ABSTRACT

CB1 RECEPTOR ANTAGONIST AM251 DIFFERENTIALLY AFFECTS ATTENTION AND IMPULSIVITY IN RATS

By Shannon J. Zimmerman-Nguyen

Recent studies suggest that the cannabinoid system may mediate attention and impulsive behavior (Arguello & Jentsch, 2004; Pattij et al., 2007). CB1 receptor antagonist SR141716A (SR) was found to dose dependently reduce the number of premature responses and slightly improved attentional performance in rats trained to perform the five-choice serial reaction time task (5-CSRTT), a measure of attention and impulsivity designed for animals. However, SR did not reduce other varieties of impulsive behavior in other tasks (Pattij et al., 2007). The aim of the current investigation was to further explore the role of CB1 receptor antagonism on attention and impulsive behavior by administering the more selective antagonist AM251 to rats trained to perform the 5-CSRTT. Furthermore, to create an attentional and behavioral challenge, rats were exposed to task variations designed to increase attentional demands and ability to inhibit impulsive behaviors.

Eight male Long-Evans rats were trained to perform the 5-CSRTT. Upon reaching a criterion level of performance in the 5-CSRTT, rats were administered (i.p.) CB1 receptor antagonist AM251 (2 and 4 mg/kg) and vehicle in the standard 5-CSRTT task and two task variations: variable long inter-trial interval and variable stimulus duration conditions. The 2mg dose significantly reduced premature responding and increased attention capability while the 4mg had little effect on performance in the standard task. The effects of AM251 on premature responding were dependent on the level of inter-trial interval. Both doses of AM251 had little effect on performance in the variable stimulus duration task. This investigation further supports CB1 receptor involvement in attention and impulsive behavior.

CB1 RECEPTOR ANTAGONIST AM251 DIFFENTIALLY AFFECTS ATTENTION AND IMPULSIVITY IN RATS

by

Shannon J. Zimmerman-Nguyen

A Thesis Submitted
In Partial of the Requirements
For the Degree of

Master of Science Psychology
Experimental

at

The University of Wisconsin Oshkosh
Oshkosh, WI 54901

May 2008

COMMITTEE APPROVAL

Advisor

8/20/08 Date Approved

Member

5/20/08 Date Approved

Member

5/20/08 Date Approved

PROVOST
AND VICE CHANCELLOR

6/4/08
Date Approved

FORMAT APPROVAL

4/22/2008
Date Approved

To my loving husband and family- Though I may be the "odd duck", I truly found something that sets my soul on fire. And although you may not completely understand the subject matter *within* these pages, you were there with your love, support, sacrifices, encouragement, and handiness *between* these pages when things got tough for me both in spirit and in person. I want you all to know that my accomplishments are also yours as you taught me: that anything worth having is worth working for; when the going gets tough, a Zimmerman keeps going; take great pride, heart, and create with our talents. I am blessed to have every one of you. This is for you.

ACKNOWLEDGEMENTS

First, I would like to thank my thesis chair Jim Koch, Ph.D. for his guidance, patience, and teaching me many valuable skills throughout two thesis projects. You are truly a fantastic teacher who set the bar high. I would also like to extend my appreciation and gratitude to Dawn Vreven, Ph.D. for her creativity, expertise, and dedication in making the 5-CSRTT apparatus and programming used for this project possible. Also, thanks to my committee members Dawn Vreven, Ph.D. Fran Rauscher, Ph.D. and Lee McCann, Ph.D. for their advice and critique of this manuscript and my first very long thesis proposal. Also, I must extent my thanks to all members of the Psychology Department- Faculty, Staff, and Graduate Students who have made learning fun, and who have made an everlasting impression that I will take with me.

