BEHAVIORAL MECHANISMS OF Δ⁹-THC IN NONHUMAN PRIMATES

BY

WILLIAM JOHN

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Approved By:

Michael A. Nader, Ph.D., Advisor
Thomas J. Martin, Ph.D., Chair
Paul W. Czoty, Ph.D.
Linda J. Porrino, Ph.D.
Jenny L. Wiley, Ph.D.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF ABBREVIATIONS</th>
<th>iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I       INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II      DETERMINANTS OF CANNABINOID SELF-ADMINISTRATION IN NONHUMAN PRIMATES</td>
<td>61</td>
</tr>
<tr>
<td>Submitted for publication to Neuropsychopharmacology, August, 2016</td>
<td></td>
</tr>
<tr>
<td>III     CHRONIC Δ⁹-THC IN RHEUS MONKEYS: EFFECTS ON COGNITIVE PERFORMANCE AND Dopamine D₂/D₃ AVAILABILITY</td>
<td>93</td>
</tr>
<tr>
<td>Submitted for publication to Biological Psychiatry, August 2016</td>
<td></td>
</tr>
<tr>
<td>IV      DISCUSSION</td>
<td>132</td>
</tr>
<tr>
<td>SCHOLASTIC VITAE</td>
<td>202</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>microgram</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>CANTAB</td>
<td>Cambridge Neuropsychological Test Automated Battery</td>
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<tr>
<td>CBR</td>
<td>cannabinoid receptor</td>
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<tr>
<td>Cd</td>
<td>caudate</td>
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<td>CD</td>
<td>compound discrimination</td>
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<td>CDC</td>
<td>Center for Disease Control</td>
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<tr>
<td>CDR</td>
<td>reversal of compound discrimination</td>
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<td>cm</td>
<td>centimeter</td>
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<td>CPP</td>
<td>conditioned place preference</td>
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<td>CS</td>
<td>conditioned stimulus</td>
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<tr>
<td>d</td>
<td>days</td>
</tr>
<tr>
<td>D2</td>
<td>dopamine D2-like receptor superfamily</td>
</tr>
<tr>
<td>D2t</td>
<td>dopamine D2 receptor subtype</td>
</tr>
<tr>
<td>D3</td>
<td>dopamine D3 receptor subtype</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DEA</td>
<td>Drug Enforcement Agency</td>
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<tr>
<td>DMS</td>
<td>delayed-match-to-sample</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
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<td>DVR</td>
<td>distribution volume ratio</td>
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<tr>
<td>ED</td>
<td>extradimensional shift</td>
</tr>
<tr>
<td>ED₅₀</td>
<td>interpolated effective dose that engenders 50% response</td>
</tr>
<tr>
<td>FI</td>
<td>fixed-interval</td>
</tr>
<tr>
<td>FR</td>
<td>fixed-ratio</td>
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<tr>
<td>FT</td>
<td>fixed-time</td>
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<td>g</td>
<td>gram</td>
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<td>h</td>
<td>hours</td>
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<tr>
<td>HCl</td>
<td>hydrochloride</td>
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<tr>
<td>icv</td>
<td>intracerebroventricular</td>
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<tr>
<td>inf</td>
<td>infusion</td>
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<tr>
<td>ID</td>
<td>intradimensional shift</td>
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<td>IRP</td>
<td>Intramural Research Program</td>
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<tr>
<td>i.v.</td>
<td>intravenous</td>
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<tr>
<td>k.g.</td>
<td>kilogram</td>
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<tr>
<td>LSD</td>
<td>least significant difference</td>
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<td>m</td>
<td>meters</td>
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<td>mm</td>
<td>millimeter</td>
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<tr>
<td>methA</td>
<td>methamphetamine</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>min</td>
<td>minute(s)</td>
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<tr>
<td>ml</td>
<td>milliliter</td>
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<td>MR</td>
<td>magnetic resonance</td>
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<td>NIDA</td>
<td>National Institute on Drug Abuse</td>
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<td>NHP</td>
<td>nonhuman primate</td>
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<td>PCP</td>
<td>phencyclidine</td>
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<td>PET</td>
<td>positron emission topography</td>
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<td>PFC</td>
<td>prefrontal cortex</td>
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<td>Abbreviation</td>
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<tr>
<td>Pt</td>
<td>putamen</td>
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<tr>
<td>PT</td>
<td>pretreatment</td>
</tr>
<tr>
<td>R</td>
<td>response</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>s</td>
<td>second(s)</td>
</tr>
<tr>
<td>S+</td>
<td>stimulus signaling reinforcement</td>
</tr>
<tr>
<td>S-</td>
<td>stimulus signaling trial termination, lack of reinforcement</td>
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<tr>
<td>S(^c)</td>
<td>consequent stimulus</td>
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<tr>
<td>S(^o)</td>
<td>discriminative stimulus</td>
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<tr>
<td>SA</td>
<td>self-administration</td>
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<tr>
<td>SAMHSA</td>
<td>Substance Abuse and Mental Health Services Administration</td>
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<tr>
<td>s.c.</td>
<td>subcutaneous</td>
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<tr>
<td>SC</td>
<td>consequence stimulus</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>SR</td>
<td>reinforcer</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>THC</td>
<td>(\Delta^9)-tetrahydrocannabinol</td>
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<tr>
<td>TO</td>
<td>timeout</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>WCST</td>
<td>Wisconsin Card Sort Test</td>
</tr>
<tr>
<td>WM</td>
<td>Working memory</td>
</tr>
</tbody>
</table>
LIST OF TABLES

