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Cannabinoid Induced Behavioral Sensitization in Pre Adolescent and Adolescent Sprague Dawley Rats

A Thesis

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by

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CHAPTER ONE

CANNABIS

Cannabis has been called by many 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. The Emperor Shen Neug of China in 2,000 B.C., was the first to record cannabis, or 'ma' as they referred to it, as a textile and for soil fertilization (Zuardi, 2006). The Materia Medica Sutra (Pen ts'ao Ching) first documented the psychotropic properties of cannabis sativa L. (Huo ma ren) and has been translated to read "the seeds could nourish intestines and relieve constipation" (Chinese Herbal medicine translation; Zuardi, 2006). Hemp rope was used by the Vikings for coarse textiles such as rope and fine household textiles as well (Skoglund, Nockert & Holst, 2013). Throughout history, various religions and cultures also noted that cannabis has medical properties and creates euphoria (Aldrich, 1997).

However, cannabis has also shown to have negative side 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; Touwn, 1981; Russo, 2007; Zuardi, 2006). Furthermore, India has prohibited cannabis resin (haras) even though, Athrava Veda considered cannabis (bhang) to be one of the five scared plants of India (Li, 1978; Touwn, 1981; Bapat, 2015; Russo, 2007; Zuardi, 2006). Although cannabis possesses both beneficial effects it also has shown to produce side effects that are deemed dangerous and have made its use outlawed in many countries.

The United States has also had a long history of cannabis controversy. In 1916 botanists by the name of 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 and continued through 1937 when the United States congress passed the Marijuana Tax Act which made anyone dealing with marijuana pay an occupational tax and register with the Internal Revenue Service(MeKenna, 2014). This act passed with the help of negative propaganda like the film "Reefer Madness" (Stringer & Maggard, 2016). Interestingly, this

happened the same year Dr. William C. Woodward of The American Medical Association proposed the study of cannabis for medical use (Newton, 2014).

The Controlled Substance Act was then passed in 1970 which placed marijuana in the schedule one category. However in 1992 the first pharmaceutical based cannabis compound, dronabinol, became legal for medical use specifically, for AIDS-wasting syndrome (Warner, 2001). Later in 1996, California became the first of 25 states to legalize medical marijuana. It became legal for persons in California suffering from cancer, glaucoma, migraines, seizures, severe nausea, muscle spasms and chronic pain to treat symptoms associated with these disorder using marijuana. In 2012, Colorado and Washington made it legal to use cannabis recreationally for people over the age of 21 and Alaska and Oregon followed suit in 2014. Most recently, California has passed recreational use as of 2016. However, as of today the federal government still considers cannabis an illicit drug and it remains listed as a schedule one drug.

Cannabis

The cannabis plant grows indigenously in many regions including Asia, India 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 indicia strain of the plant grows short and stocky with dark leaves, while the sativa strain of the plant grows tall and thin with light leaves (Russo, 2007). There are three main preparations of this plant which include; the cannabis resin (hash), the seeded plant that contains stems, flowers, and leaves, and the unfertilized female flowers which are the most commonly used to produce psychoactive effects (Russo, 2007).

The cannabis plant contains over 70 different cannabinoids that produce a range of medicinal and psychoactive effects (ElSohly & Slade, 2005). The two main constituents of this plant consist of (-)-trans-Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are believed to contribute to the majority of these effects (Russo, 2007). The psychoactive effects produced by using cannabis have been attributed to THC, and CBD is attributed

to the medicinal effects without the psychoactive properties associated with THC (Pertwee, 2014). In order for the plant to release these chemical constituents the plant must be heated, which is why smoking the plant is the most popular method of intake (Pertwee, 2014).

When used acutely cannabis intoxication can cause red eyes, sleepiness, and decrease motor coordination and slow respiratory rate (Grotenhermen, 2004). The psychological effects acutely seen with cannabis use include dysphoria, alterations to attention, concentration, and learning as well as somatic and visual sensations (Grotenhermen, 2004). However, persistent long term effects of cannabis use has been 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 before the age of 15 (Fontes et al., 2011). On the other hand cannabis withdrawal is common in adults that do not seek treatment and can include irritability, difficulty sleeping, restlessness, and changes in mood such as, depression and nervousness (Gorelick et al., 2012; Verweij et al., 2013).

Challenges of legalization

Even though cannabis is still illegal by the United States government, the legalization of cannabis for medical and recreational use 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 contents averaging around 20% compared to an average of about 4% in 1995 (Elsohly et al., 2016). Also cannabis is now processed into oils and butters that are now being processed into candies, drinks, condiments and other daily food items and without federal regulations there has been an increase in accidental exposure to these cannabis products in young children (Davis et al., 2016; Wang et al., 2014). Furthermore concentrated waxes that can be up to 100% THC have been popularized more recently (Loflin & Earlywine, 2014). These concentrated waxes knows as "dabs" have been self-reported to cause an increased tolerance and withdrawal to THC (Loflin & Earlywine, 2014). The variability in the concentrations of active constituents makes it important to understand the wide range of physical and behavioral effects that cannabis can cause along with its abuse potential.

Cannabis has become the most commonly used illicit substance in the United States. The changes in the legalization of cannabis especially in states that have made it recreational and medical, has led to an increase in the report of cannabis dependence and abuse (Cerdra, Wall, Keyes, Galea & Hasin, 2012). Cannabis use disorder is outlined in the DSM V with the criteria of, the use of cannabis for over one year and meeting two symptoms that relate to the user having difficulty controlling use, quitting use, or that their use has preoccupied their time and is significantly impairing of daily life functioning (DSMV; American Psychological Association, 2013). Although cannabis use disorder is seen at lower rates than other disorders involving illicit substances, the prevalence of cannabis use disorder is increasing largely 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 (Farmer et al., 2015). In fact, patients with a life time diagnosis of cannabis use disorder report that their first episode of cannabis dependence was around the age of 18 (Farmer et

al., 2015) Thus, making this young population vulnerable to cannabis use disorder and an issue of public health concerns (Haberstick et al., 2014).

CHAPTER TWO

THE ENDOCANNABINOID SYSTEM

After the primary psychoactive component of cannabis, THC, was identified, it was quickly discovered that this compound worked by binding to distinct receptors in the central nervous system and peripheral nervous system (Howlett et al., 2002). The receptors were labelled cannabinoid receptors and endogenous ligands were eventually found to bind to these receptors. The cannabinoid receptors include the cannabinoid one (CB1) and cannabinoid two (CB2) receptors and 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 (Howlett et al., 2002).

