, or 40 µg/kg, IP) or vehicle (50% DMSO/H_2O) once daily for 5 consecutive days on postnatal day (PD) 30- PD 34. Distance traveled and stereotyped movement was assessed for 1 h after each drug injection. After a 48 h abstinence period (i.e., on PD 36), rats were given CP (4 or 13.2 µg/kg, IP) and distance traveled and stereotyped movement was monitored for 2 h. In the second experiment, 146 male and female rats were tested with the same protocol as in Experiment 1 except that rats were given CP

(13.2 or 4 µg/kg), cocaine (20 mg/kg), or vehicle (saline or 50% DMSO/H_2O) for five days and then tested with saline or cocaine (10 mg/kg) after 48 h. In the first experiment, no dose of CP altered distance traveled scores or stereotyped movement over the five pre-exposure days nor did CP cause behavioral sensitization on the test day. In the second experiment, pretreatment with cocaine led to enhanced distance traveled scores and stereotyped movement when challenged with cocaine (behavioral sensitization) or saline (conditioned activity) on test day. In contrast, CP-pretreated rats did not show greater activity when injected with cocaine or saline on test day. These data show that cannabinoids do not act like psychostimulant drugs, since CP did not cause the same changes in drug sensitivity as cocaine. The cocaine sensitization observed in adolescent rats indicates that this age group is particularly vulnerable to the rewarding effects of cocaine, and suggests that early cocaine exposure can augment drug seeking behavior. The failure to detect cannabinoid-induced sensitization, conditioned activity, or cocaine cross-sensitization during adolescence suggests that CP, when given at a consistent dose, does not increase the addictive properties of cannabinoids or cocaine. The results also indicate that cannabinoid use does not alter drug responsivity or lead to greater drug seeking and abuse in the adolescent population.

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CHAPTER ONE

INTRODUCTION

Cannabis

Cannabis has been called by several names including hemp, hashish, ganja, and marijuana (Russo, 2007). The cannabis plant has been used for clothing, paper, rope, and as a medicine throughout many time periods in various cultures. Emperor Shen Neug of China in 2,000 B.C. was the first to record cannabis use, or 'ma' as a textile and for soil fertilization (Zuardi, 2006). Hemp rope was also used by the Vikings for both coarse textiles and fine household textiles (Skoglund, Nockert & Holst, 2013). This plant has also been used in religious and spiritual rituals for enlightenment and communication with spirits (Bapat, 2015; Russo, 2007; Touw, 1981; Zuardi, 2006). The Materia Medica Sutra (Pen ts'ao Ching) first documented the psychotropic properties of cannabis sativa L. (Huo ma ren) and refers to the "fruit of the cannabis" which if taken would cause the user to have visions of spirits and devils (Zuardi, 2006). Throughout history, various cultures and religions have noted that cannabis may have useful properties (Aldrich, 1997).

The Chinese were also the first to document the medical uses of cannabis for pain reduction, female reproductive health, and constipation relief (Touw, 1981; Zuardi, 2006). Common medical uses for cannabis that have been reported by cultures throughout Asia and the Middle East include analgesia, appetite stimulation, diarrhea relief, and mental relaxation (Bapat, 2015; Russo,

2007; Touw, 1981; Zuardi, 2006). However, cannabis also produces euphoric and psychoactive effects that have influenced cultures to outlaw its use. For example, the Chinese called the resinous seeds (Ma Fen) poisonous and made it illegal to consume them (Li, 1978; Russo, 2007; Touw, 1981; Zuardi, 2006). Furthermore, India prohibited cannabis resin (haras) despite cannabis (bhang) being considered one of the five sacred plants of India (Bapat, 2015; Russo, 2007; Touw, 1981; Zuardi, 2006).

The United States also has a long history with cannabis. In 1916 botanists Lyster H. Dewey and Jason L. Merrill of the Department of Agriculture reported that hemp would make a more efficient and environmentally safer paper compound compared to wood (Dewey & Merrill, 1916, pg. 25). However, the prohibition of marijuana had already begun in California in 1915. In 1937, the United States Congress passed the Marijuana Tax Act which made anyone selling marijuana pay an occupational tax and register with the Internal Revenue Service (McKenna, 2014; Musto, 1972). This act passed with the help of negative propaganda like the film “Reefer Madness” (Stringer & Maggard, 2016). Interestingly, Harry Anslinger and the Federal Bureau of Narcotics passed the Marijuana Tax act essentially outlawing cannabis in 1937, the same year Dr. William C. Woodward of The American Medical Association proposed the study of cannabis for medical use (McKenna, 2014; Newton, 2014).

The Controlled Substance Act was then passed in 1970 and banned the medical and recreational use of marijuana by placing it on the Schedule I drug

list, the most restrictive drug schedule. Drugs that are on this schedule are defined as drugs with no currently accepted medical use and a high potential for abuse. Nevertheless, in 1992, the first pharmaceutical based cannabis compound, dronabinol, became legal for medical use specifically for AIDS-wasting syndrome (Werner, 2001). In 1996, California became the first of 25 states to decriminalize medical marijuana for persons in California suffering from cancer, glaucoma, migraines, seizures, severe nausea, muscle spasms, and chronic pain. In 2012, Colorado and Washington decriminalized cannabis recreationally for people over the age of 21, and Alaska and Oregon followed suit in 2014. Most recently, California has passed laws for recreational use beginning in 2017. Despite these changes in state law, the federal government still considers cannabis an illicit substance and it remains listed as a Schedule I drug.

Cannabis Plant

The cannabis plant grows indigenously in many regions, in Asia and the Middle East. The two main species of the cannabis plant are indica and sativa. In addition, a number of genetic hybrids have been created through cross breeding of indica and sativa plants (Russo, 2007). The indica strain of the plant grows short and stocky with dark leaves while the sativa strain grows tall and thin with light leaves (Russo, 2007). Different types of sativa and indica strains have been shown to produce differing effects and are now being classified by their genetic makeup and reported medical benefits (Janatová et al., 2018). Cannabis indica has been reported to have pain relieving effects on the body, produce

sedation, and help with nausea and lack of appetite (Pearce, Mitsouras & Irizarry, 2014). The sativa strains, on the other hand, are self-reported to cause euphoric and psychedelic effects and are considered energizing and stimulating (Pearce et al., 2014).

There are three main preparations of the cannabis plant including the cannabis resin (hash), the seeded plant that contains stems, flowers, and leaves, and the unfertilized female flowers which are most commonly used to produce psychoactive effects (Russo, 2007). The cannabis flowers contain numerous active chemical compounds known as cannabinoids, which are responsible for the altered state that is experienced by someone who uses the cannabis plant. The two main cannabinoids of this plant, (−)-trans-Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), are believed to mediate most cannabis-induced effects (Russo, 2007).

When used acutely, cannabis can cause red eyes, sleepiness, decreased motor coordination, and slow respiratory rate (Grotenhermen, 2004). The psychological effects observed with acute cannabis use include dysphoria, alterations to attention, concentration, and learning, and somatic and visual sensations (Grotenhermen, 2004). Although the acute effects are well understood, the persistent long-term effects of cannabis use are variable and depend on age of onset and duration of use. Overall, decreases in verbal fluency, visual attention, and executive functioning have been associated with cannabis use that is initiated before the age of 15 (Fontes et al., 2011).

Conversely, onset of cannabis use after the age of 15 does not produce the same long-term consequences. Cannabis withdrawal is common in adults but people do not commonly seek treatment for these symptoms. Cannabis withdrawal can include irritability, difficulty sleeping, restlessness, and changes in mood such as depression and nervousness (Gorelick et al., 2012; Verweij et al., 2013). In sum, the cannabis plant contains cannabinoids that can acutely produce desirable effects but can also have long-term consequence, especially if the onset of use begins at an early age.

Challenges of Cannabis Legalization

Even though cannabis is still illegal according to the United States federal government, the decriminalization of cannabis by several states has led to the development of numerous marketable products with varying levels of potencies (ElSohly et al., 2016). Today the cannabis flowers sold in recreational cannabis shops can contain THC levels averaging around 20% compared to an average of about 4% in 1995 (Elsohly et al., 2016). This increase in potency means that current THC effects are no longer comparable to those observed in earlier research and, thus, the effects of this level of potency are not well understood (Vergara et al., 2017). Furthermore, concentrated waxes that can contain up to 100% THC have been popularized recently (Loflin & Earlywine, 2014). These concentrated waxes known as “dabs” are self-reported to cause increased tolerance and withdrawal to THC (Loflin & Earlywine, 2014). Cannabis is also processed into oils and butters that are turned into candies, drinks, condiments,

and other daily food items, which has led to unforeseen issues. For example, with no federal regulations to control access to these poorly labeled cannabis products there has been an increase in accidental exposure in young children (Davis et al., 2016; Wang et al., 2014).

Cannabis has become the most commonly used illicit substance in the United States (Johnston et al., 2018). The changes in the legalization of cannabis, especially in states supporting cannabis, has led to an increase in reports of cannabis dependence and abuse (Cerdá, Wall, Keyes, Galea & Hasin, 2012). Cannabis use disorder is characterized by the following criteria; the use of cannabis for over 1 year, uncontrolled craving and consumption that are related to the user having difficulty controlling use, difficulty quitting use, or significantly impairing daily life functioning (American Psychological Association, 2013). Although cannabis use disorder is seen at lower rates than other disorders involving illicit substances, its prevalence is increasing primarily among the young adult population (Haberstick et al., 2014; Peer et al., 2013). Late adolescence and early adulthood populations are at the highest risk of cannabis use disorder, with the most susceptible age of onset ranging from 14-24 years of age (Farmer et al., 2015). In fact, patients with a lifetime diagnosis of cannabis use disorder report that their first episode of cannabis dependence was under the age of 18 (Farmer et al., 2015) As a result, this young population is vulnerable to cannabis use disorder, thus, making the consequences of adolescent cannabis use an issue of public health concern (Haberstick et al., 2014).

CHAPTER TWO

THE ENDOCANNABINOID SYSTEM: RECEPTORS

After the primary psychoactive component of cannabis, THC, was identified, it was quickly discovered that this compound works by binding to distinct receptors in the central and peripheral nervous systems. These receptors were labelled cannabinoid receptors, which includes the cannabinoid one (CB1) and cannabinoid two (CB2) receptors (For review; see Howlett et al., 2002). Endogenous ligands were eventually found to bind to these receptors, the two endogenous cannabinoid ligands are anandamide and 2-arachidonoylglycerol (2-AG; Bisogno et al., 1999; Felder et al., 1996). Together, the receptors and the endogenous ligands are known to be important neuromodulators of neuronal activity. In particular, the endocannabinoid system is important for the modulation of pain, feeding, neuroprotection, and reward (For review; see Howlett et al., 2002).

Cannabinoid Receptors

CB receptors have been characterized across human, porcine, primate, and rodent brains (For review; see Howlett et al., 2002). Until recently, CB1 receptors were thought to only exist in the central nervous system whereas CB2 receptors were confined to the peripheral nervous system. Now it is known that both receptors are distributed throughout the brain and body (Gong et al., 2006). CB receptors are composed of seven hydrophobic segments that consist of N-

terminal extracellular and C-terminal intracellular domains (For reviews; see Howlett et al., 2002; Pertwee, 1997; Svíženská, Dubový & Šulcová, 2008). The CB1 receptor has been extensively studied but the CB2 receptor is not as well understood. Additionally, there has been discussion of a third CB receptor, the vanilloid receptor (TRPV1 or VR1), with similar neurological functions and expression as the endocannabinoid system (Cristino et al., 2006).