There are so many to thank for their technical expertise including my husband, Andy Nguyen, and my Dad, Dan Zimmerman, for building and modifying the 5-CSRTT chambers, not just once, but several times. A special thanks to Marty Verboomen and Keith Gonyea for coming to my aid in a time of need with their technical expertise with the pellet dispenser. Go engineers! Also, thanks to Dace Almane for being so quick when it broke again. Also, to Matthew Roberts for building the bridge task that was meant for my first thesis. Last, but not least, thanks to Jennifer Mosley, Jamie Schrauth, and the animal colony workers for taking special care of my rats. Thank you all for everything, I couldn't have done this without you.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Overview of the Cannabinoid System	2
Implications for ADHD	8
Five- Choice Serial Reaction Time Task (5-CSRTT)	11
HYPOTHESES	20
METHOD	22
Subjects	22
Materials	22
Drug	23
Procedure	24
Administration of AM251 & 5-CSRTT Testing	27
Stimulus Duration	28
Long ITI Schedule	28
Dependent Variables	29
Data Analyses	30
RESULTS	31
The Effects of AM251 in the Standard Task	31
The Effects of AM251 in the Long ITI Condition	33
The Effects of AM251 by ITI	34
ITI Duration 5 sec	34
ITI Duration 6.5 sec	35
ITI Duration 8 sec	35
ITI Duration 9.5 sec	36
The Effects of AM251 in the Stimulus Duration Task	37
The Effects of AM251 as a Function of Stimulus Duration	38
Stimulus Duration 0.25	38
DISCUSSION	40

REFERENCES..... 48

APPENDICES
APPENDIX A: Figures..... 57
APPENDIX B: Tables..... 70

INTRODUCTION

The cannabinoid system has been implicated in a wide range of behaviors including eating (for review, see Di Marzo & Matias, 2005), drug addiction (for review, see De Vries & Schoffelmeer, 2005), and learning and memory (for review, see Lichtman, Varvel, & Martin, 2002). Recently, cannabinoids have been linked to attention and impulsive behavioral processes (Arguello & Jentsch, 2004; Pattji, et al., 2007). However, although similar behavioral paradigms were used (the lateralized reaction time task and five-choice serial reaction time task (5-CSRTT)), the CB1 receptor antagonist SR141716A (SR) produced contradictory results in these two studies. Arguello and Jentsch (2004) found that SR improved attention at low doses and had no effect on measures of impulsivity, while Pattji et al. (2007) found that similar doses of SR had no effect on attention but dose-dependently decreased the number of premature responses (Pattji, et al., 2007). Although these results were mixed, they do suggest that the CB1 receptor may mediate aspects of impulsive behavior and attention.

The aim of the current study was to further assess the effects of CB1 receptor antagonism with a different ligand, AM251, in the 5-CSRTT paradigm. In addition, alterations to the 5-CSRTT paradigm were introduced to create a behavioral challenge and further assess the effects of AM251 on attention and impulsivity.

Overview of the Cannabinoid System

Cannabis sativa or marijuana has been used for centuries as a recreational drug for its mind-altering effects and as a part of spiritual ceremony in many cultures (Ameri, 1999). Feelings of enhanced well-being, euphoria, heightened sensory perception (especially to visual stimuli), disturbed time sense (i.e. feeling that time is passing more slowly), relaxation, enhanced appetite, reduced motor coordination, and disturbed attentional capability have been reported by humans while under the influence of marijuana (Grotenhermen & Russo, 2002). Although a topic of debate and controversy, several potential therapeutic applications for marijuana and/or THC have been well characterized, including treatment for pain, nausea and vomiting, appetite stimulation, and glaucoma (Grotenhermen & Russo, 2002).

It was discovered in the late 1980's that Δ^9-tetrahydrocannabinol (THC), the primary psychoactive ingredient of marijuana (Ameri, 1999), acted upon a specific type of neuronal receptor in the central nervous system, the cannabinoid receptor (Di Marzo, Meleck, Bisogno, & De Petrocellis, 1998). In 1990, the first cannabinoid receptor was successfully isolated and cloned in the rat cortex (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and later named CB1 after the discovery of a second cannabinoid receptor type (CB2) in the human spleen in 1993 (Munro, Thomas, & Abu-Shaar, 1993). Further studies have shown that CB2 receptors are located predominantly in the peripheral nervous system and have been linked to immune system function. CB1 receptors, on the other hand, are widely distributed throughout the brain and spinal cord. The CB1 receptor is one of the most abundantly expressed receptor types in the central nervous system

(Ameri, 1999), with the highest densities found in the prefrontal cortex, striatum, hippocampus, nucleus accumbens, and cerebellum (Egertova & Elpnick, 2000; Tsou et al., 1998). Following the characterization of cannabinoid receptors, the search for naturally occurring cannabinoids in the body, or endocannabinoids, ensued (Ameri, 1999).