Cannabinoid Receptors

The CB receptors have been characterized across human, porcine, primate and rodent brains (Howlett et al., 2002). Until recently, the CB1 receptors were thought to only

exist in the central nervous system whereas, the CB2 was confined to the peripheral nervous system; however, both receptors are now known to be distributed throughout the brain and body (Gong et al., 2006). The cannabinoid receptors are composed of 7 hydrophobic segments that consist of N-terminal extracellular and C-terminal intracellular domains (see Reviews Howlett et al., 2002, Pertwee, 1997; Svizenska, Dubovy & Sulcova, 2008). The CB1 receptor has been extensively studied whereas the CB2 receptor is not as well understood. Additionally, there has been discussion of a possible third cannabinoid receptor, the vanilloid receptor (TRPV1 or VR1) because the vanilloid receptor has similar neurological functions and expression as the endocannabinoid system (Cristino, 2006).

Adult Distribution and Density

The CB1 receptors are expressed in adult rats on the terminal axonal fibers of neurons, specifically on presynaptic terminals (Howlett et al., 2002). Overall, the hippocampal formation has more dense binding than the 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 (Egertova & 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 also contain sparse binding (Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992).

In adult rats the second largest site of cannabinoid receptor densities is found in the basal ganglia (Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992). Precisely, within the basal ganglia the highest densities of cannabinoid receptors are found in the globus pallidus, entopeduncular nucleus, and the substaintia nigra pars reticulate (Egertova & 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 intensity that increases from the medial to lateral regions (Egertova & 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 cannabinoid receptors in comparison

(Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992). The basal ganglia also has cannabinoid receptors in white matter tracts. Specifically, the striatonigral descending pathway contains detectable cannabinoid receptors (Herkenham et al., 1991).

The density of the cannabinoid receptors in the cerebral cortex 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, very dense 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 are found in the corpus callosum, thalamus, hypothalamus and midbrain (Egertova & Elphick, 2000; Matsuda et al., 1993; Tsou et al., 1998).

Despite earlier reports that the CB2 receptors were only found in the peripheral nervous system, the CB2 receptor has been discovered in 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, staining of the CB2 receptors was found in the thalamus, periaqueductal gray, substanitia nigra pars reticulate, 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 suggesting that the CB2 receptors are found in similar locations as the CB1 receptors; however, their distribution patterns and densities differ (Gong et al., 2006).

Gestational Development

There are similarities and differences between the gestational cannabinoid receptor system and the adult cannabinoid 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 (Berrendero et al., 1998). This binding at GD 18 has also been blocked with the use of the antagonist SR141716 (Berrendero et al., 1998). In the

hippocampus by GD 16, CB1 receptor mRNA is detectable in the dentate gyrus and by GD 21, they are localized in the subfields of the Ammon's horn. CB1 receptor mRNA can also be measured in the cerebral cortex at a progressive increase from GD 16 to GD 21. Overall, by GD21 CB1 receptor binding is identifiable in the basal ganglia, hippocampus, cerebral cortex, and cerebellum (Berrendero et al., 1999). Interestingly during the gestational periods of GD 16 to GD 21, CB1 mRNA was measurable in the midbrain, pons and brainstem, whereas adulthood, these brain regions do not contain cannabinoid receptors (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, Berrendero et al., 1999, Romero, 1997).0

Postnatal Development

The distribution of the cannabinoid receptors in early development is consistent with the adult localization of cannabinoid receptors in brain areas (Beleu, Howlett,

Westlake & Hutchings, 1994). The densities of CB1 receptors in the basal ganglia, and limbic begin to steadily increase at PD 5 to adult binding densities seen in adulthood (Berrendero et al., 1999). On the other hand, the caudate-putamen, septum nuclei and nucleus accumbens appear to decrease in binding levels from GD 21 until birth before they begin to increase (Beleu et al., 1994; Berrendero et al., 1999). The striatum then doubles from PD 0 to PD 7 and then doubles again by PD 21 before reaching full binding levels in adulthood (Beleu et al., 1994; Berrendero et al., 1999). The CB1 receptors located the on external (II-III) and internal (V-VI) layers of the cerebral cortex continue to increase after birth at a consistent rate from PD 21 until reaching full adult levels (Berrendero et al., 1998, Berrendero et al., 1999, Herkenham et al., 1991, Mailleux & Vanderhaeghen, 1992).

An increase in CB1 receptor development was also seen with receptor binding in the cerebellum and cortex, except the cortex increased less from PD 14 to adulthood than the striatum and cerebellum (Beleu et al., 1994). The hippocampus displays a gradual increase throughout development to reach adult levels of cannabinoid receptors

(Beleu et al., 1994). The densities of CB1 receptors that can be found in white matter areas such as the corpus callosum and sub-ventricular zone of the neocortex in gestational development that are no longer visible in adult development, these brain areas are known to assist with neuronal development (Berrendero et al., 1998, Berrendero et al., 1999, Romero, 1997).

Cellular Signal Transduction

The stimulation of both CB1 and CB2 receptors causes the activation of cellular signal transduction through Gi/o protein pathways (see reviews; 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. Furthermore CB1 receptors are coupled to inwardly rectifying potassium channels. Through the Gi/o protein pathway the CB1 receptors can inhibit voltage gated calcium channels which increases intracellular calcium. CB receptors can also stimulate the phosphorylation of mitogen-activated protein kinase. Lastly, CB1 and CB2 receptors facilitate immediate early gene expression as

well as, regulate protein synthesis (see reviews; Howlett, 2002; Pertwee, 1997).

Cannabinoid Receptors and Neurotransmission

The activation of cannabinoid receptors on the presynaptic terminals inhibits the release of both excitatory and inhibitory neurotransmitters (Howlett et al., 2002). The cannabinoid receptors are highly 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). On the other hand, the activation of cannabinoid receptors can also increase the release of other transmitters. Dopamine release is stimulated in the nucleus accumbens, ventral tegmental area and substintia nigra by the activation of CB1 receptors. Furthermore when presynaptic cannabinoid receptors are activated there is an increase of acetylcholine in the hippocampus (Acquas, Pisanu, Marrocu & Di chiara, 2000) and glutamate release in the cerebral cortex (Ferraro et al., 2001).

Endogenous Cannabinoids

Once the cannabinoid receptors were discovered it

became apparent that there were endogenous ligands that bind to these receptors. Currently, two compounds have been found including anandamide and 2-AG, which bind endogenously to the cannabinoid receptors. The chemical anandamide was named after the Sanskrit word for bliss (Devane et al., 1992). 2-AG though identified second, is found to be more abundant in the brain compared to anandamide (Stella, Schweitzer & Piomelli, 1997).