CHAPTER I

Table I. Cannabinoid self-administration studies in experimental animals ..........13

CHAPTER II

Table I. Ages, weights, and self-administration histories for individual rhesus monkeys ...........................................................................................................66
Table 2. Relationship between CP55,940 potency to decrease food-maintained responding and CP55,940 self-administration ........................................73

CHAPTER III

Table I. Individual parameters used throughout study that produced delay-dependent effects on DMS performance.........................................................104
Table II. Baseline percent accuracy (± SD) at each delay for individual monkeys responding under the DMS task.................................................................111
Table III. Individual and mean (±SEM) [11C]-raclopride DVRs in control and THC treated monkeys..........................................................................................116
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>FIGURE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>Dose comparisons for preclinical THC self-administration studies in nonhuman primates ..........................................19</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>Effects of CP 55,940 and THC on food maintained responding and body temperature in individual rhesus monkeys ..........................................................72</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Individual self-administration dose-response curves for CP 55,940, THC, and THC before after the development of tolerance to rate-decreasing effects on food-maintained responding under an FR 10 schedule of reinforcement ...........................................74</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Acquisition of CP 55,940 self-administration ............................................75</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Transient reinforcing effects of THC in individual rhesus monkeys ..........76</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>THC functioned as a reinforcer in three monkeys after daily THC treatment ........................................................................78</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>THC self-administration under a [FI 10-min (FR 30:S)] second-order schedule of reinforcement .........................................80</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>Experimental timeline ........................................................................102</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Effects of acute THC on food-maintained responding and body temperature before and during chronic THC (1.0 mg/kg, s.c.) treatment (A) and mean (±SD) food-reinforced response rates for sessions before (BL, baseline) during, and after chronic THC treatment for individual monkeys (B) .................................................................................109</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Effects of acute THC on cognitive performance before and during chronic THC 1.0 mg/kg, s.c.) treatment ................................................................................113</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Residual effects (i.e., 22 hrs after administration) of chronic THC treatment on delayed match-to-sample performance (A) and discrimination and reversal learning and attentional set-shifting performance (B) ........................................................................115</td>
</tr>
</tbody>
</table>
Figure 5. Effects of chronic THC treatment on dopamine D_2/D_3 receptor availability as measured with [^{11}C]-raclopride .......................................................... 117

CHAPTER IV

Figure 1. Hypothetical dose-response curve under a concurrent THC-food schedule of reinforcement ........................................................................................................ 145

Figure 2. Effects of THC (A) and CP 55,940 (B) pretreatment on cocaine-drug choice in rhesus monkeys ................................................................. 161

Figure 3. Rate dependency of THC’s potency to decrease rates of food-maintained responding ................................................................. 178
ABSTRACT

John, William S.

BEHAVIORAL MECHANISMS OF Δ⁹-THC IN NONHUMAN PRIMATES

Dissertation under the direction of Michael A. Nader, Ph.D., Professor of Physiology and Pharmacology