Endocannabinoids

Endocannabinoids are classified as neuromodulators, as they help control the release of neurotransmitters (NTs) (Di Marzo et al., 1998). Endocannabinoids are similar to NTs, in that they stimulate their respective receptor types and subtypes, also known as having an agonist action (or being classified as an "agonist"). In general, neurotransmitters act as "master keys" because they have the ability to "unlock" or activate all their respective subtypes. For endocannabinoids, this agonist action can occur at both CB1 and CB2 type receptors (Stahl, 2000).

Currently, five different endocannabinoids have been identified, but only two have been extensively studied (van der Stelt & Di Marzo, 2003). The first endogenous compound was discovered in the porcine brain in 1992 (Devane et al., 1992) and named anandamide from the Sanskrit word "ananada" meaning "bliss" (Di Marzo et al., 1998). Later, 2-arachidonylglycerol or 2-AG was discovered (Mechoulam et al., 1995). Both anandamide and 2-AG have properties similar to those of THC in that they are lipid molecules (van der Stelt & Di Marzo, 2003) and produce similar behavioral effects in rodents (Di Marzo et al., 1998), yet they are far less potent than THC and the effects do not last as long (Julien, 2005). Both these and other endocannabinoids are not stored in

vesicles inside of nerve terminals like typical neurotransmitters but are produced "on demand" from postsynaptic neurons by activation of their precursors (see Figure 1, Appendix A) (van der Stelt & Marzo, 2003).

Cannabinoid Signaling

Cannabinoid receptors belong to the super family of G-protein coupled receptors and are located primarily on presynaptic nerve terminals of neurons that release GABA, the primary inhibitory neurotransmitter of the mammalian brain. GABA neurons serve as interneurons and act to inhibit or decrease the release of NTs other neurons (Julien, 2005). Unlike most neurotransmission in which the chemical signal (NT) flow travels from presynaptic neuron to postsynaptic neuron, cannabinoids utilize retrograde signaling (Alger, 2005) (see Figure 1, Appendix A). Thus, cannabinoids travel from postsynaptic neuron to presynaptic cannabinoid receptors (Ameri, 1999).

When an endocannabinoid is released from the postsynaptic neuron and binds to a presynaptic cannabinoid receptor it activates a G-protein, which in turn inhibits adenylyl cyclase and various other effectors inside the presynaptic neuron. This process acts to decrease the GABA neuron's excitation by stimulating potassium (K+) and inhibiting calcium (Ca2+) ion channels. The release of NTs is dependent upon Ca2+ influx into the neuron. Since Ca+2 channels are inhibited, this results in decreased GABA release. The decreased GABA release ultimately results in increased neurotransmitter release from other neurons, as these are released from GABA-induced inhibition (van der Stelt & Di Marzo, 2003; Stahl, 2000; see Figure 1, Appendix A).

CB1 Receptor Agonists

The discovery and cloning of cannabinoid receptors led to the development of a class of pharmaceuticals that have increased the understanding of cannabinoid function and modulation of behavior. Several synthetic agonists (CB1 receptor stimulators) such as WIN55, 212-22 (D'Ambra, et al. 1992), HU-211 (Feigenbaum et al., 1989), and CP55 940 (Devane, Breuer, Sheskin, et al., 1992) exist for research purposes, while others, Dronabinol and Sativex are used for medicinal purposes for stimulating appetite in wasting diseases such as AIDS, and treatment of chronic pain disorders such as multiple sclerosis (MS) (Julien, 2005; for pain review see Ashton & Milligan, 2008).

CB1 receptor Antagonists

Shortly after the development of synthetic agonists, Sanofi-Aventis Labs introduced the first selective CB1 antagonist SR141716A (Barth & Rinaldi-Carmona, 1999). Another antagonist developed by Alexandros Makriyannis, AM251 has properties similar to those of SR141716A, but is more potent and has a greater affinity for the CB1 receptor (Gatley, Gifford, Volkow, Lan, & Markriyannis, 1996). The current study focused on AM251.