Anandamide

Distribution.

The distribution of anandamide in the brain coincides with the distribution of cannabinoid receptors (Bisogno et al., 1999; Felder et al., 1996). 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 seen in high concentrations in the brainstem, striatum, and hippocampus and low concentrations in the cerebellum, however the levels of NArPE are much higher

overall compared to 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 catalyzes NArPE hydrolysis (Cadas, Di Tomaso & Piomelli, 1997; Di Marzo et al., 1994, Basavarajappa, 2007). This calcium dependent phospholipid precursor NArPE leads to the activation of N-acyltransferase (NAT) which causes the movement of an acyl group from phospahatidylcholine to the ethanolamine portion of phosphatidylethanolamine which produces N-acyl phosphatidylethanolamine (NAPE), (Cadas et al., 1997, Di Marzo et al., 1994, Basavarajappa, 2007). Anandamide and phosphatic acid are then released into the synaptic cleft after cleavage by NAPE specific phospholipase D (Basavarajappa, 2007). However, 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, 2001). After anandamide is removed, intracellular degradation occurs by enzymatic hydrolysis (Deutsch 1993, Di Marzo 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 breakdown of anandamide occurs when FAAH breaks the amide bond which causes the release of arachidonic acid and ethanolamine.

Behavioral effects.

Anandamide can cause a wide range of behavioral effects. In rats increases in the motivation to eat, frequency of eating, food intake and reduced eating latency are seen after injections of anandamide (Martinez-Gonzalez, 2004; Williams, 2001). When rats are given injections of anandamide the frequency of ejaculation changes in a dose dependent manner, with a low dose decreasing ejaculations and a high doses increasing ejaculation. In addition the latency to ejaculate increases with high doses of anandamide (Martinez-Gonzalez et al., 2004). The injection

of anandamide also reduces pain behavior in the formalin test (Guindon, De Léan, & Beauliue, 2006). Changes in anandamide levels induces sleep wake cycles by causing rapid eye movement and slow-wave sleep II, which in rats regulates their level of alertness (Murillo-Rodriguez et al., 1998). Furthermore injections of anandamide also produces similar effects to motor behavior as the exogenous cannabinoid THC (Romero et al., 1995). Both THC and anandamide can decrease grooming, rearing, and motor behavior in the open field test but also increase inactivity similarly (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, 2006). 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 can decrease serotonin in the hippocampus and increase it in the hypothalamus (Hao, Avraham, Mechoulam & Berry, 2000).

Anadamide can also increase dopamine in the hippocampus and hypothalamus as well as, increase cortisol levels (Hao et al., 2000). Overall, anandamide can modulate hunger, sexual activity, alertness, and neurotransmission, as well as, produces rewarding effects Martinez-Gonzalez et al., 2004; Guindon et al., 2006; Kirkham & Williams, 2001; Romero 1995 Solinas et al., 2006; Justinova et al., 2005; Hao et al, 2000).

2-AG

Distribution.

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). Furthermore the lowest levels of 2-AG are seen in the hypothalamus of the diencephalon, cerebellum and the 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 includes a few possible routes (See review, 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 phosphatidylinositol from phosphatidylinositol - specific phospholipase A1 which is converted into 2-AG by lyso- phosphatidylinositol specific phospholipase C (Sugiura et al., 1995). Overall, it is understood that the phospholipase C and diacylglycerol lipase are important to 2-AG synthesis.

Inactivation and Metabolism.

The inactivation of 2-AG occurs through the anandamide membrane transporter. This inactivation of 2-AG occurs by reuptake through this membrane transporter molecule. 2-AG is then metabolized into 2-arachidonyl LPA by monoacyl glycerol kinase, which is then converted into 1-steoyl-2arachidonyl PA (See review, Basavarajappa, 2007).

Behavioral effects.

2-AG has been associated in multiple behavioral and neurological functions. Elevated levels of 2-AG have been found after head injury and it has been suggested this elevation helps to reduce brain edema, hippocampal cell death and improve the level of recovery (Panikashvili et

al., 2001). Moreover, 2-AG serves as the immediate response to reduce inflammation and is formed when there is a pro-inflammatory immune response, to act as a negative feedback loop for the inflammation (Berdyshev, Schmid, Krebsbach & Schmid, 2001). Also, 2-AG has been found to inhibit invasive prostate cancer cells (Nithipatikom et al., 2004). Futhermore, 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). As well as, 2-Ag levels are elevated after chronic stress exposure suggesting a role in preventing the development of anxiety (Sumislawski, Ramikie, & Patel, 2011).

2-AG has also been linked to the rewarding properties experienced with stimuli such as food and drugs. Mice that are given high fat diets show an increase in hypothalamic 2-AG which was attributed to increase the rewarding and reinforcing effects of the high fat diet (Higuchi et al., 2012). Moreover, squirrel monkeys self-administer 2-AG which shows, it has reinforcing properties like drugs of abuse (Justinova, Yasar, Godfrey, Redhi & Goldberg, 2011). Overall, 2-Ag is produced as a neuro-protectant and cancer

growth inhibitor, has anti-inflammatory and analgesic properties, reduces stress, and augments rewarding circuitry (Panikashvili et al., 2001; Berdyshev et al., 2001; Nithipatikom et al., 2004; Hohman et al., 2005; Sumislawski et al., 2011; Higuchi et al., 2012; Justinova et al., 2011; Vigano et al., 2003).

CHAPTER THREE

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 the cannabinoid receptors and have given insight into the appeal of cannabis use recreationally and medically.

THC

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 effects of THC have been outline in human's and thoroughly, please refer to figure below (Grotenhermen, 2004).

Psyche and perception	Fatigue, euphoria, enhanced well-being, dysphoria, anxiety, reduction of anxiety, depersonalization, increased sensory perception, heightened sexual experience, hallucinations, alteration of time perception, aggravation of psychotic states, sleep.
Cognition and psychomotor performance	Fragmented thinking, enhanced creativity, disturbed memory, unsteady gait, ataxia, slurred speech, weakness, and deterioration or amelioration of motor coordination.
Nervous system	Analgesia, muscle relaxation, appetite stimulation, vomiting, anti-emetic effects, neuroprotection in ischemia and hypoxia.
Cardiovascular system	Tachycardia, enhanced heart activity, increased output, increase in oxygen demand, vasodilation, orthostatic hypotension, hypertension, and inhibition of platelet aggregation.
Hormonal system	Influence on LH, FSH, testosterone, prolactin, somatotropin, TSH, glucose metabolism, reduced sperm count and sperm motility, disturbed menstrual cycle and suppressed ovulation.
Immune system	Impairment of cell-mediated and humoral immunity, immune stimulation, anti-inflammatory and anti-allergic effects.
Respiratory system	Bronchodilation, hypo-salivation and dry mouth

Figure 1. This information was adapted from Grotenhermen (2004) from TABLE 1. Effects of THC explained above were observed in both human and animal studies.