Adult Distribution and Density

CB1 receptors are expressed in adult rats on the terminal axonal fibers of neurons, specifically on presynaptic terminals (Howlett et al., 2002). CB1 receptors are located throughout the limbic system, are involved in the formation of memories and play an important role in behavior and cognition (Egertová & Elphick, 2000; Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992; Rajmohan & Mohandas, 2007). Overall, the hippocampal formation has more dense binding than other areas of the brain (Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992). The densest binding is found in the molecular layer of the dentate gyrus and the CA1 and CA3 regions of Ammons horn (Egertová & Elphick, 2000; Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992). In contrast, binding in the granule cell layer of the dentate gyrus is scarce (Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992). Similarly, the septum and amygdala exhibit sparse binding (Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992).

In adult rats, the second densest site of CB1 receptors is the basal ganglia, which controls movement, coordination, and procedural learning (Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992). Specifically, within the basal ganglia the highest densities of CB1 receptors are found in the globus pallidus, entopeduncular nucleus, and substantia nigra pars reticulata (Egertová & Elphick, 2000; Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992; Matsuda, Bonner & Lolait, 1993; Tsou, Brown, Sanudo-Pena, Mackie & Walker, 1998). These areas of the basal ganglia show a gradient of binding intensity that increases from the medial to the lateral regions (Egertová & Elphick, 2000). In the striatum, the dorsolateral region has denser binding than the ventromedial area, while the nucleus accumbens has moderate to low densities of CB1 receptors (Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992). The basal ganglia also has CB1 receptors in white matter tracts. Specifically, the striatonigral descending pathway contains detectable CB receptors (Herkenham et al., 1991).

The density of CB1 receptors in the cerebral cortex, the portion of the brain that controls higher level functioning, displays a two-layer pattern. Receptor autoradiography shows high densities in layers I and IV and lower densities in layers II and III (Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992). The hindbrain has low staining in the pons and medulla but intense staining in the cerebellum (Matsuda et al., 1993). Within the cerebellum, which is involved in motor coordination and movement, very dense CB1 binding can be

identified throughout the molecular layer of the cerebellar cortex, but sparse labeling occurs in the cerebellar granular layer (Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992). Furthermore, low densities of CB1 binding occur in the corpus callosum, thalamus, hypothalamus, and midbrain (Egertová & Elphick, 2000; Matsuda et al., 1993; Tsou et al., 1998).

Despite earlier reports that CB2 receptors were only found in the peripheral nervous system, the CB2 receptor has been discovered in brain areas including the orbital, visual, motor, and auditory cortices (Gong et al., 2006). Furthermore, CB2 receptors are found in the anterior olfactory nucleus and the pyramidal neurons of the hippocampus, specifically, the CA2 and CA3 regions. In addition, CB2 receptor staining was found in the thalamus, periaqueductal gray, substantia nigra pars reticulata, midbrain and medulla. There is also intense staining of the Purkinje cell bodies and moderate staining of their dendrites in the cerebellum (Gong et al., 2006). Thus, CB2 receptors are found in similar locations as the CB1 receptors; however, their distribution patterns and densities within these regions differ (Gong et al., 2006).

Gestational Development

There are similarities and differences between the gestational CB receptor system and the adult CB receptor system. The expression of CB1 receptor mRNA has been measured in rats as early as gestational day (GD) 14 (Berrendero et al., 1998). Specific binding at GD 18 is detected in areas such as the hippocampus, cerebral cortex, and cerebellum, which is similar to adults

(Berrendero et al., 1998). CB1 receptor mRNA is detectable in the dentate gyrus of the hippocampus by GD 16 and is localized in the subfields of the Ammon's horn by GD 21. CB1 receptor mRNA progressively increases in the cerebral cortex from GD 16 to GD 21. By GD 21, CB1 receptor binding can be identified in the basal ganglia, hippocampus, cerebral cortex, and cerebellum (Berrendero et al., 1999). Interestingly, CB1 mRNA was measurable in the midbrain, pons, and brainstem from GD 16 to GD 21 whereas these brain regions do not contain cannabinoid receptors in adulthood (Berrendero et al., 1998; Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992). The distribution of CB1 receptors found in the corpus callosum, anterior commissure, fornix, fimbria, fasciculus retroflexum, and the stria medullaris and terminalis are also inconsistent with the distribution of CB1 receptors in adulthood (Berrendero et al., 1998; 1999; Romero et al., 1997).

Postnatal Development

The distribution of CB1 receptors in early postnatal development is consistent with the adult neuronal localization of CB1 receptors (Belue, Howlett, Westlake & Hutchings, 1995). The densities of CB1 receptors in the basal ganglia and limbic system steadily increase from PD 5 to adulthood (Berrendero et al., 1999). On the other hand, binding levels in the caudate putamen, septum nuclei, and nucleus accumbens appear to first decrease from GD 21 until birth, before they begin to increase postnatally (Belue et al., 1995; Berrendero et al., 1999). The striatal levels of CB1 receptors double from PD 0 to PD 7 and then

double again by PD 21 before reaching adult levels (Belue et al., 1995; Berrendero et al., 1999). CB1 receptors located on external (II-III) and internal (V-VI) layers of the cerebral cortex continue to increase at a consistent rate from PD 21 until reaching adult levels (Berrendero et al., 1998; Berrendero et al., 1999; Herkenham et al., 1991; Mailleux & Vanderhaeghen, 1992).

An increase in CB1 receptor levels across development is also observed with receptor binding in the cerebellum and cortex, except that CB1 binding in the cortex increases less from PD 14 to adulthood than in the striatum and cerebellum (Belue et al., 1995). CB1 receptors in the hippocampus display a gradual increase throughout development before reaching adult levels (Belue et al., 1995). Lastly, the densities of CB1 receptors found in white matter areas, such as the corpus callosum and sub-ventricular zone of the neocortex, during gestational development, are no longer visible during postnatal development and in adulthood (Berrendero et al., 1998; Berrendero et al., 1999; Romero et al., 1997).

Cellular Signal Transduction

Stimulation of both CB1 and CB2 receptors causes the activation of cellular signal transduction through $G_{i/o}$ protein pathways (For reviews; see Howlett, 2002; Pertwee, 1997). These receptors, when activated, cause the inhibition of cyclic AMP formation. Through this inhibition, CB receptors modulate intracellular cyclic AMP, which regulates ion channels via protein kinase A (for reviews; see Howlett, 2002; Pertwee, 1997). CB receptors are

coupled to inwardly rectifying potassium channels. Through the $G_{i/o}$ protein pathway, CB receptors inhibit voltage-gated calcium channels which increases intracellular calcium (For reviews; see Howlett, 2002; Pertwee, 1997). CB receptors also stimulate the phosphorylation of mitogen-activated protein kinase. Lastly, CB1 and CB2 receptors facilitate immediate early gene expression and, regulate protein synthesis (For reviews; see Howlett, 2002; Pertwee, 1997).

Cannabinoid Receptors and Neurotransmission

Activation of CB1 receptors on the presynaptic terminals inhibits the release of both excitatory and inhibitory neurotransmitters (For review; see Howlett et al., 2002). CB1 receptors are heavily involved in GABAergic inhibition in the globus pallidus, substantia nigra and hippocampus (Maneuf, Crossman, & Brotchie, 1996; Maneuf, Nash, Crossman & Brotchie, 1996; Hoffman & Lupica, 2000; Romero, De Miguel, Ramos & Fernández-Ruiz, 1997). Furthermore, activation of cannabinoid receptors can also increase the release of other transmitters. For example, dopamine release is stimulated in the nucleus accumbens, ventral tegmental area, and substantia nigra by the activation of CB1 receptors (Oleson & Cheer, 2012). In addition, when presynaptic CB1 receptors are activated there are increases in acetylcholine in the hippocampus (Acquas, Pisanu, Marrocu & Di Chiara, 2000) and glutamate release in the cerebral cortex (Ferraro et al., 2001). Overall, CB1 receptors modulate synaptic transmission across the brain (Kreitzer, 2005).

CHAPTER THREE

THE ENDOCANNABINOID SYSTEM: ENDOGENOUS CANNABINOIDS

Once CB receptors were discovered, it was apparent that there were endogenous ligands that must bind to these receptors. Anandamide was identified first and named after the Sanskrit word for bliss because its effects were similar to THC (Devane et al., 1992). Next, 2-AG was identified and was found to be more abundant in the brain than anandamide (Stella, Schweitzer & Piomelli, 1997). These two endocannabinoids are responsible for the stimulation of CB receptors and the neuromodulatory actions of the endocannabinoid system (For review, see Howlett, 2002).

Anandamide

Distribution

The distribution of anandamide in the brain coincides with the known distribution of cannabinoid receptors (Bisogno et al., 1999; Felder et al., 1996). Thus, rats have the highest levels of anandamide in the hippocampus, brainstem, medulla, and striatum (Bisogno et al., 1999; Felder et al., 1996); whereas, low levels of anandamide are found in the cerebellum, thalamus, diencephalon, and cortex (Bisogno et al., 1999; Felder et al., 1996). The precursor for anandamide, N-arachidonoyl phosphatidylethanolamine (NArPE), is also measured in high concentrations in the brainstem, striatum, and hippocampus while low

concentrations occur in the cerebellum. The levels of NArPE are much greater than anandamide (Bisogno et al., 1999).

Synthesis and Release

Anandamide is created from free arachidonic acid and ethanolamine (Sugiura et al., 1996). Anandamide is produced on demand and its biosynthesis is controlled by intracellular calcium levels that activate phospholipase D and catalyze NArPE hydrolysis (For review, see Basavarajappa, 2007, Cadas, Di Tomaso & Piomelli, 1997; Di Marzo et al., 1994). NArPE activates N-acyltransferase (NAT), which causes the movement of an acyl group from phospahatidylcholine to the ethanolamine portion of phosphatidylethanolamine, thus producing N-acyl phosphatidylethanolamine (NAPE; Basavarajappa, 2007; Cadas et al., 1997; Di Marzo et al., 1994,). Anandamide and phosphatic acid are then released into the synaptic cleft after cleavage by NAPE specific phospholipase D (For review, see Basavarajappa, 2007). It is unclear if the rate limiting step is the cleavage by NAPE-specific phospholipase D or the activation of NAT (Hansen, Hansen, Schousboe & Hansen, 2000; Maccarrone et al., 1998).

Metabolism

After the release of anandamide into the extracellular space, it experiences selective and rapid uptake through the anandamide membrane transporter (Deutsch et al., 2001). After anandamide is removed, intracellular degradation occurs by enzymatic hydrolysis (Deutsch & Chin, 1993; Di Marzo et al., 1994). The enzyme that causes anandamide hydrolysis, fatty acid amide

hydrolase (FAAH), is properly known as arachidonoylethanolamide amidohydrolase (Deutsch et al., 2001; Maccarrone & Finazzi-Agró, 2002). The metabolism of anandamide occurs when FAAH breaks the amide bond, which causes the release of arachidonic acid and ethanolamine (Deutsch et al., 2001; Maccarrone & Finazzi-Agró, 2002).

Behavioral Effects

Anandamide can cause a wide range of behavioral effects. In rats, it increases in the motivation to eat, frequency of eating, and food intake as well as reduced eating latency are observed after injections of anandamide (Martínez-González et al., 2004; Williams & Kirkham, 2002). Sexual behavior also changes when rats are given injections of anandamide. Specifically, the frequency of ejaculation changes in a dose-dependent manner with a low dose decreasing ejaculations and a high dose increasing ejaculation. Anandamide also reduces pain in the formalin test (Guindon, De Léan, & Beauliue, 2006). In addition, anandamide modulates sleep wake cycles by causing rapid eye movement and slow-wave sleep II, which regulates alertness in rats (Murillo-Rodriguez et al., 1998). Furthermore, anandamide and the exogenous cannabinoid THC affect open field behavior similarly because both THC and anandamide decrease grooming, rearing, and motor behavior while increasing periods of inactivity (Romero et al., 1995).