Cannabinoids, Behavioral Pharmacology, and the Dopamine Pathways

Endocannabinoids interact with many neurotransmitter systems including GABA, glutamate, acetylcholine, serotonin, norepinephrine, and dopamine (Grotenhermen & Russo, 2002). Endocannabinoids have shown to modulate the release of dopamine in mesocortical, mesolimbic, and corticostriatal pathways (Cheer, Wassum,

Heien, Phillips, Wightman, 2004; Szabo, Muller, & Koch, 1999). Central administration of THC and other exogenous CB1 receptor agonists into the brain of rats increases extracellular dopamine release in the ventral tegmental area, nucleus accumbens, and striatal regions (see figure 2, Appendix A) (Cheer et al., 2004; Szabo et al., 1999).

Dopamine has been implicated in a wide range of behaviors including movement, cognition, attention, learning, memory, and reward (Julien, 2005). In addition, the dopaminergic system is thought to play a major role in several psychiatric and neurological disorders (van der Stelt & Di Marzo, 2003). This close relationship between the endocannabinoid system and dopaminergic systems has become the focus of many behavioral studies and potential therapeutic effects in regard to addiction, impulsivity, and behavioral reinforcement (Ameri, 1999).

Recently, CB1 receptor antagonist SR141716A, under the trade name Acomplia (rimonabant), received approval in Europe for the treatment of obesity. At low doses (20mg), Acomplia can significantly reduce body weight and improve cardiovascular risk (Sanofi-Aventis press release, 2006). Recent evidence suggests that Acomplia inhibits food intake through two neural substrates. The first action is at the level of the hypothalamus, which is responsible for appetite control (Jamishidi & Taylor, 2001), and the second action is at the mesolimbic dopamine pathway, which is responsible for the motivational and rewarding properties of eating and translating the motivation to eat into action (Kirkham, Williams, Fezza, & Di Marzo, 2002). Acomplia not only reduces intake of normal foods, but also highly palatable, sweet and fattening foods, as rewarding properties of these foods may be blocked (for review, see Di Marzo & Matias, 2005).

Cannabinoids also act in the mesolimbic/mesocortical dopamine pathways to mediate conditioned drug seeking behavior (for review see De Vries & Schoffelmeer, 2005). Animal models of addiction or relapse have become a widely accepted research tool. Rats are operantly trained to intravenously self-administer a drug of abuse by pressing a bar (i.e. heroin, cocaine, amphetamines, nicotine, or alcohol) associated with an external cue (i.e. a light or sound stimulus, for several weeks). After stable self-administration is established, both the drug and drug-associated cues are removed. After several weeks of this condition, few or no bar presses are made (a period of extinction), the drug is made available again, and the rat is administered the CB1 receptor antagonist. The rat is then re-introduced to either the drug associated cue, a stressor (foot shocks), or a priming dose of the drug of abuse. SR141716A inhibited relapse (bar pressing for drug) when the rats were reintroduced to drug associated cues, priming doses, and in some cases under stressors for heroin (Fuchs & See, 2002), amphetamines, cocaine, and alcohol (Gonzalez, Cascio, Fernandez-Luiz, Fezza, Di Marzo, & Ramos, 2002; McGregor & Gallate, 2004; for review see De Vries & Schoffelmeer, 2005).

Thus, the modulatory effect of endocannabinoids within dopamine pathways has been well established. This close connection between these two systems has led to theories that endocannabinoids play an important role in pathology of many physiological and psychological disorders including Attention Deficit Hyperactivity Disorder (ADHD) (van der Stelt & Di Marzo, 2003).

Implications for ADHD

Attention Deficit Hyperactivity Disorder (ADHD) often is marked by age inappropriate and chronic symptoms of inattention, distractibility, disorganization, impulsivity and hyperactivity (American Psychiatric Association, 1994). It is estimated to affect 3-5% of school age children (American Psychiatric Association, 1994) and 5% of adults as symptoms typically continue into adulthood (Seidman et al., 2004). Diagnosis of ADHD is more common for males (four males are diagnosed for every female), as they are more likely to display hyperactive behaviors that are disruptive to school and home settings (American Psychiatric Association, 1994). Clinicians emphasize that ADHD is a multifaceted disorder and the current theoretical model of ADHD includes deficits in executive functions mediated by the prefrontal cortex such as planning, working memory, cognitive flexibility, organization and behavioral control (Seidman et al., 2004).