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 for smoked THC ranges from 2-56 percent since there is variability in the frequency and quantity of

THC use depending on individual. After smoke inhalation, the blood plasma levels of THC peak quickly ranging anywhere from 3 to 10 minutes (Huestis et al., 2005). In contrast an oral administration of THC, has a much slower onset of effects and erratic effects when compared to the inhalation of THC (Law, Mason, Moffat, Gleadle & King, 1984). The bioavailability of THC taken orally is about 10 to 20 percent 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 minutes and 120 minutes and last an average of four to six hours (Ohlsson et al., 1982).

Once absorbed in to the body, THC binds to lipoproteins and 90 percent of THC is found in blood plasma and 10 percent is found in red blood cells, as seen is rats (Fehr & Kalant, 1974). There are over 80 different metabolites of THC and is primarily metabolized by cytochrome P450 (Huestis, 2005; Sharma, Murthy & Barath, 2012). THC is eliminated from plasma at low doses (16 mg) within three to six hours, whereas high doses (34 mg) can take six to 27 hours (Huestic, 2005). The half-life of THC is 25 hours to 26 hours but in heavy users the half-life

can range from 19 hours to 53 hours (Hunt & Jones; Wall et al., 1983). Within five days of use up to 90 percent of THC has been eliminated from the body, over 65 percent is excreted in the fecal matter and around 25 percent through urine (Huestic, 2005, Wall et al., 1983). Overall, THC can be detected in the body up to 12 days with a single dose and around 30 days for moderate use and in heavy users THC can be detected up to 77 days (Ellis, Mann, Judson, Schramm & Tashchian, 1985). A lethal human dose of cannabis has not been reported but animal models report a lethal oral dose of cannabis to be 800 to 1900 (Thompson et al., 1973; Grotenhermen, 2007).

THC is a partial agonist of both CB1 and CB2 receptors but with lower efficacy for the CB2 receptor than the CB1 (Howlett et al., 2002; Pertwee, 2008). THC activates CB1 receptors in the central nervous system located on presynaptic terminals and modulates the release of neurotransmitters (Pertwee & Ross, 2002). When THC binds to CB1 receptor they influence the release of glutamate, Gamma-Amino Butyric acid (GABA), dopamine and acetylcholine. These neuro-modulatory actions can been seen within the nucleus accumbens and the synaptic projections

that extend to the ventral tegmental area, hippocampus and prefrontal cortex (Pertwee, 2008; Pertwee & Ross, 2002). THC is a non-lethal drug that takes an average of 30 days to be excreted from the body. This partial cannabinoid agonist is involved in numerous behavioral and neurological process that can cause euphoria as well as have medical value.

Cannabidiol

The plant cannabinoid cannabidiol (CBD) does not give the euphoric effects seen with THC, however, CBD can elicit 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 percent after a single use (Ohlsson et al., 1986). CBD can bind to CB1 and CB2 receptors where it works as both an antagonist and inverse agonist at these receptors (Pertwee, 2008; Thomas et al., 2007). Interestingly, CBD has been found to inhibit the response of the synthetic cannabinoid agonists CP55940 and R-(+)-WIN55212 (Pertwee et al., 2002). CBD has therapeutic value for the treatment of symptoms associated with cancer, arthritis, anxiety, diabetes and

immune disorders (see review Mechoulam, Peters, Murillo-Rodriguez & Hanus, 2007). Overall, CBD possess value as a medical treatment for a variety of ailments without the psychoactive side effects experienced with THC

Synthetic Cannabinoids

Synthetic cannabinoids such as CP55940, HU-210 and R-(+)-WIN55212 bind to both CB1 and CB2 receptors and have been used characterize the CB1 receptor system (Howlett et al., 2002). These synthetic CB receptor agonists exhibit similar behavioral effects as seen with THC and endogenous cannabinoids including hypothermia, analgesia, catalepsy, and locomotor suppression (Tai & Fantegrossi, 2014). These agonist modulate neurotransmission by inhibiting GABA transmission in the substantia nigra and hippocampus and increasing acetylcholine in the hippocampus, dopamine in the nucleus accumbens and glutamate in the cerebral cortex (Howlett et al., 2002). Overall, synthetic cannabinoid agonists have similar physiological and behavioral effects as THC.

The creation of CB antagonists such as SR14716A, AM281 and LY320135 have been used to 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. Whereas, LY320135 blocks the effects of CB receptor agonists and works as an inverse agonist at the CB1 signal transduction pathway (Howlett et al, 2002). However the most highly 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 can be seen with SR14716A include an increase in locomotor activity, hyperalgesia and pro-inflammatory responses (Pertwee, 2005). SR14716A has been found to increase 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 work to reverse the actions of the cannabinoid system.

CHAPTER FOUR

BEHAVIORAL SENSITIZATION

The prevalence of cannabis use disorder has led to the inescapable 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 such as self-administration, drug discrimination, condition place preference and behavioral sensitization have been invaluable tools for studying the addictive properties of drugs such as caanbis (Maldonado & Rodriguez de Fonseca, 2002; Sanchis-Segura & Spanagel, 2006). In particular, behavioral sensitization is a paradigm that examines the motor-stimulant response to a given drug (Steketee & Kalivas, 2001). In terms of addiction the sensitized response to the given drug can be related to the idea of drug craving and may play a role in relapse to drug seeking behavior in the beginging of drug use (Markou, 1993; Robinson & Berridge, 1993; Kalivas, Pierce, Cornish & Song, 1998).

Behavioral sensitization often occurs through a two-step process of induction and expression. Induction is the

pre-exposure phase where the animal is exposed to the drug either once or numerous times (Steketee & Kalivas, 2001). Expression is the test phase where the animal is exposed to the drug after a period where the drug is discontinued (Steketee & Kalivas, 2001). Behavioral Sensitization can be assessed by monitoring changes in stereotype and non-stereotype behaviors during both the induction and expression phase (Rubino, Vigano, Massi & Parolaro, 2001; reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). Stereotyped behaviors are actions such as gnawing, licking and undirected sniffing whereas non-stereotyped behavior are considered exploratory sniffing, locomotor activity, and rearing (Rubino et al., 2001).