Interestingly, both non-human primates and rats will intravenously self-administer anandamide (Justinova, Solinas, Tanda, Redhi & Goldberg, 2005;

Solinas, Justinova, Goldberg & Tanda, 2005). This self-administration behavior is accompanied by an elevation of dopamine levels in the accumbens shell and suggests that anandamide may have rewarding properties (Solinas et al., 2006). Additionally, anandamide modulates the release of other neurotransmitters. For example, anandamide decreases serotonin in the hippocampus and increase it in the hypothalamus (Hao, Avraham, Mechoulam & Berry, 2000). Anandamide also increases dopamine in the hippocampus and hypothalamus as well as increases cortisol levels (Hao et al., 2000). Overall, anandamide modulates hunger, sexual activity, alertness, and neurotransmission and, like THC, produces rewarding effects (Guindon et al., 2006; Hao et al, 2000; Justinova et al., 2005; Kirkham & Williams, 2001; Martínez-González et al., 2004; Romero et al., 1995; Solinas et al., 2006;).

2-Arachidonoylglycerol

Distribution

Distribution of 2-AG has the highest levels in the brainstem and hippocampus and moderate to high levels in the limbic forebrain and striatum (Bisogno et al., 1999). The lowest levels of 2-AG are found in the hypothalamus, cerebellum, and anterior pituitary (Bisogno et al., 1999; Sugiura & Waku, 2000). Interestingly, 2-AG levels are found to fluctuate with the light/ dark cycle in rats (Valenti et al., 2004).

Synthesis and Release

The biosynthetic pathways of 2-AG include a few possible routes (For review, see Basavarajappa, 2007). The first pathway is mediated by phospholipase C hydrolysis to produce diacylglycerol which, in turn, is converted into 2-AG by diacylglycerol lipase (Sugiura et al., 1995). The second possible 2-AG biosynthesis route is through the hydrolysis of phosphatidylinositol from phosphatidylinositol-specific phospholipase A1, which is converted into 2-AG by lyso-phosphatidylinositol specific phospholipase C (Sugiura et al., 1995). Therefore, it is clear that phospholipase C and diacylglycerol lipase are important for 2-AG synthesis but it is unclear the main biosynthetic pathway.

Inactivation and Metabolism

The inactivation of 2-AG occurs by reuptake through the anandamide membrane transporter and is then metabolized into 2-arachidonyl LPA by monoacyl glycerol kinase, which is then converted into 1-stearoyl-2 arachidonoyl PA (For review, see Basavarajappa, 2007).

Behavioral effects

Multiple behavioral and neurological functions are modulated by 2-AG. Elevated levels of 2-AG have been found after head injury and this elevation may help to reduce brain edema and hippocampal cell death and improve the level of recovery (Panikashvili et al., 2001). 2-AG serves as the immediate response to reduce inflammation (Berdyshev, Schmid, Krebsbach & Schmid, 2001) and 2-AG inhibits invasive prostate cancer cells (Nithipatikom et al., 2004). Furthermore, 2-

AG is involved in stress-related behaviors. For example, the formation of 2-AG is triggered by stress and helps enhance stress-induced analgesia (Hohman et al., 2005). 2-AG levels are also elevated after chronic stress exposure suggesting a role in preventing the development of anxiety (Sumislawski, Ramikie, & Patel, 2011).

2-AG has been linked to the rewarding properties of stimuli such as food and drugs. Mice given high fat diets show an increase in hypothalamic 2-AG, which may increase the rewarding and reinforcing effects of the high fat diet (Higuchi et al., 2012). Squirrel monkeys self-administer 2-AG, which shows that it has reinforcing properties like drugs of abuse (Justinova, Yasar, Godfrey, Redhi & Goldberg, 2011). Overall, 2-AG is a neuroprotectant and cancer growth inhibitor, it has anti-inflammatory and analgesic properties, reduces stress, and augments reward circuitry (Berdyshev et al., 2001; Higuchi et al., 2012; Hohman et al., 2005; Justinova et al., 2011; Nithipatikom et al., 2004; Panikashvili et al., 2001; Sumislawski et al., 2011; Vigano et al., 2003).

CHAPTER FOUR

EXOGENOUS CANNABINOIDS

The cannabis plant includes over 70 chemicals that are responsible for the psychoactive and medical properties experienced by the user (ElSohly & Slade, 2005). The actions of the chemical constituents in cannabis have been mimicked and inhibited with synthetic compounds that can bind to CB1 and CB2 receptors (Pertwee et al., 2010). Both plant and synthetic cannabinoids have allowed for a more in-depth examination of the behavioral outcomes associated with CB receptors and have given insight into the appeal of cannabis use recreationally and medically.

Tetrahydrocannabinol

THC is the most commonly studied exogenous cannabinoid and produces a wide range of actions. The psychological aspects of THC can be separated into four categories: affective (euphoria), sensory (increased perception of stimuli), somatic (body sensations), and cognitive (problems with concentration, perception and time estimation; Perez-Reyes, 1999). The psychological and physiological effects of THC have been thoroughly assessed. This cannabinoid can influence cognition by altering perception and psychomotor performance as well as regulate emotional states (For review, see Grotenhermen, 2007). Futhermore, THC modulates functions of the nervous system, cardiovascular system, hormonal system, immune system and respiratory system (For review, see Grotenhermen, 2007)

THC can be absorbed into the body via multiple routes of administration. Inhalation is the most common way THC enters the human body (Agurell et al., 1986). The bioavailability of inhaled THC ranges from 2-56% because there is variability in the frequency and quantity of THC use depending on the individual. After smoke inhalation, the blood plasma levels of THC peak quickly within anywhere from 3 to 10 min (Huestis, 2005). In contrast, the oral administration of THC has a much slower onset of effects and more erratic effects compared to inhalation (Law, Mason, Moffat, Gleadle & King, 1984). The bioavailability of THC taken orally is about 10-20% after it is absorbed into the gastrointestinal tract and liver (Wall, Sadler, Brine, Taylor & Perez-Reyes, 1983). When ingested, the effects of THC peak between 60 and 120 minutes and last an average of 4-6 hr (Ohlsson et al., 1982).

As shown in rats, once absorbed into the body, THC binds to lipoproteins and 90% of THC is found in blood plasma and 10% is found in red blood cells (Fehr & Kalant, 1974). THC is primarily metabolized by cytochrome P450, with over 80 different metabolites produced (Huestis, 2005; Sharma, Murthy & Bharath, 2012). At low doses (16 mg), THC is eliminated from plasma within 3 to 6 hours whereas high doses (34 mg) can take 6-27 hr (Huestis, 2005). The half-life of THC is 25-26 hr but in heavy users the half-life can range from 19-53 hr (Hunt & Jones, 1980; Wall, Sadler, Brine, Taylor & Perez-Reyes, 1983). Within 5 days of use, up to 90% of THC has been eliminated from the body, over 65% is excreted in the fecal matter, and around 25% through urine (Huestis, 2005; Wall

et al., 1983). Overall, a single dose of THC can be detected in the body for up to 12 days, moderate use can be detected for around 30 days, and THC can be detected up to 77 days in heavy users (Ellis, Mann, Judson, Schramm & Tashchian, 1985). A lethal human dose of cannabis has not been reported but an oral dose of 800-1900 mg/kg THC is lethal to rodents (Grotenhermen, 2007; Thompson, Rosenkrantz, Schaeppi & Braude, 1973).

THC is a partial agonist of both CB1 and CB2 receptors, but with lower efficacy for the CB2 than CB1 receptor (Howlett et al., 2002; Pertwee, 2008). THC activates CB1 receptors located on presynaptic terminals in the central nervous system, thus modulating the release of neurotransmitters glutamate, GABA, dopamine and acetylcholine (Pertwee & Ross, 2002). These neuromodulatory actions have been observed in the nucleus accumbens and synaptic projections that extend to the ventral tegmental area, hippocampus, and prefrontal cortex (Pertwee, 2008; Pertwee & Ross, 2002). In sum, THC is a non-lethal drug that takes an average of 30 days to be excreted from the body (Ellis et al., 1985). This partial cannabinoid receptor agonist is involved in numerous behavioral and neurological processes that induce reward as well as medical value.

Cannabidiol

The plant cannabinoid, cannabidiol (CBD), does not produce the euphoric effects observed with THC but it can have a wide range of effects, including antipsychotic, antiepileptic, anxiolytic, and anti-inflammatory actions (Izzo,

Borrelli, Capasso, Di Marzo & Mechoulam, 2009). Inhalation of CBD has an average bioavailability of 31% after a single use (Ohlsson et al., 1986). CBD binds to CB1 and CB2 receptors where it acts as both an antagonist and inverse agonist (Pertwee, 2008; Thomas et al., 2007) and inhibits the effects of the synthetic cannabinoid agonists CP55940 and R-(+)-WIN 55,212 (Pertwee et al., 2002). CBD has therapeutic value for the treatment of symptoms associated with cancer, arthritis, anxiety, diabetes, and immune disorders (For review, see Mechoulam, Peters, Murillo-Rodriguez & Hanuš, 2007). Overall, CBD has value as a medical treatment for a variety of ailments without the psychoactive side-effects experienced with THC.

Synthetic Cannabinoids

Synthetic cannabinoids such as CP, HU-210, and R-(+)-WIN 55,212 bind to both CB1 and CB2 receptors and have been used to characterize the CB1 receptor system (For review, see Howlett et al., 2002). These synthetic CB receptor agonists have similar behavioral effects as THC and endogenous cannabinoids including hypothermia, analgesia, catalepsy, and locomotor suppression (Tai & Fantegrossi, 2014). These agonists modulate neurotransmission by inhibiting GABA release in the substantia nigra and hippocampus whereas synthetic cannabinoids increase acetylcholine in the hippocampus, dopamine in the nucleus accumbens, and glutamate in the cerebral cortex (For review, see Howlett et al., 2002). Overall, synthetic cannabinoid agonists have similar physiological and behavioral effects as THC.

CB antagonists, such as SR14716A, AM281, and LY320135, block the effects of the CB receptor system (Pertwee, 2005). The antagonist AM281 can reduce food intake in rats, increase locomotor activity in mice, and increase glutamate release in the cerebellum (Pertwee, 2005). In contrast, LY320135 blocks the effects of CB receptor agonists and works as an inverse agonist in the CB1 signal transduction pathway (Howlett et al, 2002). The most studied antagonist is SR14716A because this potent CB1 ligand is able to inhibit CB receptor agonists as well as reverse the effects of the CB1 and CB2 receptors (Howlett et al, 2002). The behavioral effects that are observed with SR14716A include enhanced locomotor activity, hyperalgesia, and pro-inflammatory responses (Pertwee, 2005). SR14716A increases the release of acetylcholine, epinephrine, and GABA in the hippocampus as well as increase glutamate in the prefrontal cortex and striatum (Pertwee, 2005). Overall, these antagonists reverse the actions of the cannabinoid system.