Historically, ADHD was defined by hyper-motor symptoms. Formerly, it was known as hyperactivity, hyperkinesis disorder of childhood, or minimal brain dysfunction. In fact, deficits in attention did not become the defining feature until the 1970's. Attention refers to a complex set of mental processes that allow the brain to filter and encode information from the world for use in cognitive and motor operations (Seidman et al., 2004). ADHD patients often have difficulty focusing attention on mundane or uninteresting tasks, continuously allocating attention to the task at hand, and once attention has been allocated, often have difficulty disengaging or shifting focus (Barkley, 1997).

Another key feature of ADHD is impulsivity or a lack of behavioral control (Barkley, 1997). ADHD patients often show response impairments such as difficulty waiting for a turn, interrupting others, saying or doing things that are inappropriate for the situation, failure to stop an action once it has been initiated and an inability to filter-out disrupting competing events and responses (Seidman et al., 2004). However, impulsivity is a collective concept that has both cognitive and motor components that that can be measured independently. Several forms of impulsivity have been identified behaviorally and pharmacologically including the inability to delay gratification, impulsive choice, and response inhibition. These behaviors can be modified by manipulations to serotonergic neurons (Soubrie, 1986) and dopaminergic reward systems (Evenden, 1998). Measures of these types of impulsivity include the delayed-reward and stop-signal paradigms and the five-choice serial reaction time task (discussed below) (Evenden, 1999).

Research into the pathophysiology of ADHD has focused on dysfunctions of the prefrontal cortex and striatum. Behavioral symptoms of ADHD often mirror the behaviors seen in patients and lower animals with lesions to these areas (Valera et al., 2005; Barkley, 1997). Structural MRI studies support this view showing reduced basal ganglia and prefrontal cortical volumes in ADHD patients (Seidman et al., 2004). In addition, the striatum is the proposed site of action for stimulant medications such as the dopamine and norepinephrine agonists methylphenidate (Ritalin) and amphetamine (Adderall) which are the first-line treatments for ADHD (Seidman et al., 2004). Dopaminergic and noradrenergic systems are known to play an extensive role in

executive function including problem solving, motor control, and impulsive and reward seeking behaviors (Pliszka, 2005).

However, exactly how dopaminergic and noradrenergic systems are dysfunctional in ADHD patients, either hyper- or hypo-functioning, remains unclear. Indeed, even though specific mechanisms and brain regions where Adderall and Ritalin exert their therapeutic actions are unknown, their success in the treatment of ADHD has driven clinical acceptance of decreased dopaminergic activity of mesocortical and corticostriatal systems as the main cause of ADHD (Pliszka, 2005). Furthermore, studies of methylphenidate have supported this view. When healthy subjects were administered methylphenidate, an increase in the release of dopamine in the striatum occurred only when performing tasks that required attention and reward (Volkow, Wang, Fowler, Telang, Maynard, et al., 2004).

Recent research indicates a combination of both hyper- and hypo-functioning of dopaminergic systems as the cause (Pliszka, 2005). Often ADHD patient symptoms worsen when over-exerting concentration or focus (Seidman et al., 2004). This may result from an overabundance of dopamine release in the corticostriatal, mesocortical, and mesolimbic systems that could disorganize thought processes and overstimulate reward centers. It would seem counterintuitive that stimulant medications would reduce ADHD symptoms as they increase the amount of dopamine available in the synaptic cleft. However, further increasing the pool of dopamine with stimulant medications stimulates autoreceptors (sensory mechanisms located on presynaptic neurons) which decreases dopamine release and returns the dopamine balance to a more favorable state (Pliszka,

2005). As stated previously, the endocannabinoid system also plays a role in the regulation of the dopamine pathways (Ameri, 1999; De Vries & Schoffelmeer, 2005; Cheer et al., 2004). Recent studies suggest that cannabinoids can mediate executive functions including behavioral flexibility (Hill, Froese, Morrish, Sun, & Floresco, 2006) attention, visuospatial capability and impulse control (Pattij et al., 2007; Arguello & Jentsch, 2004).