Sensitization can also be affected by associative processes such that, the enhanced behavioral effect is only seen when the animal is given the drug in the same environment in which it is tested (reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). This type of sensitization that only occurs when the enviorment stays constant during induction and expression is called associative or context-dependent sensitization. Whereas sensitization that is apparent without a consistant context

is known as non-associative or context-independent sensitization. This means the drug can cause sensitization when enviorment is different during the induction and expression phases(reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). Behavioral sensitization has been used to examine drugs of abuse and is can be better explained using psychostimulants, a class of drugs that is known to cause behavioral sensitization during different periods of development.

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, usually low doses of psychostimulants can produce sensitization but the intensity of the sensitized behavior becomes more robust with higher doses (Davidson, Lazarus, Lee & Ellinwood, 2002; Frantz, O'Dell, & Parsons, 2007).

Multi-trial verses Single-trial Sensitization

The enhanced behavioral resposse can be seen after the induction of senstization which can occur after a number of pre-exposures or after only one pre-exposure to the drung.

Both, multi-trial and single-trial sensitization have been extensively examined using psychostimulants. With 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 a withdrawal period (see reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). For example, multi trial sensitization to psychostimulants can be seen in adult rats after six days of pretreatment with amphetamine (Leith & Kuzenski, 1982). However multiple days of pre-exposure are not nessacary to cause an enhanced behavioral response, adult rats given a single pretreatment injection of cocaine show a sensitized response to the drug 24 hours later (McDougall, Baella, Stuebner, Halladay & Crawford, 2007). Though, these sensitized responses in single-trial sensitization typically require a relatively high dose of the drug (Battisti, Chang, Uretsky & Wallace, 1999).

Futhermore the duration of the sensitized response can occur long after the animal has been exposed to the drug (Leith & Kuzenski, 1982). For example multi-trial sentization to psychstimulants can been seen after a 12 week withdrawl period (Leith & Kuzenski, 1982). This means

that multi-trial behavioral sensitization is a long lasting phenomena that can occur long after the discontinuation of use (Leith & Kuzenski, 1982). However young rats given a single exposure to cocaine do not show a persistence of sensitization through young adulthood (McDougall, Charntikov, Cortez, Amodeo, Martinez & Crawford, 2009). This enhanced behavioral response that is seen after one or many drug exposures may relate to drug relapse and the continuation of drug use (Robinson & Berridge, 1993).

Associative verses Non-Associative Sensitization

Differences in sensitization can occur depending on the environment the drug was given (see review, Tirelli, Laviola & Adriani, 2003). Multi-trial behavioral sensitization is stronger when induction and expression of psychostimulant senstitization are conducted in the same environment but is apparent if the environments are not the same (McDougall et al., 2007; McDougall et al., 2009). For example an animal given psychostimulants show context independent senitization although it is not as robust as it is with context dependent sensitization, when the drug is paired with a distinct testing enviorment (Crombag, Badiani, Maren & Robinson, 2000). 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; McDougall et al., 2007; McDougall et al., 2009). One-trial sensitization however, is completely context dependent in adult rats (McDougall et al., 2007). This means that a single pre-exposure to a psychostimulants such as cocaine will not cause behavioral sensitization if the rat is challenged with cocaine in a different environment (McDougall et al., 2007). Therefore the assicative context of the drug exposure in adult rats is important to the intensity of the sensitized response.

Developmental Sensitization

Multi-trial vs. Single-trial sensitization

Young rodents will also show an enhanced behavioral response after exposure to some drugs. However the sensitized response of these pups differ both quantitatively and qualitatively from adult rodents (McDougall, Duke, Bolanos & Crawford, 1994). Preweanling rats have expressed both a short term sensitization to psychostimulant drugs and a persistant sensitization to these drugs. In example, when preweanling rats aged PD 11

and PD 17 are given four days of pre-exposure to indirect and direct dopamine agonists they show sensitization after two days of abstinence to these drugs however after eight days after drug exposure the sensitized response is not apparent (McDougall et al., 1994). Where as, the ability for stimulants to cause long term sensitization may not occur with all types of psychostimulants it has been seen with cocaine exposure during the pre-weanling period. When rats were treated with cocaine from PD 14 to PD 20 sensitization to cocaine was seen the strongest up to seven days after exposure (Snyder, Katovic, & Spear, 1998). Although the sensitized response was not as robust prewealing rats still showed sensitization to cocaine up to 21 days after the intitial exposure period (Snyder, et al., 1998). One-trial sensitization occurs in young rats (PD 19) as well. When give a single pretreatment injection of cocaine, a sensitized locomotor response can be seen the next day after treatment and up to five days after exposure to the drug (McDougall et al., 2009). However a single pretreatment with cocaine did not cause sensitization seven or 14 days after exposure but interestingly after 61 days of abstinence sensitization began to reemerge as these

animals began adulthood (McDougall et al., 2009). Therefore the development and persistence of sensitization in young rodents is dependent on how many times the animal is exposed to the drug and how long that animal has gone without exposure to that drug.

Associative vs Non-Associative Sensitization

Interestingly, context is not as important for sensitization in young rats as it for adult rats. Preweanling rats (PD 19) given a single pretreatment injection of cocaine show both context-dependent and context-independent sensitization with a 10 mg/kg challenge of cocaine (McDougall et al., 2007). Additionally, these young rats actually showed an increase in sensitization a context independent environment when compared to a context dependent environment (McDougall et al., 2007). Furthermore one-trial sensitization assessed in preweanling rats given cocaine, showed both context-dependent and context-independent behavioral sensitization one, three and five days after pre-exposure (McDougall et al., 2007). Interestingly, cocaine caused stronger sensitization one and three days after exposure in a context-independent environment (McDougall et al., 2009). Although young rats

experience non associative sensitization after a short abstinent period, environmental conditioning is important for sensitization in these pups after a longer drug free period (Zavala, Nazzarian, Crawford & McDougall, 2000). Specifically, after a five day pretreatment with cocaine preweanling rats only show context-dependent sensitization after a seven day drug free period. Whereas after one day of abstinence, preweanling pups show both non-associative and associative sensitization (Zavala et al., 2000).

Behavioral Sensitization using Cannabinoids

While behavioral sensitization has become a common tool for studying abused drugs, relatively little has been done with cannabinoids. This is especially true when examining early and late adolescent rats.