CHAPTER FIVE

BEHAVIORAL SENSITIZATION

The prevalence of cannabis use disorder has led to the conclusion that cannabis has addictive properties (Hasin et al., 2015). Therefore, it is important to have a better understanding of how cannabis alters behavior and leads to compulsive drug taking. Animal models, including self-administration, drug discrimination, conditioned place preference, and behavioral sensitization, have been invaluable tools for studying the addictive properties of drugs such as cannabis (Maldonado & de Fonseca, 2002; Sanchis-Segura & Spanagel, 2006). In particular, behavioral sensitization examines incentive value or "craving" for a given drug (For reviews, see Robinson & Berridge, 1993, 2000, 2001, 2003; Steketee & Kalivas, 2011).

Behavioral sensitization is a phenomenon in which prior exposure to a drug leads to an enhanced behavioral response to later administration of that drug (For reviews, see Robinson & Berridge, 1993, 2000, 2001, 2003; Steketee & Kalivas, 2011). In animals, this occurs through the process of induction, or the pre-exposure phase, in which the animal is exposed to the drug either once or numerous times; and expression, or the test phase, when the animal is exposed to the drug after a period of abstinence (For reviews, see Robinson & Berridge, 1993, 2000, 2001, 2003; Steketee & Kalivas, 2011). Behavioral sensitization can be assessed by monitoring changes in stereotyped and non-stereotyped behaviors during both the induction and expression phases (For reviews, see

Robinson & Berridge, 1993, 2000, 2001, 2003; Pierce & Kalivas, 1997; Steketee & Kalivas, 2011). Stereotyped behaviors include actions such as gnawing, licking, and undirected sniffing whereas non-stereotyped behaviors include exploratory sniffing, locomotor activity, and rearing (Rubino, Viganò, Massi & Parolaro, 2001). Sensitization can be affected by associative processes such that the enhanced behavioral effect is stronger or exclusively observed when the animal is tested in the same environment in which the drug was initially given (For review, see Robinson, Browman, Crombag & Badiani, 1998). This type of sensitization is referred to as context-dependent sensitization whereas sensitization that is apparent without a consistent context is known context-independent sensitization (For review, see Robinson et al., 1998).

Adult Sensitization

Dose-dependence

Sensitization can be dependent on the amount of drug that is given. For example, psychostimulants cause a dose-dependent enhanced behavioral response such that low doses of psychostimulants produce sensitization but the intensity of the sensitized behavior becomes more robust with higher doses (Browman, Badiani, & Robinson, 1998a, 1998b; Davidson, Lazarus, Lee & Ellinwood, 2002; Frantz, O'Dell, & Parsons, 2007).

Multi-trial vs. Single-trial Sensitization

An enhanced behavioral response may occur after a number of pre-exposures or only one pre-exposure to a drug. Both psychostimulant induced

multi-trial and single-trial sensitization have been extensively examined. During multi-trial sensitization, the animal is pretreated with the drug repeatedly over a period of time (typically at daily intervals) and then examined for changes in behavior after an abstinence period (for reviews, see Pierce & Kalivas, 1997; Steketee & Kalivas, 2011). The sensitized response in single-trial sensitization tends to require a relatively high dose of the drug (Battisti, Chang, Uretsky & Wallace, 1999; Fontana, Post & Pert, 1993). The expression of the sensitized response can persist long after the animal has been exposed to the drug (Leith & Kuczenski, 1982). This enhanced behavioral responsiveness after one or many drug exposures may be relevant to drug relapse and the continuation of drug use (Robinson & Berridge, 1993).

Psychostimulant induced multi-trial sensitization can been seen after short and long periods of abstinence. For example, adult rats given cocaine for 4 consecutive days show a sensitized response 48 hr later (Laviola, Wood, Kuhn, Francis & Spear, 1995). Sensitization to 5 days of repeated cocaine exposure is observed in adult rats after a 3 week abstinence period (Ujike, Tsuchida, Akiyama, Fujiwara, & Kuroda, 1995). Furthermore, the persistence of multi-trial sensitization can also be seen after a 12 week abstinence period when pretreated for 6 days with amphetamine (Leith & Kuczenski, 1982).

Nonetheless, multiple days of pre-exposure are not necessary to cause an enhanced behavioral response because adult rats given a single pretreatment injection of cocaine show a sensitized response to the drug 24 hr later

(McDougall, Baella, Stuebner, Halladay & Crawford, 2007). This can also be observed in adult mice when given single pretreatment injection of amphetamine and examined for sensitization 24 hr later (Battisti et al., 1999). Although the persistence of single-trial psychostimulant sensitization is not as apparent as is it with multiple exposures, mice exposed to single injection of methamphetamine have shown a sensitized response up to 21 days after exposure (Jing et al., 2014).

Context-dependent vs. Context-independent Sensitization

Differences in sensitization occur depending on the environment in which the drug was given (For review, Robinson et al., 1998). Multi-trial behavioral sensitization is stronger when the induction and expression of psychostimulant sensitization are conducted in the same environment but is apparent if the environments are not the same (For review, Robinson et al., 1998). Adult rats given psychostimulants show context-independent sensitization although it is not as robust as with context-dependent sensitization (Crombag, Badiani, Maren & Robinson, 2000; Badiani, Browman & Robinson, 1995; Browman et al., 1998a; 1998b; Laviola et al., 1995). This demonstrates that the enhanced behavioral response associated with multi-trial sensitization is sensitive to, but not dependent on, the context that the drug is given in (Crombag et al., 2000; Baldiani et al., 1995; Laviola et al., 1995). One-trial sensitization, however, is completely context-dependent in adult rats (McDougall et al., 2007). This means that a single pre-exposure to a psychostimulant, such as cocaine, will not cause

behavioral sensitization if the rat is challenged with cocaine in a different environment (Fontana, Post & Pert, 1993; McDougall et al., 2007; Weiss, Post, Pert, Woodward & Murman, 1989). Therefore, the associative context of drug exposure modulates the intensity of the sensitized response in adult rats.

Adolescent Sensitization

Multi-trial vs. Single-trial Sensitization

Multi-trial psychostimulant induced behavioral sensitization is also apparent early adolescent (PD 28) and late adolescent (PD 42) rats (Caster, Walker & Kuhn, 2005). Specifically, when these male Sprague-Dawley rats are exposed to escalating doses of cocaine both age groups express sensitization after multiple drug exposures but early adolescent animals show a more robust sensitization compared late adolescent rats (Caster et al., 2005). Adolescent animals also display gender differences after repeated treatment with the psychostimulant methylphenidate, as Sprague-Dawley female rats show enhanced locomotor activity compared to males (Chelaru, Yang & Dafny, 2012). Furthermore, repeated exposure to cocaine during adolescence causes a persistent sensitized response that can be observed after a short (PD 37) and/or long (PD 64) abstinence period (Marin, Cruz & Planeta, 2008).

The effects of psychostimulants using one trial sensitization are similar to what has been observed with multiple trial sensitization during adolescent development. Young rats (PD 19) given a single pretreatment injection of cocaine show a sensitized locomotor response for up to 5 days after initial drug

exposure (McDougall et al., 2009). Both early adolescent (PD 28) and late adolescent (PD 42) rats show a sensitized behavioral response to a single high dose of cocaine, although this response is more robust in the younger animals (Caster, Walker & Kuhn, 2007). Additionally, amphetamine sensitization is observed in early adolescent rats (PD 30) when tested 2 or 30 days later (PD 60; Mathews, Kelly & McCormick, 2011). Overall, psychostimulant induced behavioral sensitization is observed in pre-adolescent and adolescent rats using both single-trial and multi-trial procedures.

Associative vs Non-associative Sensitization

Context is not as important for sensitization in young rats as it is in adult rats. Preweanling rats (PD 19) given a single pretreatment injection of cocaine show both context-dependent and context-independent sensitization for up to 5 days with a 10 mg/kg challenge of cocaine (McDougall et al., 2007). In fact, these young rats actually showed stronger sensitization in a context-independent environment relative to a context-dependent environment (McDougall et al., 2007). Stronger sensitization in a context-independent environment can be seen 1 and 3 days after exposure (McDougall et al., 2009).

Although young rats experience context-independent sensitization after a short abstinence period, environmental conditioning is important for sensitization in these pups after a longer drug-free period (Zavala, Nazarian, Crawford & McDougall, 2000). Specifically, rat pups pretreated for 5 days with cocaine only show context-dependent sensitization after a 7-day drug free period; whereas,

after 1 day of abstinence, preweanling pups show both context-independent and context-dependent sensitization (Zavala et al., 2000).

Behavioral Sensitization using Cannabinoids

Although behavioral sensitization has become a common tool for studying abused drugs, relatively little sensitization research has been conducted with cannabinoids. This is especially true when examining early and late adolescent rats. Cannabinoids, like psychostimulants, can produce behavioral sensitization that persists after the discontinuation of drug use (Rubino et al., 2001). In the first report of cannabinoid multi-trial sensitization, adult Sprague-Dawley male rats were pretreated with THC twice a day for 5 days (Rubino et al., 2001). All animals received a dosage regimen in which the amount of THC administration increased over the 5-day period (5, 10, 20, 40 and 40 mg/kg) and sensitization was assessed after a long abstinence period of 20 days (Rubino et al., 2001). Pretreatment with THC sensitized behaviors associated with stereotyped activity including gnawing, licking, and undirected sniffing after a 5 mg/kg THC challenge injection. Furthermore, a trend was noticed in non-stereotype activities, including a slight increase in forward locomotion, sniffing, and rearing (Rubino et al., 2001).

Different patterns of sensitization occur depending on the length of exposure and the dose of THC administered. After 3 days of an increasing THC regime (2, 4, and 8 mg/kg), adult male Sprague-Dawley rats exhibited enhanced non-stereotyped activity when challenged 14 days later with THC (150 µg/kg intravenous; Cadoni, Pisanu, Solinas, Acquas & Chiara, 2001; Cadoni, Valentini

& Di Chiara, 2008). This enhancement may have occurred due to the short exposure period to THC and short abstinence period (Cadoni et al., 2001; Cadoni, Valentini & Di Chiara, 2008). Overall, pretreatment with THC can cause changes to both non-stereotyped and stereotyped behaviors that are associated with an increased sensitized response to the drug.

Like THC, the synthetic cannabinoid agonist WIN 55,212-2 can cause the induction of behavioral sensitization after a single exposure or multiple exposures (Enayatfard et al., 2013). Specifically, an enhanced behavioral response to WIN 55,212-2 was seen after a single pretreatment administration of a low dose (0.1 kg/mg, IP) when compared to saline-pretreated rats. After a 10-day regimen of WIN 55,212-2 pretreatment rats also showed a sensitized response that persisted 48 hr after the final pretreatment day (Enayatfard et al., 2013). Furthermore a single exposure to WIN at a low dose has been shown to increase locomotor responses whereas a single exposure to a high dose of WIN did not enhance motor activity in the open field test (Drews et al., 2005).

Cross-sensitization with Other Drugs

Interestingly, the ability of cannabinoids to cross-sensitize with other drugs has been examined more thoroughly than it has been for cannabinoid-induced sensitization. For example, in adults, a single pre-exposure to either THC or WIN 55,212-2 increases sensitization to amphetamine when given 30 min later (Gorriti, de Fonseca, Navarro & Polomo, 1999; Muschamp & Siviy, 2002). Also, multiple exposures of THC or WIN 55,212-2 increases the sensitized response to

amphetamine (Gorriti et al., 1999; Muschamp & Siviy, 2002). WIN 55,212-2, when acutely injected, affects both ambulatory and rearing activity of animals that are given amphetamine 30 min later (Muschamp & Siviy, 2002). When these adult rats are exposed to WIN 55,212-2 for 10 days, an increase in ambulatory movement and rearing activity is observed after a challenge injection of amphetamine (Muschamp & Siviy, 2002). Additionally, adult rats treated with morphine show an increased behavioral response when pretreated with a low or high dose of either THC or CP (Cardoni et al., 2001; Cardoni et al., 2008; Norwood, Cornish, Mallet & McGregor, 2003).