Five- Choice Serial Reaction Time Task (5-CSRTT)

The study of ADHD pathology has relied on the use of animal models, which allow experimental manipulations and treatments that are otherwise impossible in human research while eliminating many of the confounding environmental and individual difference factors (Paule et al., 2000). Thus, a valid and reliable assessment tool for psychopharmacological ADHD treatments and animal models was needed (Robbins, 2002). Some assessment tools which consistently demonstrate distinctions between persons with ADHD and normal control subjects examine selective and sustained attention and impulse control. These include tests of continuous performance (Rosvold et al., 1956) and tests of visuopatial attention such as Leonard's five-choice serial reaction time test for humans (Seidman et al., 2004). A modified five-choice serial reaction time task, or 5-CSRTT, was developed for rodents by Carli, Robbins, Evenden, and Everitt (1983) and integrates the benefits of both tests of continuous performance and Leonard's task for humans (Robbins, 2002). The 5-CSRTT has become a common and well-

regarded behavioral paradigm used to assess attention and impulsivity in rodents (Robbins, 2002).

The five-choice task is typically performed in a 9-hole box with four of the hole locations always covered (Figure 3 in Appendix A; see procedure for further details of the 5-CSRTT). A stimulus light is presented in a random order for a brief period in one of five holes. A rat must learn to respond to the stimulus with a nose-poke in the corresponding location within 5 sec, detected by an infra-red photo receptor within the entrance, in order to receive a food reward over the course of 100 trials (Carli et al., 1983). Upon performance to a criterion level, the procedure may be modified to assess several forms of attention and impulse control based on response latencies and performance measures (Robbins, 2002). These include:

Sustained attention- the ability to continuously allocate sensory and information processing resources to detect the stimulus and initiate proper responses over the course of the 100-trial test session. Deficits of sustained attention are typically detected toward the end of a test session.

Selective attention- the ability to allocate attention to a fixed number of stimulus locations while ignoring others. Variations to the stimulus brightness or duration of presentation or length of inter-trial intervals can ensure the rat is using focused attention rather than simple reflexive orienting responses. In addition, interpolating white noise at varying intervals prior to stimulus presentation can further test distractibility.

Divided attention- the ability to monitor five spatial locations simultaneously and correctly respond to the stimulus. Variations of stimulus duration can increase the difficulty of divided attention.

Impulsivity

Premature responses- responses made to spatial locations prior to stimulus presentation within the inter-trial interval period. Increasing the duration of inter-trial interval can further assess inhibitory control.

Perseverative responses- responses made to any spatial location after the first response has been made within the ITI period.

In an attempt to investigate the cannabinoid system's role in impulsive behavior, Pattij et al. (2007) assessed the impact of SR141716A (SR) and CB1 receptor agonist WIN 55,212-2 (WIN) on three distinct measures of impulse control: the 5-CSRTT, the delayed-reward paradigm, and the stop-signal paradigm. For the 5-CSRTT, two groups of rats were trained with the parameters of a 1 sec stimulus duration and a 5 sec inter-trial interval (ITI) to a stable level criterion performance of $\geq$ 80% correct responses and $\leq$ 20% errors of omission. Rats were then administered intraperitoneal (i.p.) injections of either SR (0.3, 1, and 3 mg/kg) or WIN (0.3, 1, 3 mg/kg) and vehicle (Pattij et al., 2007). SR dose-dependently decreased the number of premature responses compared to vehicle. SR also increased attentional performance as the number of correct responses increased from 77% accuracy with vehicle to 82% at the 0.3 mg/kg dose. Yet, SR had no effect on perseverative responses, omissions, or latency to collect the food reward. However, the 3

mg/kg dose significantly increased the latency to respond correctly (Pattij et al., 2007). In contrast, WIN had no effect on premature or perseverative responding at any of the doses tested. In addition, there was no difference in choice accuracy or feeder latency. Yet, the 1 and 3 mg/kg doses of WIN impaired attentional capability as the number of omissions significantly increased and latency to make a correct choice increased (Pattij et al., 2007). These results suggest that blockade of the CB1 receptor with SR can mediate premature responding but not perseverative responding in the 5-CSRTT. Furthermore, a low dose of SR was also found to improve attention by increasing correct choice accuracy. However, other forms of impulsive behavior and attention were not found to be mediated by the cannabinoid system (Pattij et al., 2007).