Multi-trial vs. One-trial Sensitization

Cannabinoids like psychostimulants, produce behavioral sensitization that persists after the discontinuation of drug use (Rubino et al., 2001). The first report of multi-trial sensitization with THC used adult Sprague-Dawley male rats and pretreated all rats with THC twice a day for five days (Rubino et al., 2001). All animals recived a dose regimen in which the amount of THC given to the them

increased over the five day period (5, 10, 20, 40, 40 mg/kg) and sensitization was assessed after a long withdrawal period of 20 days (Rubino et al., 2001). the pre-treatment with THC increased behaviors associated with stereotyped activity including gnawing, licking and undirected sniffing after a 5mg/kg THC challenge injection. Furthermore, a slight increase was noticed in non-stereotype activities including forward locomotion, sniffing and rearing (Rubino et al., 2001).

However different changes in behavior are seen depending on the length of exposure and dose of THC. After only three days of pretreatment, in which, the level of THC the animal is exposed to increase each day using low doses of THC (2,4,8 mg/kg), adult male Sprague-Dawley rats exhibited increased non-stereotyped activity when challenged with THC (150 µg/kg i.v) 14 days later (Cadoni, Pisanu, Solinas, Acquas & Chiara, 2001; Cadoni, Valentini & Di Chiara, 2008). The short exposure period to THC and short withdrawal period caused increased non-stereotyped activity such as sniffing, locomotion and gnawing. Overall, the pretreatment with THC can cause changes to both non-stereotyped and stereotyped behaviors that are associated

with an increased sensitized response to a drug. In contrast to multi trial sensitization, one trial sensitization has not been conducted using cannabinoids.

Cross sensitization with amphetamine and morphine

Interestingly the ability of cannabinoids to cross sensitize with other drugs has been examined more so than sensitization to cannabinoids. For example, a single pre-exposure to either THC or WIN 55,212-2 can cause an increase in sensitization to amphetamine when given 30 minutes later (Gorriti, de Fonseca, Navarro & Polomo, 1999; Muschamp & Siviy, 2002). The use multi-trial cross sensitization when THC or WIN 55,212-2 is given has also shown sensitization amphetamine (Gorriti et al., 1999; Muschamp & Siviy, 2002). In that, chronic exposure to THC sensitized animals to the stimulatory properties of amphetamine including locomotion, exploratory and stereotype behaviors (Gorriti et al., 1999). Moreover WIN 55,212-2, when acutely injected, effects both ambulatory and rearing activity of amphetamine given 30 minutes later and after WIN 55,212-2 is given for 10 days it caused an increase in ambulatory movement and rearing activity after a challenge injection of amphetamine (Muschamp & Siviy,

2002). Rats pretreated with either THC or CP 55,490, show an increase in sensitization to morphine as well (Cardoni et al., 2001; Cardoni et al., 2008; Norwood, Cornish, mallet & McGregor, 2003). Likewise, pretreatment with morphine increased sensitivity to both a low and high dose of THC causing an enhanced behavioral response (Cardoni et al., 2001; Cardoni 2008). Although cross sensitization with THC and morphine cause reciprocal changes in drug sensitivity, it is unclear the effects of behavioral sensitization using cannabinoids and a better understanding is essential.

Developmental effects of Cannabinoid Sensitization

Unfortunately, limited research has been conducted on the ontogeny of cannabinoid sensitization. At present it is unclear whether young rats would show similar differences in sensitization from adult rats as demonstrated with psychostimulant induced sensitization.

CHAPTER FIVE

SUMMARY THESIS STATEMENT

Both medical and recreational use of cannabis is now legal in California and many other states. Since the change in these state laws, there has been an increase in availability and use of this drug (Cerda, Wall, Keyes, Galea & Hasin, 2012; Hasin et al., 2015 a). A major concern with the increased societal acceptance of cannabis and the increased availability of this drug, is higher use rates in adolescent populations (Kosterman, Hawkins, Guo, Catalano, & Abbott 2000). This increased use of cannabis in adolescents is important because the risk of developing cannabis use disorder is stronger in people who start drug use earlier (Richter, Brandie, Pugh & Ball, 2017). The use of cannabis before the age of 15 also increases the likelihood of becoming a chronic user and enhances the probability of experimenting with other illicit drugs (Richter et al., 2017; Nelson, Van Ryzin, & Dishion, 2015; van Leeuwen et al., 2013). Moreover, early adolescent use of cannabis can cause long-term consequences that are not apparent in users that begin after the age of 15 (Fontes et al., 2011).

The cause for the increase in problematic cannabis use with early onset is unknown, but an increase in the addictive properties of cannabis during this developmental period may be partially to blame. To this end, this thesis will focus on age-dependent differences in the addictive response to cannabis using the behavioral sensitization paradigm. Behavioral sensitization is an animal model used to study craving, an important component of drug addiction (Berridge & Robinson, 1995; Robinson & Berridge, 1993). In this model, drugs with addictive properties induce an augmented behavioral response in animals after prior exposure to the drugs (reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). Behavioral sensitization can be measured as changes in stereotypic and non-stereotypic behaviors (reviews Steketee & Kalivas, 2011; Pierce & Kalivas, 1997). Behavioral sensitization is seen in a wide number of abused drugs and 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 during this developmental period of adolescences (see reviews Jacobus & Tapert, 2014; Viveros, Llorent, Moreno, & Marco, 2005). Thus the purpose of this thesis project is to examine the effects of cannabinoid CP 55,490 (10, 33, or 100 µg, IP) in preadolescent (PD 17- PD 21) and adolescent sprague dawley rats (PD 30- PD 36) using the behavioral sensitization paradigm. CP 55,490 will be used because it mimics the effects of THC, the primary psychoactive ingredient in cannabis (Gurney, Scott, Kacinko, Presley & Logan, 2014). Specifically, this project will assess whether the number of drug exposures (1 vs 5), environmental context, or length of the abstinence period (48 hr vs 14 days), has an age-dependent effect on CP 55,490-induced behavioral sensitization. In addition we will determine if there are gender differences in the sensitization to CP 55, 940.

It is imperative to understand the development of cannabis use during a critical period of development in a youth population since little data is available. Therefore, based on the limited data on developmental of effects of cannabinoids and behavioral sensitization studies in young

rats using other illicit drug, we have formulated the five following hypotheses:

1) Pre-adolescent vs. Adolescent

Based on evidence that early use of cannabis increases the risk of cannabis use disorder (Farmer et al., 2015), we hypothesize that preadolescent rats will show an increased sensitized response compared to adolescent rats. We expect, however, that this sensitized response will differ depending on context, length of the abstinence period, and number of drug exposures.