Ontogeny of Cannabinoid Sensitization

Unfortunately, very limited research has been conducted on the ontogeny of cannabinoid sensitization. At present, it is unknown whether cannabinoid sensitization, like psychostimulant sensitization, would differ between adolescent and adult rats. Interestingly, there has been one investigation that showed, that an 8-day THC pretreatment induced a sensitized response to a cocaine challenge in late adolescent rats but not in adult rats (Dow-Edwards & Izenwasser, 2012).

CHAPTER SIX

SUMMARY THESIS STATEMENT

Both the medical and recreational use of cannabis are now decriminalized in California and many other states. Since the change in these state laws, there has been surges in the availability and use of cannabis (Cerdá, Wall, Keyes, Galea & Hasin, 2012; Hasin et al., 2015). A major concern with the rise in societal acceptance of cannabis and the increased availability of this drug is higher use rates in adolescent populations (Kosterman, Hawkins, Guo, Catalano, & Abbott, 2000). Escalation of cannabis use in adolescents is important because the risk of developing cannabis use disorder is stronger in people who start drug use early (Richter, Pugh & Ball, 2016). The use of cannabis before the age of 15 increases the likelihood of becoming a chronic user and enhances the probability of experimenting with other illicit drugs (Nelson, Van Ryzin, & Dishion, 2015; Prince van Leeuwen et al., 2014; Richter et al., 2016). Moreover, adolescent use of cannabis can cause long-term consequences, including problems with executive functioning, that are not apparent in users that begin after the age of 15 (Fontes et al., 2011).

The cause for the inflation in problematic cannabis use with early onset is unknown but an increase in the addictive properties of cannabis during this developmental period may partially account for this. To this end, the present thesis focused on the adolescent response to cannabis using the behavioral sensitization paradigm. Behavioral sensitization is an animal model used to study

craving, which is an important component of drug addiction (Berridge & Robinson, 1995; For reviews, see Robinson & Berridge, 1993, 2000, 2001, 2003). In this model, drugs with addictive properties induce an augmented behavioral response in animals after prior exposure to the drug (for reviews, see Robinson and Berridge, 1993, 2000, 2001, 2003; Steketee & Kalivas, 2011). Behavioral sensitization can be measured as changes in stereotypical and non-stereotypical behaviors (For reviews, see Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). Behavioral sensitization is sensitive to changes in environmental contexts, number of drug pre-exposures, and developmental stage.

Currently, no studies have examined developmental differences in behavioral sensitization to cannabinoids and very little data exists on the acute effects of cannabinoids adolescence (For reviews, see Jacobus & Tapert, 2014; Viveros, Llorent, Moreno, & Marco, 2005). Thus, the purpose of this thesis was to examine the effects of the cannabinoid agonist CP in early adolescent Sprague-Dawley rats (PD 30-36) using the behavioral sensitization paradigm. CP was used because it mimics the effects of THC, the primary psychoactive ingredient in cannabis (Gurney, Scott, Kacinko, Presley & Logan, 2014). Specifically, this study assessed the ability of CP to induce behavioral sensitization in adolescent rats and determine if there are gender differences in the sensitization to CP. In addition, we also examined the ability of CP to induce cross-sensitization to cocaine in adolescent rats.

Despite the limited data available on developmental sensitization to cannabinoids, the following four hypotheses were formulated:

1. Repeated treatment with CP would induce a sensitized behavioral response in male and female adolescent rats.

As mentioned above, there is limited data on the response of adolescent rats to cannabinoid agonists. However, based on the adult literature assessing behavioral sensitization with cannabinoid agonists (Cadoni et al., 2001; Cadoni et al., 2008; Enayatfard et al., 2013; Rubino et al., 2001) and literature showing that adolescent rats are more often more sensitive to drug exposure than adults (For review, see Izenwasser, 2005), it was predicted that repeated treatment with the CP compound would result in a augmented behavioral response in adolescent rats.

2. Female adolescent rats would show a greater behavioral response to CP than male adolescent rats.

Gender differences are common in the behavioral response to drugs of abuse. Adult female rats are more sensitive to the behavioral effects of cannabinoids because female rats given either THC or CP have a greater antinociceptive response, show increased catalepsy, and display more spontaneous locomotor activity compared to male rats (Tseng & Craft, 2001). Additionally, female rats display greater sensitization to psychostimulants (Chelaru et al., 2012). Based on the evidence that female rats display greater sensitivity to cannabinoids in other behavioral measures using cannabinoids and

show enhanced behavioral sensitization to psychostimulants, it was hypothesized that female rats would show stronger behavioral sensitization to the cannabinoid agonist CP than male rats.

3. Cocaine would induce behavioral sensitization and conditioned activity in both male and female adolescent rats.

Cocaine-induced sensitization and conditioned activity are both well documented phenomenon in rats. Cocaine sensitization can be observed in rats as early as PD 19 and has been previously demonstrated in adolescent rats (Caster et al., 2005, 2007; McDougall et al., 2007, 2009). In contrast, conditioned activity is not found in preweanling and preadolescent rats but is quite robust and persistent in adult rats (McDougall et al., 2014). Based on this literature, it was expected that adolescent rats would show a cocaine sensitized response. We also believed that both the male and female adolescent rats would respond in an adult like manner and show conditioned activity.

4. Repeated treatment with CP would induce a sensitized response to cocaine.

Early adolescent exposure to cannabis increases the likelihood of using other drugs of abuse in human adolescents (Haberstick et al., 2014; Peer et al., 2013; Farmer et al., 2015). The reason for this increased vulnerability is unclear as it is not known whether environmental/social issue drive the increased drug use or changes in neuronal functioning after cannabis exposure. In adult and later adolescent rats there is data showing that exposure to THC or WIN can cause a

sensitized response amphetamine, cocaine and morphine (Dow-Edwards & Izenwasser, 2012; Cardoni et al., 2001; Cardoni et al., 2008; Gorriti et al., 1999; Muschamp & Siviy, 2002). We predicted that early adolescent rats given repeated injections of CP would also show a sensitized response to cocaine.

CHAPTER SEVEN

MATERIALS AND METHODS

Subjects

Male and female rats (*N* = 283) of Sprague-Dawley descent (Charles River; Hollister, CA) were used. Rats were in early adolescence at the time of pretreatment (PD 30- PD 34) and testing (PD 36). All rats were bred and raised in the vivarium of the Psychology Department of California State University, San Bernardino. Dams and pups were housed in maternity cages that were large polycarbonate clear boxes (56 × 34 × 22 cm) with a wire lid. Litters were culled on PD 3 to 10 pups per dam and pups were group housed (3-4 per cage) away from dams on PD 23. All cages had Tek-Fresh® bedding (Harlan, Indianapolis, IN). All animals received food and water ad libitum and kept on a12 hour light and 12 hour dark cycle. Behavioral testing took place during the light cycle with subjects returned to their home cage after testing. All subjects were handled according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of California State University, San Bernardino.

Apparatus

All behavioral testing was performed in commercially available activity monitoring chambers (41 × 41 × 41 cm) from Coulbourn Instruments (Allentown, PA, USA). These chambers were kept in a separate testing room away from the animal colony. The activity chambers consist of four acrylic walls, a gray plastic

floor, and an open top. To measure horizontal locomotor activity or distance traveled, each chamber included an X-Y photobeam array with 16 photocells and detectors with a photobeam resolution of 0.76 cm. The position of each rat was determined every 100 ms (i.e., the sampling interval). Each chamber was equipped with a video camera centered above the chamber.

Drugs

CP and cocaine were purchased from Sigma Aldrich (St. Louis, MO). The CP drug was mixed in a 50% dimethyl sulfoxide (DMSO)/ water solution and cocaine was mixed in saline. All injections were given intraperitoneally (IP) at a volume of 1.0 ml/kg.

Procedure and Statistical Analysis

Experiment 1. Effect of Dose on CP-induced Multi-trial Sensitization in Early Adolescent Male and Female Rats. Adolescent rats (N= 137) were given five pretreatment days (PD 30-PD 34), a 48 hr abstinence period, and one test day (PD 36; see Table 1). During pretreatment, rats were given CP (4, 13.2, or 40 µg/kg, IP) or vehicle in the testing room and then placed immediately into the activity chambers. Following a 10 min habituation period, distance traveled and stereotyped movements were measured for 60 min. After the 48 hr abstinence period, half of the rats in each drug group received 4 µg/kg CP and the other half received 13.2 µg/kg CP. Test day injections of CP were given in the testing room and rats were placed in activity chambers for 120 min. Similar to the conditioning days, behavioral monitoring began after a 10 min habituation period.

Table 1.

Experiment 1 Design and Timeline

	Pretreatment age	Pretreatment doses CP 55,940	Test age	Test dose of CP 55,940
Male or Female	PD 30-PD 34	0, 4, 13.2 or 40 µg/kg	PD 36	4 or 13.2 µg/kg

Experiment 1 Analyses. Distance traveled and repetitive movement data for the pretreatment sessions were analyzed by separate three-way (sex × pretreatment drug × day) mixed factorial ANOVAs. Test day data was analyzed by separate four-way (sex × pretreatment drug × test day drug × time block) mixed factorial ANOVAs. Significant higher-order interactions were further analyzed using two- or one-way ANOVAs. Post hoc analysis were made using Tukey tests ($p < 0.05$). Litter effects were controlled through experimental design, with no more than one subject per litter being assigned to a particular group.

Experiment 2. Cross-sensitization between CP 55,940 and Cocaine in Early Adolescent Male and Female Rats. Adolescent rats (N= 146) were given five pretreatment days (PD 30- PD 34), a 48 h abstinence period, and one test day (PD 36), (see Table 2). The CP pretreatment doses (13.2 or 40 µg/kg) were determined from Experiment 1, cocaine (20 mg/kg) and vehicle (DMSO or Saline). All rats were given one daily IP injection in the experimental chamber. After rats received their injection in the experimental room they were placed in activity chambers. After a 10 min habituation period distance traveled and

stereotyped movements were measured for 60 min. On the test day (PD 36), all animals received an IP injection of cocaine (10 mg/kg) or saline and after 10 min distance traveled and stereotyped movement were measured for 120 min.

Table 2.

Experiment 2 Design and Timeline

	Pretreatment age	Pretreatment dose	Test age	Test dose
Male or Female	PD 30- PD 34	13.2 µg/kg CP 55,940 40 µg/kg CP 55,940 20 mg/kg Cocaine Vehicle	PD 36	10 mg/kg Cocaine Saline

Experiment 2 Analyses. Distance traveled and stereotyped movement data for the pretreatment sessions were analyzed by separate three-way (sex × pretreatment drug × day) mixed factorial ANOVAs. Test day data was analyzed by separate four-way (sex x pretreatment drug× test drug × time block) mixed factorial ANOVAs. Significant higher-order interactions were further analyzed using two- or one-way ANOVAs. Post hoc analysis of data was made using Tukey tests ($p < 0.05$). Litter effects were controlled through experimental design, with no more than one subject per litter being assigned to a particular group.

CHAPTER EIGHT

RESULTS

Experiment 1 examined the dose-response relationship of cannabinoid-induced sensitization. It was predicted that female rats would display an overall greater sensitivity to the cannabinoid doses and that both males and females would show cannabinoid induced sensitization through increased distance traveled and stereotyped movement.