A comprehensive understanding of the reinforcing effects and cognitive consequences of Δ⁹-tetrahydrocannabinol (THC), the primary psychoactive constituent in marijuana, is critical for developing treatments in cannabis dependent patients. Despite the fact that marijuana is the most commonly abused illicit substance in the U.S., self-administration (SA) of THC has only been established in one laboratory using New World primates (squirrel monkeys). In Chapter 2, THC and the synthetic cannabinoid agonist CP55,940 were made available for SA in Old World monkeys, a species more closely related to humans, by using similar parameters deemed successful in squirrel monkeys. CP55,940 functioned as a reinforcer in a subset (3 of 8) of rhesus monkeys, with an inverse relationship between magnitude of SA and sensitivity to rate-decreasing effects. On the other hand, THC did not function as a reinforcer in any monkey. To test the hypothesis that attenuating the rate-decreasing effects of THC would increase the likelihood THC would function as a reinforcer, monkeys were administered THC daily until tolerance developed. When THC SA was reexamined, it functioned as a reinforcer in a subset (3 of 8) of subjects. Further, the reinforcing effects of THC were noted in two of four cynomolgus monkeys responding under a second-order schedule of reinforcement in which behavior was maintained by conditioned stimuli. These studies indicate that sensitivity to rate-decreasing effects and schedule of reinforcement are important determinants for cannabinoid-maintained behavior.
In Chapter 3, monkeys were chronically treated with THC and the residual effects (22 hrs after THC administration) were assessed on cognitive performance. Impairments were found in working memory (WM) but not in other aspects of executive function. These cognitive deficits were independent of changes in dopamine D_{2/3} receptor availability as measured with PET and [$^{11}$C]-raclopride. THC-induced impairments to WM recovered during abstinence. Moreover, chronic THC treatment resulted in tolerance to the acute impairments in compound discrimination learning but only to impairments in working memory when the cognitive demand was low.

Overall, these studies provide insight on the behavioral mechanisms underlying the reinforcing, unconditioned, and cognitive effects of THC, which is important for understanding the abuse-related properties of cannabis. Such knowledge should aid in the development of treatments for cannabis use disorder.
CHAPTER I
INTRODUCTION

Drug abuse is a major public health problem worldwide, with an estimated 21.6 million individuals that meet DSM IV criteria for substance dependence or abuse in the United States alone (SAMSHA, 2013). Drug abuse is a chronic relapsing brain disease characterized by compulsion to seek and take a drug, loss of control in limiting intake, and the emergence of negative affect (e.g., dysphoria, anxiety) when access to the drug is withheld. In addition to the deleterious effects on the individual, drug abuse poses an enormous economic cost to society including costs from crime, loss in productivity, health care, incarceration, and drug enforcement operation costs. Estimates indicate that overall, these costs exceed $600 billion annually in the United States (National Drug Intelligence Center, 2011; Rehm et al., 2009; CDC, 2014).

Marijuana (Cannabis sativa) is the most commonly abused illicit substance in the United States with more than 4 million Americans that meet DSM IV criteria for marijuana dependence (SAMSHA, 2013). In fact, this number is greater than dependence on painkillers, cocaine, heroin, and stimulants combined (SAMSHA, 2013). Furthermore, the second most substance abuse treatment admissions in the U.S. were for cannabis-related disorders, with alcohol-related disorders being the first (SAMSHA, 2013). The number of marijuana users has been steadily increasing among 12th graders, which is associated with a decrease in the perceived risks of regular use (Johnston et al., 2014). Moreover, marijuana is now legal in 4 states, suggesting that use will increase. The behavioral therapies that have been evaluated as treatments for marijuana dependence are only modestly effective while there are currently no effective pharmacotherapies (Nordstrom and Levin, 2007). As a result, there is a compelling need for more research on the impact of marijuana on neurobiology and behavior in order to develop more targeted therapeutic strategies for cannabis dependence.
Cannabis contains more than 400 different chemicals including phytocannabinoids, terpenoids and essential oils (ElSohly and Slade, 2005); however, the behavioral effects of cannabis (i.e. euphoria, relaxation, anxiety, confusion, memory loss, and paranoia) are primarily the result of the psychoactive ingredient, Δ9-tetrahydrocannabinol (THC) (Adams and Martin, 1996; Wachtel et al., 2002; Hall and Degenhardt, 2009). The behavioral effects of THC are largely mediated by its action as a partial agonist at the G-protein coupled cannabinoid-type 1 (CB₁) receptor, which is the most-abundantly expressed G-protein-coupled receptor in the brain. In particular, CB₁ receptors have dense expression in brain regions involved in reward, addiction, and cognitive function, including the amygdala, cingulate cortex, prefrontal cortex, ventral pallidum, caudate-putamen, nucleus accumbens, ventral tegmental area, and lateral hypothalamus (Glass et al., 1997; Wang et al., 2003). The endocannabinoid system also contains cannabinoid type 2 (CB₂) receptors, at which THC is a partial agonist as well. CB₂ receptors are known to mainly be expressed by immune cells; however, recent evidence suggest CB₂ receptors are also found centrally, albeit at much lower levels than CB₁ receptors, and may mediate some of THC’s actions as well (Atwood and Mackie, 2010). CB₁ and CB₂ receptors are coupled to similar transduction systems, primarily through Gᵢ or Gₒ proteins, which directly inhibit the release of GABA, glutamate, and acetylcholine. Moreover, THC has been demonstrated to stimulate neuronal firing of dopamine (DA) through the mesolimbic DA system in animals (Tanda et al., 1997) and humans (Bosson et al., 2009; Voruganti et al., 2001; Bosson et al., 2015; Van de Giessen et al., 2016), which has been proposed as a contributing factor to its reinforcing effects.