2) Context-Dependent vs. Context-Independent

In adult rats, substantial context conditioning occurs during exposure to drugs such as psychostimulants which increase the strength of the sensitized response (Tirelli et al., 2003). Specifically, rats tested in the same chamber that the drug was given, have a greater behavioral response than rats tested in a novel chamber (Crombag et al., 2000). Interestingly, context conditioning is not as important in pre-weanling rats when sensitized with psychostimulant drugs (McDougall et al., 2007; McDougall et al., 2009). Therefore we hypothesize that CP 55,940-induced sensitization, similar to psychostimulants, will not depend

on the context in which the drug exposures occurred in our preadolescent rats. In contrast, we expect, adolescent sensitization to the CP 55,940 to be context-dependent.

3) Multi-trial vs. One-trial

In adult rats, the number of drug exposures alters the sensitized response because one drug exposure leads to a completely context-dependent response while multiple exposures induces both context dependent and context-independent sensitization (McDougall et al., 2007). In preweanling rats, context-independent sensitization is found after both one or multiple drug exposures (McDougall et al., 2007). We expect in the present study, that young rats will show sensitization to the CP 55,940 compound after one and multi-trial sensitization and that the response will be context-independent regardless of the number of drug exposures. We also expect that the adolescent rats will show a more adult-like profile and show only context-dependent sensitization with one drug exposure and both context-dependent and context-independent with multiple exposures.

4) Persistence

We hypothesize that the sensitized response to CP 55,940 will persist for at least 14 days in our adolescent rats because of the long lasting effects of cannabinoids (Rubino & Parolaro, 2008). However, the hypotheses about our younger rats are less clear. In humans, cannabinoids have longer lasting effects in early adolescent age groups while the effects of these drugs appear to be more transient in adults (Fontes et al., 2011; Gorelick et al., 2012; Verweij et al., 2013). In contrast, the sensitization literature in rats using psychostimulant compounds show that younger rats have a more short-lived sensitized response (McDougall et al., 2009).

5)Gender

Adult female rats are more sensitive to the behavioral effects of cannabinoids because female rats given either THC or CP 55,490 have a greater antinociceptive response, show increased catalepsy, and display more spontaneous locomotor activity when compared to male rats (Tseng, 2001). Based on this greater sensitivity in other behavioral measures, we hypothesize that female rats will show increased behavioral

sensitization to the cannabinoid agonist. We also expect this sex effect to be more prominent in our older age groups as compared to our younger rats.

CHAPTER SIX
MATERIALS AND METHODS

Subjects

Subjects will include 480 male and female rats Sprague- Dawley descent (Charles River, Hollister, CA) will be used for this experiment. Rats be either preadolescent (PD 17- PD 21) or early adolescent (PD 30- PD 35) at the time of testing. All rats will be bred from dams and raised in the vivarium of the Psychology Department of California State University, San Bernardino. The maternity cages will be a large polycarbonate clear box (56 × 34 × 22 cm) and consist of a wire lid. Litters will culled on PD3 to ten pups per dam and pups will be group housed (3-4 per cage) away from dams on PD 23. All cages will have Tek-Fresh® bedding (Harlan, Indianapolis, IN). All animals will receive food and water ad libitum and kept on a 12 hour light and 12 hour dark cycle. All behavioral testing will take place during the light cycle with subjects returned to their home cage after testing. All subjects will be 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 will be performed in commercially available activity monitoring chambers (preweanling 25 × 25 × 41 cm, young adults 41×41×41 cm) from Coulbourn Instruments, Allentown, PA, USA. These chambers are 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 the distance traveled, each chamber includes an X-Y photobeam array, with 16 photocells and detectors with photobeam resolution of 0.76 cm. The position of each rat is determined every 100 ms (i.e., the sampling interval). Each chamber is equipped with a video camera centered above the chamber.

Drugs

CP 55,490 will be purchased from Sigma Aldrich and mixed in a 50% dimethyl sulfoxide (DSMO) water solution. All injections will be given intraperitoneally (IP) at a volume of 5 ml/kg (PD 15-PD 21) or 2.5 ml/kg (PD 30- PD 36).

Procedure

All experiments will take place in the same set of experimental chambers, all trials will be recorded by video camera and the activity monitoring chambers. If necessary video tapes will be watch and scored for behavior.

Experiment 1a: Effect of dose on CP 55,940-induced multi-trial sensitization in preadolescent rats.

Rats will be given five pre-treatment days (PD 15-PD 19), a 48 hour abstinence period, and one test day (PD 21), (see Figure 2). During pretreatment rats will be given CP 55, 490 (10, 33, or 100 µg, IP). All injections will occur in the testing room and 10 minutes later placed in activity chambers and distance traveled will be measured for 60 minutes. After the 48 hour abstinence period half of the rats in each drug group will receive a 10 µg/mg CP 55,490 injection and the other half will receive 33 µg/kg CP 55,490. Test day injections of CP 55,490 will be given in the testing room and rats will be placed in activity

chambers 10 min late for 120 min test day session.

Experiment 1b: Effect of dose on CP 55,940-induced multi-trial sensitization in adolescent rats.

Experimental procedures will be the same as experiment 1a however pretreatment will start at PD 30 and the test day will be on PD 36 (see Figure 2).

Experiment	Pretreatment age	Pretreatment dose CP 55,490	Test age	Test dose CP 55,490
1a	PD 15- PD 19	0, 10, 33 or 100 µg/kg	PD 21	10 or 33 µg/kg
1b	PD 30- PD 34	0, 10, 33 or 100 µg/kg	PD 36	10 or 33 µg/kg

Figure 2. Experiment 1a and 1b are picture above. These two experiments will examine the dose response cure for cannabinoid use in early and late adolescent Sprague Dawley rats. Both groups will receive one intraperitoneal injection daily of the same CP 55, 490 dose depending on the group during the pretreatment phase and one intraperitoneal injection for the testing day depending on the group.

Experiment 2a: The effects of context on CP 55,490-induced multi-trial behavioral sensitization in pre-adolescent rats.

Rats will be given five pre-treatment days (PD 15-PD 19), a 48 hour abstinence period, and one test day (PD 21), (see Figure 3). The pretreatment doses will be the best pretreatment dose determined from experiment 1 and a vehicle. All animals will be given two daily

intraperitoneal injections one in the experimental chamber and one in the home cage the two injections will occur 6 hours apart, when animals receive their injection in the experimental room they will be placed in activity chambers 10 min later and distance traveled will be measured for 60 min. On the test day (PD 21) all animals will receive a 100 mg/kg CP 55,490 intraperitoneal injections in the experimental room and animals placed in activity chambers 10 min later to monitor distance traveled for 120 minutes.

Experiment 2b: The effects of context on CP 55,490-induced multi-trial behavioral sensitization in adolescent rats.