During the pretreatment period a significant main effect of sex was observed in distance traveled scores, $F(1,127) = 5.478$, $p = .02$. Specifically, females had greater distance traveled scores compared to their male counterparts. However, there was no significant main effect of sex in stereotyped movement, $F(1,127) = 3.128$, $p = .11$ (Fig. 1). There was also a trend for a main effect of conditioning drug for distance traveled, $F(3,127) = 2.485$, $p = .06$. This trend was primarily between the vehicle and 40 µg/kg CP group, as animals treated with the high cannabinoid dose showed less activity than rats given vehicle. There was no main effect of the conditioning drug observed in stereotyped movement, $F(3,127) = 2.076$, $p = .11$. Lastly, no interaction occurred between sex and conditioning drug for distance traveled, $F(3,127) = 1.400$, $p = .25$ or stereotyped movement, $F(3,127) = 0.934$, $p = .43$. In summary, there was no difference in the behavioral response of rats given cannabinoids or vehicle during the conditioning phase (Fig. 2).

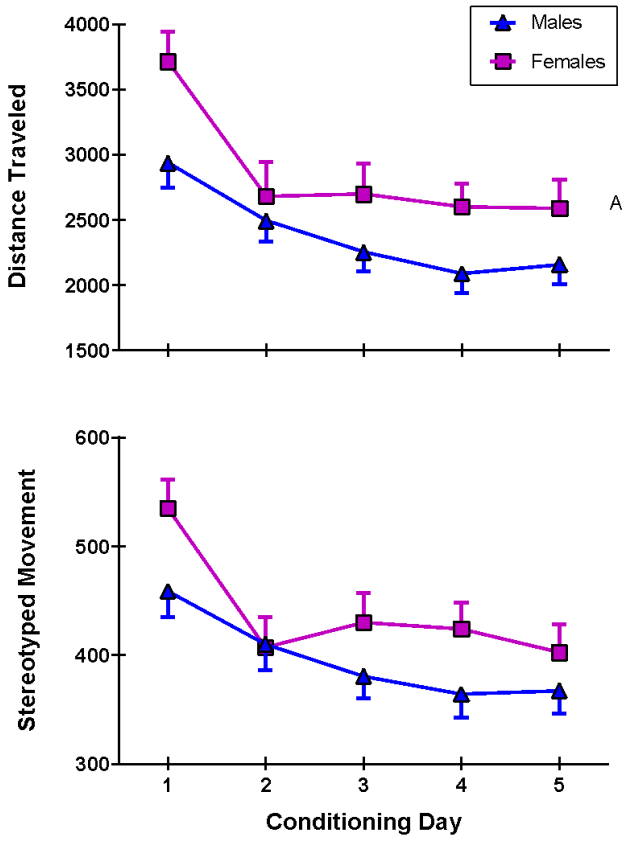

Figure 1. Experiment 1 Conditioning Days by Gender
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of male and female adolescent rats injected with CP 55,940 (4, 13.5, 40 µg/kg, IP) or vehicle and placed in photobeam activity chambers for five conditioning days (PD 30-34). Data on this graph were collapsed across conditioning drug group. [A] Indicated a significant difference from male rats over the 5 day conditioning phase.

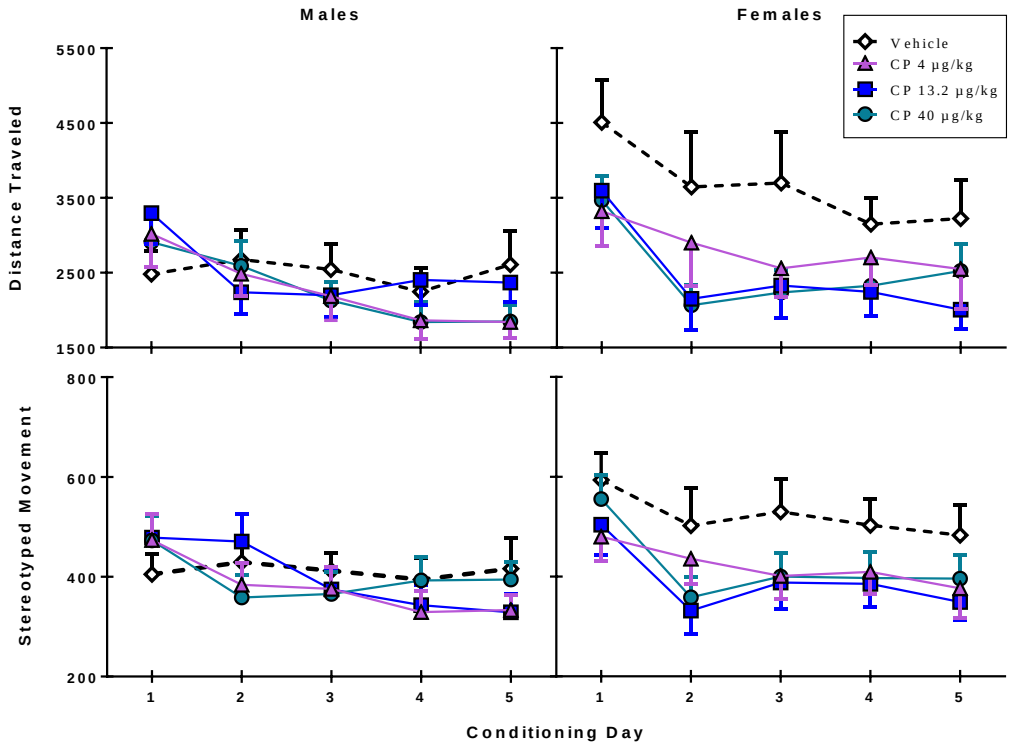

Figure 2. Experiment 1 Conditioning Days by Conditioning Drug
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of male and female adolescent rats injected with CP 55,940 (4, 13.2, 40 µg/kg, IP) or vehicle and placed in photobeam activity chambers for 5 conditioning days (PD 30-34).

On the test day, treatment with CP differentially altered stereotyped movements, $F(1,121) = 7.58$, $p < 0.001$, as rats treated with the low dose of CP (4 µg/kg) had more stereotyped movements ($M = 683.81$, $SEM = 29.14$) than rats given the higher dose of CP (13.2 µg/kg) ($M = 570.73$, $SEM = 26.42$). Distance traveled scores did differ for the two CP drug doses, $F(1,121) = 2.755$, $p = 0.10$ (figure not shown). Pretreatment with CP at all doses failed to significantly enhance distance traveled scores, $F(3,121) = 1.099$, $p = 0.35$, or stereotyped

movements, $F(3,121) = .753$, $p = 0.52$ on the test day. Lastly, there was no interaction between sex, condition drug and test day drug for distance traveled, $F(3,121) = .878$, $p = 0.45$; (Fig. 3) or stereotyped behaviors, $F(3,121) = .581$, $p = 0.63$ (Fig. 4). These results indicate that cannabinoid pre-exposure did not increase the behavioral response to a cannabinoid agonist in either female or male adolescent rats.

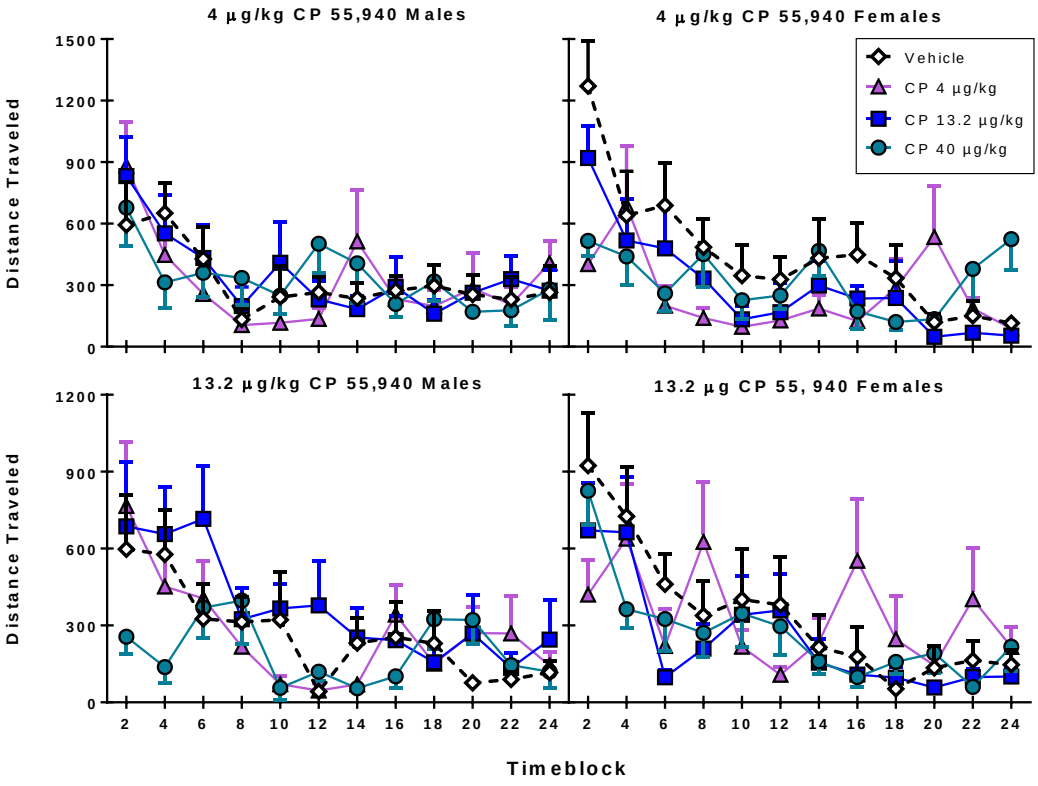

Figure 3. Experiment 1Test Day Distance Traveled
Mean distance traveled scores (± SEM) of male and female adolescent rats injected with CP 55,940 (4, 13.2, 40 µg/kg, IP) or vehicle and placed in photobeam activity chambers on the five conditioning days (PD 30-34) and then challenged with CP 55,940 (4 or 13.2 µg/kg, IP) on test day (PD 36).

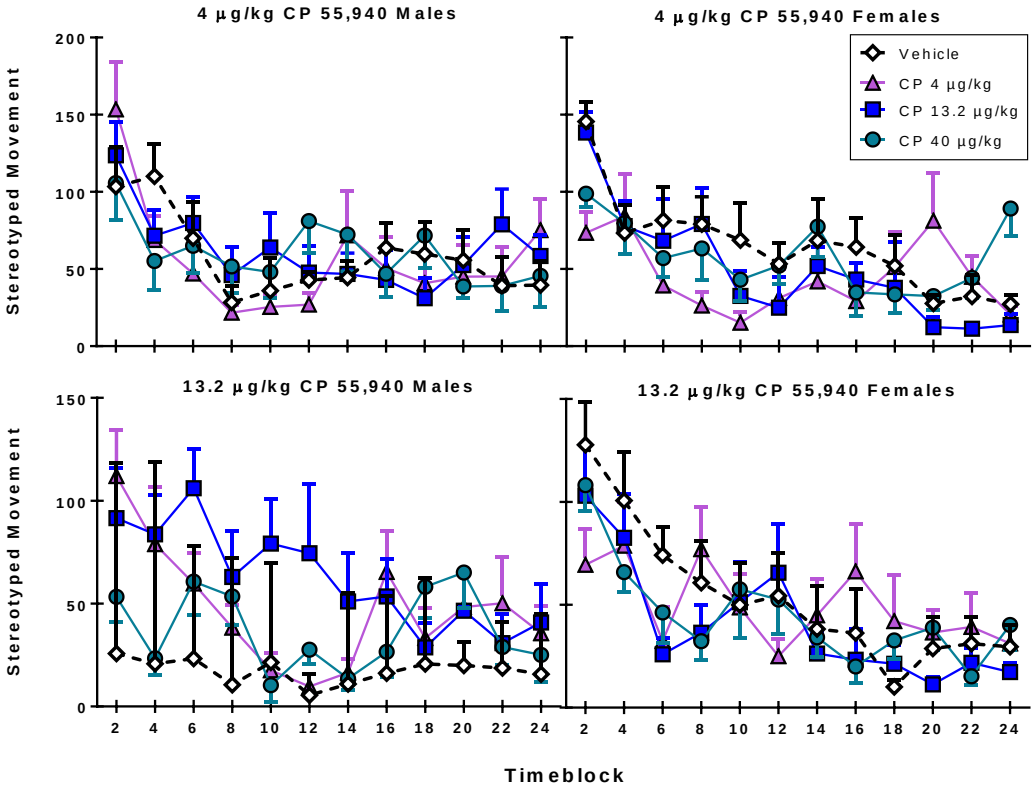

Figure 4. Experiment 1 Test Day by Stereotyped Movement
Mean stereotyped movements (± SEM) of male and female adolescent rats injected with CP 55,940 (4, 13.2, 40 µg/kg, IP) or vehicle and placed in photobeam activity chambers on the five conditioning days (PD 30-34). On PD 36, rats were injected with (4 or 13.2 µg/kg, IP) and placed in photobeam activity chambers for 2 h.