In a second experiment, rats were trained to perform the delay-reward paradigm, which is a measure of delay of gratification and/or impulsive choice. The task was performed in a similar box as that used for the 5-CSRTT, but only two holes were available for nose poke responses. One location resulted in an immediate small reinforcement (one pellet) and the second location resulted in a larger reinforcement (four pellets) but delayed progressively over trials (0, 10, 20, 40, and 60 sec). After 30 training sessions, the rats were randomly assigned to two groups that were administered the same doses of WIN, SR or vehicle as described above. Results indicated that no doses of WIN or SR were ineffective in shifting the preference for the larger reward as delays progressed. Thus, cannabinoids do not appear to be involved in the delay of impulsive choice (Pattij et al., 2007).

In a third experiment, rats were trained in the stop-signal paradigm, which assesses the ability to inhibit an ongoing or already initiated response at the sound of a tone (duration 50 ms, intensity 80 db). Rats were trained to a criterion level of performance (80% correct responses, 20% omissions). Measures of impulse control: premature responding, perseverations, omissions and correct responses were recorded. As in the experiments described previously, rats were administered SR, WIN, or vehicle prior to testing. SR had no effect on premature responses, perseverations, correct responses, or omissions, compared to the vehicle group. However, both the 1 and 3 mg/kg doses of SR significantly increased reaction time. WIN also had no measurable effects on impulse control measures but significantly increased reaction time (Pattij et al., 2007).

Overall, it appears that CB1 blockade has a role in mediating a specific form of impulsive behavior: the ability to inhibit a response before the stimulus is available, i.e., premature responding. In addition, there was a slight improvement in attention following the low dose of SR (Pattij et al., 2007). However, an earlier study investigating the role of CB1 receptors and attention produced conflicting results (Arguello & Jentsch, 2004).

A study by Arguello and Jentsch (2004) was one of the first investigations into the involvement of the cannabinoid system in attention and utilized the lateralized reaction time task. The lateralized reaction time task is similar to the 5-CSRTT with all the same behavioral measures, but simplified by using only two hole locations. In experiment 1, a group of rats that had received five months of training (considered "well-trained") were subjected to two task variations: stimulus duration and stimulus brightness. For the

stimulus duration condition, the stimulus was varied between 0.5, 1, 2, and 4 sec and for the stimulus brightness condition the brightness was decreased to 20-80% of full intensity. Rats were subjected to the task variations while challenged with (i.p.) injections of WIN (1, 2.5 mg/kg) and vehicle. Results indicated that in both stimulus intensity and duration conditions, the highest dose of WIN (2.5 mg/kg) decreased the number of correct choices, increased omissions and slowed response times compared to vehicle. No dose of WIN affected measures of impulse control (Arguello & Jentsch, 2004).

In a second experiment, rats received different training to avoid potential ceiling effects of high baseline performance and to see potential improvements in performance produced by SR. One group of rats was trained in one session (considered "minimally" trained) while another group was "well-trained" in a variable stimulus duration condition. After training, rats received (i.p.) SR (0.1, 0.5, or 1.0 mg/kg) or vehicle. SR had no effects on measures of attention, impulsivity, or reaction time (Arguello & Jentsch, 2004).

A third experiment attempted to demonstrate that the doses of SR used in experiment 2 were effective in blocking the CB1 receptor. Two groups of rats, well-trained and minimally trained, were pretreated with 0.5 mg/kg of SR or vehicle 5 min before they were challenged with WIN (2.5 mg/kg) or vehicle in the variable stimulus duration task. Results indicated that the 0.5 mg/kg dose of SR was able to reverse or inhibit some of the effects produced by the 2.5 mg/kg dose of WIN. The SR/WIN groups had significantly more correct responses, fewer incorrect responses, and had fewer omissions than the vehicle/WIN group. Again, there were no significant effects on

measures of impulsivity for vehicle/WIN or SR/vehicle compared to vehicle/vehicle groups.

The lack of behavioral effects of SR in the Arguello & Jentsch (2004) study could be the result of several factors. First, the variable stimulus duration condition included only one stimulus duration that was shorter (0.5 sec) than the trained target duration of 1 sec. The other two durations were longer or the same as the target duration (1 and 2 sec). The attentional load was easier for the rats because the number of correct responses increased as stimulus durations increased. Response times also were well within 1 sec of stimulus onset. Thus, the stimulus duration condition possibly lacked enough challenge, especially for well-trained rats, to see behavioral effects (Arguello & Jentsch, 2004).