The experimental procedure will be the same as experiments 2a however pretreatment will start on PD 30 and the test day will be on PD 36 (see Figure 3).

Experiment 2a Groups	Pretreatment PD 15- PD 19		Test Day PD 21
	Experiment Room	Home cage	Experiment Room
Context Dependent Group	CP 55,490	Saline	CP 55,490 100 mg/kg
Context Independent Group	Saline	CP 55,490	CP 55,490 100 mg/kg
Control Group	Saline	Saline	CP 55,490 100 mg/kg

Experiment 2b Groups	Pretreatment PD 30- PD 34		Test Day PD 36
	Experiment Room	Home cage	Experiment Room
Context Dependent Group	CP 55,490	Saline	CP 55,490 100 mg/kg
Context Independent Group	Saline	CP 55,490	CP 55,490 100 mg/kg
Control Group	Saline	Saline	CP 55,490 100 mg/kg

Figure 3. All groups in experiment 2a and 2b will receive two daily intraperitoneal injections, one of the best pre-treatment dose from experiment one and the other saline and the same dose for experiment two.

Experiment 3a: The effects of withdrawal and persistence on CP 55,490-induced multi-trial behavioral sensitization preadolescent rats.

Rats will be examined with five pre-treatment days (PD 15- PD 19) and either given 48 hours abstinence (PD 21) or a ten day abstinence (PD 29) period (see Figure 4). The pretreatment dose used for the five days will be the best pretreatment dose determined from experiment 1. All animals will be given their injection in the experimental room then, rats will be placed in

activity chambers 10 min later and distance traveled will be measured for 60 minutes. After either 48 hours or 10 days of abstinence all animals will receive a 100 µg/kg of CP 55,490 and be placed in activity chambers 10 min later to examine distance traveled for 120 minutes.

Experiment 3b: The effects of withdrawal and persistence on CP 55,490-induced multi-trial behavioral sensitization adolescent rats.

The same procedures will be used from experiment 3a except animals will begin pretreatment on PD 30 and be tested either at PD 36 or PD 44 (see Figure 4).

Experiment	Age groups	Pretreatment	Abstinence period	Test day age	Test day drug
3a	PD 15 to PD 19	CP 55,490 or Saline	2 days or 10 days	PD 21 or PD 29	CP 55,490 100 µg/kg
3b	PD 30 to PD 34	CP 55,490 or Saline	2 days Or 10 days	PD 36 or PD 44	CP 55,490 100 µg/kg

Figure 4. Experiment 4a and 4b will be injected with the best pretreatment dose determined from experiment one for a five day period then given either 48 hours or 10 days of abstinence and then tested for behavioral sensitization with the high dose of CP 55,490.

Experiment 4a: Effect of dose on CP 55,940-induced single-trial sensitization in preadolescent rats

All rats will be examined using a single pre-

treatment day (PD 19) and tested 24 hours later (PD 20), (see Figure 5). On the pretreatment day animals will receive a CP 55,490 (100 µg/kg) or vehicle intraperitoneal injection given in the experimental room and will be placed in activity chambers 10 minutes later and distance traveled will be measured for 60 min. All rats will be given a CP 55,490 (10, 33 or 100 µg/kg) or vehicle on the testing day in the experimental room and animals will be placed in activity chambers 10 minutes later for 120 minutes.

Experiment 4b: Effect of dose on CP 55,940-induced single-trial sensitization in adolescent rats

This experiment will use the same procedures from experiment 4a to examine will be examined using a single pre-treatment day (PD 34) and tested 24 hours later (PD 35) (see Figure 5).

Experiment	Pretreatment age	Pretreatment dose CP 55,490	Test age	Test dose CP 55,490
4a	PD 20	Saline or 100 µg/kg	PD 21	10, 33 or 100 µg/kg
4b	PD 35	Saline or 100 µg/kg	PD 36	10, 33 or 100 µg/kg

Figure 5. Experiment 4a and 4b will examine one-trial cannabinoid sensitization. Animals will experience either a single saline or a high CP 55,490 dose and then be give one of three doses of CP 55,490 24 hours later.

Experiment 5a: The effects of context on CP 55,490-induced single-trial behavioral sensitization

All rats will be examined using a single pre-treatment day (PD 20) and tested 24 hours later (PD 21), (see Figure 6). Animals will be divided in the context independent group the context dependent group and the control group animals (see Figure 6). All animals will be given two daily intraperitoneal injections one in the experimental chamber and one in the home cage the two injections will occur 6 hours apart, when animals receive their injection in the experimental room after 10 minutes they will be placed in activity chambers and distance traveled will be measured for 60 minutes. On the test day all animals will be given 100 µg/mg of CP 55,490 in the experimental room and animals will be placed in

activity chambers 10 min later for 120 min.

Experiment 5b: Single trial dose associative and non-associative cannabinoid-induced behavioral sensitization

All rats will be examined using a single pre-treatment day (PD 34) and tested 24 hours later (PD 35) using the same steps in experiment 5a with adolescence rats (see Figure 6).

Experiment 5a Groups	Pretreatment PD 20		Test Day PD 21
	Experiment Room	Home cage	Experiment Room
Context Dependent Group	CP 55,490	Saline	CP 55,490 100 µg/kg
Context Independent Group	Saline	CP 55,490	CP 55,490 100 µg/kg
Control Group	Saline	Saline	CP 55,490 100 µg/kg

Experiment 5b Groups	Pretreatment PD 35		Test Day PD 36
	Experiment Room	Home cage	Experiment Room
Context Dependent Group	CP 55,490	Saline	CP 55,490 100 µg/kg
Context Independent Group	Saline	CP 55,490	CP 55,490 100 µg/kg
Control Group	Saline	Saline	CP 55,490 100 µg/kg

Figure 6. All groups in experiment 5a and 5b will receive two intraperitoneal injections in one day, one of the best pre-treatment dose from experiment four and the other saline and the test day dose will be given 24 hours later and consist of the high CP 55,490 dose.

Analyses

To examine cannabinoid induced behavioral sensitization all experiments will use mixed-measures analyses of variance (ANOVAs) for the statistical analysis of distance traveled. A (sex x drug x time block) mixed measures ANOVA will examine pre-treatment for all experiments. A (sex x drug x time block) mixed ANOVA will be used to analyze test day data. For all experiments Tukeys tests ($p < 0.05$) will be used for the Post hoc analysis of distance traveled data. Furthermore, any litter effects will be controlled through the experimental design and in most experiments no more than one subject per litter will be assigned to a particular group, if more than one animal is associated to a group a mean of their data will be used.

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