Moreover, a fundamental principle governing the field of behavioral pharmacology is the notion that the behavioral action of a drug cannot be confined to the inherent pharmacological properties of the drug itself. Rather, the behavior-environment
contingencies must be considered in determining the effects of a drug in addition to its pharmacological properties. These conditions include, but are not limited to, the schedule of reinforcement, the stimulus context, the type and level of the establishing operation, the type of consequent event, and the behavioral history of the subject. For instance, the same dose range of nicotine has been shown to maintain or punish responding that produces its injection or to maintain responding that prevents its injection (Goldberg et al., 1983). Similarly, cocaine has been shown to simultaneously maintain responding by scheduled injections of the drug and by termination of the schedule of cocaine injection (Spealman, 1979). These pioneering behavioral pharmacological studies demonstrate that drugs affect behavior in many different ways depending on the prevailing environmental conditions and that these effects cannot be predicted simply on the basis of their pharmacological nature.

The overarching goal of this dissertation was to apply this concept of behavioral mechanisms and extend it to an examination of the factors that influence the reinforcing effects of THC and the specificity and temporal nature of the cognitive consequences of THC by using a within-subject study design in nonhuman primates. A better understanding of these behavioral mechanisms of THC will be important for 1) identifying variables that may render certain individuals more vulnerable to cannabis abuse, 2) discovering parametric manipulations that can be used to advance the cannabinoid self-administration methodology in preclinical animal models and 3) for identifying specific attributes of cannabis dependent patients that may be targeted by pharmacological and behavioral interventions to improve treatment outcome.

In the context of these aims, the following chapter will present an overview of previous studies that have examined the effects of THC in 1) preclinical animal models of abuse liability, 2) human laboratory models of reinforcement, and 3) studies assessing the neurocognitive effects of cannabis/THC. This overview will serve to identify the
recent advances made in these areas of research as well as the current gaps in knowledge and how that information has shaped the studies within this dissertation.

**Drug discrimination**

Much of the information we know regarding the abuse-related effects of cannabinoids comes from preclinical studies using the drug discrimination procedure (Balster and Prescott, 1992; Wiley, 1999). The drug discrimination procedure is based on the ability of a drug to induce a specific set of interoceptive stimulus conditions perceived by animals that might be predictive of the subjective reports of perceptions/feelings induced by the same drug in humans. As a result, the drug discrimination procedure does not measure reinforcing effects of drugs; however, there is usually a good correlation between those drugs belonging to a particular pharmacological class that serve as reinforcers and those that produce subjective/discriminative effects characteristic of that pharmacological class (Schuster and Johanson, 1988). Therefore, studies of the subjective effects of new drugs in both humans and animals have been relatively good predictors of whether or not a drug will be abused.

Animal drug discrimination procedures consist of an initial period of training that consists of alternating the administration of either a specific drug dose or the drug’s vehicle before each session. Typically, sessions take place within an experimental chamber, which has two different response manipulanda. Animals learn to make a response on one manipulandum when sessions are preceded by injection of the training drug and make a response on the other manipulandum during sessions preceded by a vehicle injection. In order to maintain this behavior, responding is intermittently reinforced by food pellets or by postponement or escape from electric shocks. Once subjects reach a criterion level of correct performance (e.g. 80% drug appropriate
responding), test sessions are occasionally scheduled, during which they receive either the training drug at a different dose or a different drug, to test whether or not such a drug/dose will produce a discriminative stimulus effect similar to the training drug. Generally, if the training drug is an agonist, only agonist drugs belonging to the same pharmacological class will produce full substitution to the training stimulus and only antagonists belonging to the same pharmacological class will selectively block the training stimulus.