Second, effects of drugs can be masked when rats already have a high level of performance. Both the well and minimally trained rats had relatively few premature and perseverative responses in the Arguello and Jentsch (2004) study. This floor effect also was evident for perseverative responses in the Pattij et al. (2007) study, and overall this restricted range leaves little room for improvement (Arguello & Jentsch, 2004).

Hill et al. (2006) compared a cannabinoid agonist and an antagonist for differential effects on executive functioning. A cross-maze was used to demonstrate cognitive flexibility in the form of set-shifting: the ability to abandon a previously learned and successful strategy and adopt a new strategy. This behavior is thought to be mediated by the medial prefrontal cortex (Hill et al., 2006; Ragozzino, Detrich, & Kesner, 1999).

One group received visual cue training. They were required to enter the arm containing a visual cue (a black and white striped piece of laminated cardboard) which was placed randomly in one of the two choice arms to successfully collect a food reward. A second group received response training in which a rat always had to make either a right or left turn to collect the a food reward regardless of the location of the visual cue.

The following day, rats were then tested in the other paradigm. Thus, visual cue trained rats were tested in the response discrimination paradigm and vice versa. Prior to testing, rats were administered intraperitoneal (i.p.) injections of CB1 receptor agonist HU (5 or 20 mg/kg) or antagonist AM251 (2 or 5 mg/kg) and assessed on the following measures: the number of trials to criterion, the number of probe trials required to get one correct choice, perseverative errors (the number of incorrect arm entry errors) and the time required to successfully complete testing (Hill et al., 2006).

The high dose of HU (20mg/kg) significantly increased the number of sessions to criterion and number of perseverative errors compared to vehicle. The low dose of HU (5mg/kg) significantly decreased perseverative errors compared to vehicle. The AM251 (2mg/kg) group also had significantly fewer perseverative errors (Hill et al., 2006). Overall, administration of low doses of AM251 and HU enhanced behavioral flexibility by decreasing perseverative errors and had fewer trials to a successful set-shift, while those administered high doses HU had difficulty shifting response strategy.

As can be seen from the above, the effects of CB1 receptor blockade on attention, executive function, and impulsivity still remain unclear. In addition, there are modifications to the 5-CSRTT task that can further challenge attention and impulse

control, such as varying stimulus durations and inter-trial intervals, which may serve to clarify the role of CB1 receptors in these behaviors. These task variations can help eliminate ceiling and floor effects when rats are trained to a high level of performance. Longer inter-trial intervals can increase both attentional demand and premature responding. Shorter stimulus durations can increase selective attentional demands. Furthermore, making the stimulus less predictable by varying stimulus durations and inter-trial intervals can further assess behavioral flexibility (Robbins, 2002).

The purpose of the current study was to further investigate the role of CB1 receptor blockade in attention and impulsive behavior by administering a selective CB1 receptor antagonist, AM251. Similar to previous research, rats were trained to perform the five-choice serial reaction time task. Additionally, in order to create a behavioral challenge, rats were tested in two task variations: variable long inter-trial interval and short stimulus duration conditions.

Hypotheses & Predictions

1. Variations of the 5-CSRTT task will further challenge attention and impulse control.
    a. The variable stimulus duration condition will decrease correct choice accuracy, increase the number of omissions and incorrect responses and increase the number of premature and perseverative responses.
    b. The variable long inter-trial interval (ITI) condition will decrease the number of correct responses and increase the number of omissions, premature responses and perseverative responses.
2. AM251 will dose dependently decrease impulsive behavior.
    a. The 2 mg/kg dose of AM251 will decrease the number of premature responses in the standard task, the variant long ITI and the stimulus duration conditions.
    b. The 4 mg/kg dose will further decrease the number of premature responses made in all conditions.
    c. The 2 and 4 mg/kg dose will decrease the number of perseverative responses in the variable stimulus duration and long ITI conditions, but not in the baseline condition.
3. AM251 will increase attentional capabilities.
    a. Both the 2 and 4 mg/kg doses will increase the number of correct responses and decrease omissions in the variable long ITI and stimulus duration conditions, but not the baseline condition.

b. Both the 2 and 4 mg/kg doses will increase latency to respond correctly, but will not affect the latency to collect food reward (motivation).