Experiment 2 examined conditioned activity and behavioral sensitization in adolescent rats after pretreatment with either cocaine or CP and then a challenge injection of cocaine or vehicle. During the pretreatment phase, the conditioning drug significantly altered distance traveled scores, $F(3,131) = 561.359$, $p < .001$ and stereotyped movement, $F(3,131) = 160.957$, $p < .001$. That is, male and female rats given cocaine had greater activity levels than rats treated with CP (40

or 13.2 ug/kg) or vehicle (Tukey test, $p < .01$). The increase in stereotyped movement occurred across all 5 days of conditioning (conditioning drug × day interaction), $F(9,413) = 8.021$, $p < .001$. In contrast, conditioning drug effects were not altered by day for distance traveled, $F(8,364) = 1.760$, $p = .08$ (Fig. 5).

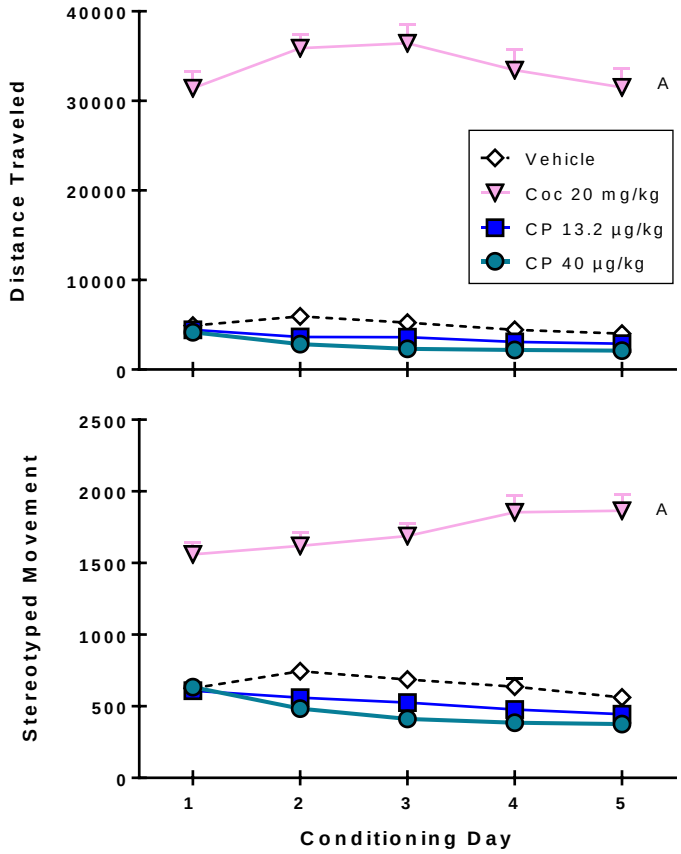

Figure 5. Experiment 2 Conditioning Days by Conditioning Drug
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of adolescent rats injected with CP 55,940 (13.2 or 40 µg/kg), cocaine (20 mg/kg) or vehicle and placed in photobeam activity chambers on the five conditioning days (PD 30-34). [A] Indicates a significant difference in activity for cocaine-pretreated animals compared to CP 55,940 and vehicle-treated animals.

On the test day, treatment with the test drug differentially altered distance traveled scores, $F(3,129) = 91.575$, $p < .001$ and stereotyped movements $F(3,129) = 110.520$, $p < .001$. Specifically, rats challenged with cocaine displayed significantly more locomotor activity compared to rats challenged with vehicle. This enhancement in activity remained significant across all 24 time blocks for both distance traveled, $F(3, 366) = 30.132$, $p < .001$, and stereotyped movement, $F(6,775) = 7.652$, $p < .001$ (Fig. 6).

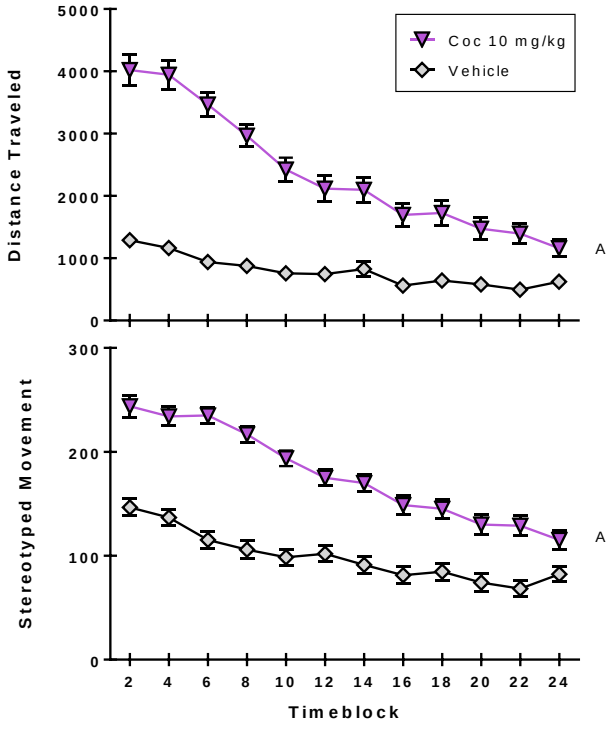

Figure 6. Experiment 2 Test Day by Test Drug
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of male and female adolescent rats injected with CP 55,940 (13.2 or 40 µg/kg, IP),

cocaine (20 mg/kg, IP) or vehicle and placed in photobeam activity chambers on five conditioning days (PD 30-34). On PD 36, rats were injected with cocaine (10 mg/kg, IP) or vehicle and placed in photobeam activity chambers for 2 h. Data in this graph were collapsed across conditioning drug group. [A] Indicated a significant difference between cocaine- and saline- challenged animals on the test day.

Rats given saline on the test day were analyzed separately to assess conditioned activity. Both distance traveled scores, $F(3,57) = 10.49$, $p < .001$ and stereotyped movement, $F(3,57) = .59$, $p < .001$ were altered depending on the conditioning drug given. Specifically, rats pretreated with cocaine had significantly greater locomotor activity than vehicle-pretreated rats (Tukey test, $p < .01$). Pretreatment with the CP drug did not alter saline-induced activity (Fig. 7). Sex did not alter the behavior of rats given saline on the test day for distance traveled, $F(3,57) = 1.31$, $p = .25$ or stereotyped movements, $F(3,57) = 1.87$, $p = .17$.

Rats given cocaine on the test day showed significant differences in distance traveled, $F(3,72) = 3.16$, $p = .03$ and stereotyped behaviors, $F(3,72) = 3.05$, $p =. 03$ depending on the conditioning drug given because, rats pretreated with cocaine had significantly greater distance traveled scores and stereotyped movements compared saline-pretreated rats (Tukey test, $p < .01$; Fig. 8). Sex did not significantly alter either distance traveled $F(3,72) = 1.80$, $p = .18$ or stereotyped movements, $F(3,72) = .02$, $p = .88$ of rats given cocaine on the test day. In contrast to cocaine-pretreated animals, rats pretreated with CP (13.2 or

40 µg/kg) did not have significantly enhanced distance traveled scores (Tukey test, $p > 0.05$) and stereotyped movement (Tukey test, $p > 0.05$) compared to vehicle-pretreated animals on the test day (Fig. 7 and 8).

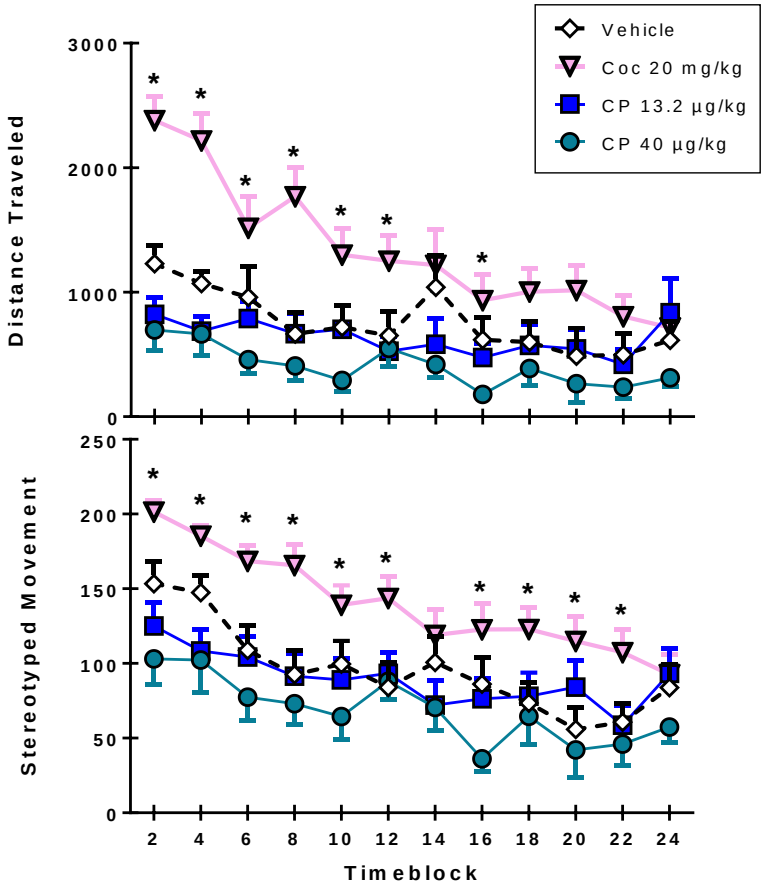

Figure 7. Experiment 2 Test Day Saline by Conditioning Drug.
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of adolescent rats injected with CP 55,940 (13.2 or 40 µg/kg, IP), cocaine (20 mg/kg, IP) or vehicle and placed in photobeam activity chambers on five conditioning days (PD 30-34). On PD 36, rats were injected with saline and placed in photobeam activity chambers for 2 h. *Indicates significant difference between cocaine-treated rats and rats treated with either saline or CP 55,940.

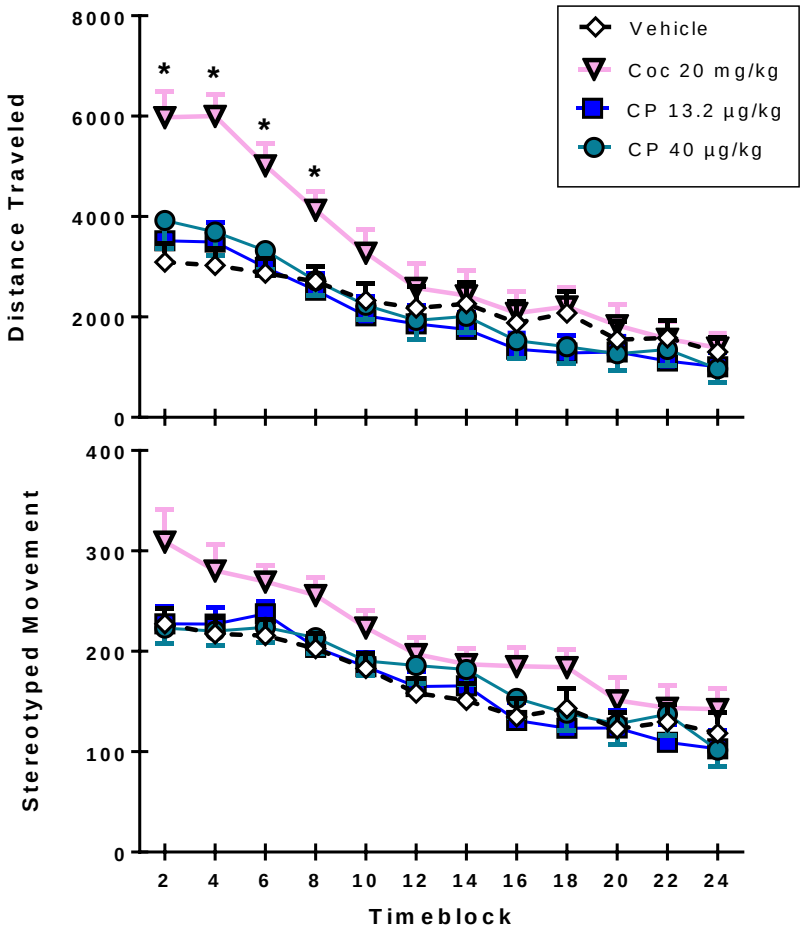

Figure 8. Experiment 2 Test Day Cocaine by Conditioning Drug
Mean distance traveled scores (± SEM) and stereotyped movements (± SEM) of adolescent rats injected with CP 55,940 (13.2 or 40 µg/kg, IP) cocaine (20 mg/kg, IP), or vehicle and placed in photobeam activity chambers on five conditioning days (PD 30-34) On PD 36, rats were injected with cocaine (10 mg/kg, IP) and placed in photobeam activity chambers for 2 h. *Indicates significant difference between cocaine-treated rats and rats treated with either saline or CP 55,940.

CHAPTER NINE

DISCUSSION

In two experiments, the present thesis examined the effects of repeated exposure to CP in early adolescent SD male and female rats using the behavioral sensitization paradigm. In this paradigm, the behavioral response to a drug is assessed after prior exposure to that drug. If the behavioral response is augmented when re-exposed to the drug, then sensitization has occurred. Behavioral sensitization has become an important tool in preclinical investigations, as a sensitized response to a drug is indicative of its abuse potential and is believed to be particularly associated with drug seeking behavior (Robinson & Berridge, 2003). The first experiment examined whether repeated CP exposure, over a range of doses, would differentially affect behavior after a later challenge dose of CP. The second experiment assesed effects of repeated CP exposure on later cocaine-induced behavioral sensitization and conditioned activity. It was hypothesized that repeated CP would induce an augmented (i.e., sensitized) response to both CP (Experiment 1) and cocaine (Experiment 2) in male and female rats. It was also predicted that female rats would have a greater sensitized response, when compared to males, and that both CP and cocaine would induce conditioned activity (Experiment 2).

In contrast to the predictions, repeated exposure to CP in Experiment 1 did not induce sensitization in adolescent rats at any dose or in either sex. Female rats were, however, more active than male rats during the pretreatment

phase. Similar to Experiment 1, pretreatment with CP did not enhance cocaine-induced activity or produce conditioned activity in adolescent rats. In comparison, repeated exposure to cocaine did induce an augmented response to both cocaine (behavioral sensitization) and saline (conditioned activity) challenge in male and female adolescent rats.

The failure to find CP-induced behavioral sensitization was somewhat surprising as a sensitized response to both synthetic cannabinoids and THC has been observed in adult rats (Cadoni et al., 2001, 2008; Rubino et al., 2001; Varvel, Martin & Lichtman, 2007). For example, 10 days of treatment with the synthetic cannabinoid WIN (0.1 mg/kg) produced an augmented locomotor response after a 48 hr abstinence period (Enayatfard et al., 2013). Adult rats also show behavioral sensitization after treatment with THC; however, a sensitized response is only observed when the dose of THC is increased each day during the pretreatment phase (Cadoni et al., 2001, 2008; Rubino et al., 2001). The use of an increasing dose regimen is believed to mimic recreational cannabis users who are likely to increase their dose over time (Ellickson, Martino, & Collins, 2004). Adolescent rats are often more sensitive to psychoactive drugs than adult rats (For review, see Izenwasser, 2005), so it was expected that they too would show a sensitized response. A possible explanation is that cannabinoids may be different from other psychoactive compounds, with adolescents showing less behavioral activation after exposure (McKinney et al., 2008).

Alternately, the inability to observe sensitization to CP may be a consequence of our procedures. In behavioral sensitization studies the drug is typically given once a day for a 1 to 10 day period (Caster et al., 2005; Enayatfard et al., 2013; Kozanian, Gutierrez, Mohd-Yusof & Mc Dougall, 2012; McDougall, Duke, Bolanos & Crawford, 1994; McDougall et al., 2007; Robinson & Berridge, 1993; Vezina, 1996). In many of the investigations showing cannabinoid sensitization, the drug was either given in an escalating dose or multiple doses were given each day (Cadoni et al., 2001, 2008; Enayatfard et al., 2013; Rubino et al., 2001). This pattern suggests that the neuronal modifications necessary to induce sensitization after cannabis exposure requires a more intense treatment than drugs such as cocaine and amphetamine, which produce sensitization after a single exposure (Caster et al., 2007; McDougall et al., 2009). Therefore, it is likely that cannabis sensitization in adolescents may be possible with a more intense dosing paradigm (Cadoni et al., 2001, 2008; Enayatfard et al., 2013; Mathews et al., 2011; Rubino et al., 2001). Importantly, these data may suggest that enhanced drug sensitivity can only be seen in populations that escalate their dose; whereas, consistent dose regimens used for medicinal reasons (Häuser et al., 2018; Sulak, Saneto & Goldstein, 2017) may not have the same detrimental impact (Kononoff et al., 2018; Melas et al., 2018).

It is also possible that the inability to find sensitization in adolescent rats is specific to the cannabinoid chosen for this investigation and is not indicative of other cannabinoids. CP is a potent agonist of the CB1 receptor system

(Pertwee, 1997) and may not produce effects that are similar to other cannabinoids which cause sensitization (Cadoni et al., 2001, 2008; Enayatfard et al., 2013; Rubino et al., 2001). The cannabis plant contains a number of cannabinoids that are not well understood and may contribute to the addictive properties of cannabis use (Andre, Hausman & Guerriero, 2016). The synergistic relationship that of cannabinoids may contribute to the rewarding effects experienced by a user, and are not observed when a synthetic full CB1 agonist is examined (McLaughlin, 2018; Russo & Guy, 2006). Thus, research focused on THC alone or synthetic cannabinoids may not be sufficient to understand the full behavioral response to cannabis. It may be necessary to compare how animals respond to the effects of vaporizing the cannabis plant to gain a better understanding of cannabinoid sensitivity (Baxter-Potter, Lugo, Fuchs, & McLaughlin 2017; McLaughlin, 2018).

This study also examined cocaine-induced sensitization and found that cocaine pre-exposure led to behavioral sensitization in adolescent rats. Cocaine-induced behavioral sensitization develops early in rats and can be observed in rats at PD 19 (McDougall et al., 2007), and thus, sensitization was not unexpected. However, previous research has demonstrated a number of age-dependent effects in the sensitization to cocaine (McDougall et al., 1994, 1999, 2007; Zavala et al., 2000). For instance, preweanling rats show cocaine sensitization at doses similar to adults; however, the sensitized response in preweanling rats does not show persistence and is not apparent after 7 days

(Zavala et al., 2000). While sensitization in adult rats can be observed for months after the last drug exposure (Leith & Kuczenski, 1982). Previous research also indicates that the adolescent sensitized response is not the same as an adult or preweanling rats (McDougall et al. 1994, 1999, 2007; Zavala et al., 2000), as preweanling and adult rats show cocaine-induced sensitization after only one drug exposure while adolescent rats do not. As predicted, this study also found conditioned activity in rats that were pretreated with cocaine and challenged with saline on the test day. This finding showed that early adolescent rats respond in an adult-like fashion to the conditioning effects of cocaine.

Although this thesis found that pre-exposure to cocaine led to sensitization after a cocaine challenge, there was no evidence that adolescent rats showed cannabinoid-induced cross-sensitization to cocaine. Unlike CP, THC does induce cross-sensitization to cocaine in older adolescent rats, but only after increasing doses of cocaine (Dow-Edwards & Izenwasser, 2012). Similarly, an escalating dose of WIN given to adolescent rats (PD 42) over an 11-day period causes cross-sensitization to cocaine (10 mg/kg) 24 h later (Melas et al., 2018). The present investigation showed that cross-sensitization to cocaine does not occur after a five-day consistent-dose regimen of CP, suggesting that a more intense drug exposure is necessary for cannabinoid sensitization to occur. Thus, these data propose that changes in drug sensitivity may not occur in cannabis users that do not increase their doses. For example, early high users that use a

consistent high dose of cannabis and stable light users that use a consistent low dose of cannabis (Ellickson et al., 2004).

The failure to find sensitization or cocaine cross-sensitization after exposure to CP is inconsistent with the cannabis gateway theory of drug use. The gateway theory postulates that cannabis use increases the likelihood of later experimentation with illicit substances (Fergusson, Boden & Horwood, 2005; Secades-Villa, Garcia-Rodiguez, Jin, Wang, & Blanco, 2015). A sensitized response to CP or cocaine would support the hypothesis that adolescent exposure to a cannabinoid causes an enhanced response when the individual is later exposed to these drugs. Instead, we found that CP exposure did not lead to an enhanced behavioral response to CP or cocaine suggesting that the gateway theory does not apply with this combination or compounds. In addition the lack of behavioral sensitization to CP suggests that moderate consistent treatment with a cannabinoids does not activate in physiological processes that drive drug seeking behavior. This finding suggests that other factors, such as environment, mental health and economic status, may play a more vital role in an adolescent's motivation to continue to use cannabis or experiment with other illicit substances (Fergusson et al., 2005; Secades-Villa et al., 2015).

In conclusion, little is known about the adolescent response to cannabis exposure. Acquiring this information is important to fully understand the abuse potential, addictive properties, and long term consequences of cannabinoid-based therapies. The present study examined the repeated exposure to the

synthetic cannabinoid agonist CP in adolescent male and female rats to assess if it would alter drug responsivity. Our data indicated that repeated treatment with CP in adolescent rats does not alter drug responsivity to a later drug challenge of CP or cocaine. These data suggest that the use of a consistent dose CP by adolescents does not alter drug sensitivity and is supportive of the medicinal use of cannabinoids; however, additional research, testing other cannabinoids, is wanted.

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