Cannabinoid drugs, indeed, show a pharmacological specificity in this behavioral procedure. For example, in animals trained to discriminate injections of THC from injections of its vehicle, only drugs that selectively activate CB₁ receptors fully substitute for the THC training stimulus (Wiley et al. 1993a, 1993b, 1995c; Barret et al. 1995). Furthermore, CB₁ receptor agonists have been shown to substitute for the THC training stimulus with a potency that is consistent with their in vitro affinity for the CB₁ receptors (Balster and Prescott 1992; Gold et al. 1992; Wiley et al. 1995b).

In addition to THC, other cannabinoid CB₁ receptor agonists have also been used as the training stimulus (Wiley et al. 1995a, 1995b; Perio et al. 1996; Järbe et al. 2001). For example, Wiley et al. (1995b) trained rats to discriminate the synthetic CB₁ receptor agonist CP 55,940 from saline and found that THC, the synthetic CB₁ receptor agonist WIN 55,212-2, and cannabinoil all substituted for the CP 55,940 training stimulus and did so at potencies similar to those that displace CP 55,940 in binding studies. Similarly, other investigators have trained rats to discriminate WIN 55,212-2 from vehicle (Perio et al. 1996), and found that WIN 55,212-2 completely generalized to THC and CP 55,940. Moreover, the discriminative stimulus effects of THC and other synthetic CB₁ agonists have been shown to be antagonized by the selective CB₁ receptor antagonist SR 141716A, further demonstrating that the cannabinoid discrimination is mediated by CB₁ receptors (Mansbach et al. 1996; Perio et al. 1996). It has been demonstrated that
these discriminative stimulus effects are centrally mediated behaviors through studies showing that a selective CB₁ receptor antagonist that does not cross the blood-brain barrier, SR 140098, does not antagonize the discriminative stimulus effects of cannabinoids (Perio et al. 1996).

**Conditioned place preference/aversion**

Another animal model of abuse liability that has provided much information on the behavioral effects of cannabinoids is the conditioned place preference (CPP) procedure. This procedure is based on the principles of classical conditioning and provides an indication of drug-related reward/aversion effects in animals. The rewarding/aversive stimulus properties of a drug assessed under these procedures refer to the appetitive nature of the stimulus as opposed to the ability of a drug to increase the probability of a given behavior (i.e. reinforcing effects). Although methodological details differ among laboratories, CPP procedures typically begin by allowing animals to freely explore two distinct environmental contexts within a chamber, which differ in color, size, or texture, and are connected by an open door. The time spent in each context is then recorded. During conditioning sessions, a drug injection (unconditioned stimulus) is repeatedly paired with one of the two environmental contexts while access to the other context is prohibited. On alternate sessions, vehicle injections are paired with the other environmental context. On the final test day, no injections are given and the relative time spent in each environmental context is measured and the difference is taken in order to provide a measure of preference. Using this dependent variable, the CPP model has been shown to have high predictive validity in that most drugs abused by humans are able to increase the time spent in the drug-paired context (i.e., produce a place preference); however, many interpretations of results are limited due to the frequency of false positives and the difficulties encountered in obtaining systematic dose-response
relationships over a limited range of doses (Bardo and Bevins 2000; Tzschentke 1998). CPP studies with cannabinoids have been largely ineffective at producing positive place preferences and the studies that have shown cannabinoid place preference have done so at various doses and magnitudes depending on the training procedure used across studies. Although very specific dosing parameters are required to produce place preferences for many drugs in the CPP paradigm (Bardo and Bevins 2000; Tzschentke 1998, the ability of cannabinoids, such as THC and the synthetic CB1 receptor high-efficacy agonist CP 55,940, to produce a place preference in rats and mice are particularly sensitive to the timing of injections as well as on the range of doses used (Lepore et al. 1995; Valjent and 2000; Braida et al. 2001; Ghozland et al. 2002). For instance, Lepore et al. (1995) demonstrated that when a standard schedule of daily training injections was used (i.e., vehicle, drug, vehicle, drug, etc.), a relatively low dose of THC, 1.0 mg/kg, produced a conditioned place aversion, however, higher doses of 2.0 and 4.0 mg/kg THC produced positive place preferences. When the schedule of daily injections was changed and a day where no drug or vehicle injection was administered was added in between test sessions (i.e., vehicle, day off, drug, day off, vehicle, day off, drug, etc.), the results were opposite such that THC produced a conditioned place preference at 1.0 mg/kg but place aversions were found at the 2.0 and 4.0 mg/kg doses of THC. These findings indicated that the rewarding effects of THC as assessed under the CPP paradigm might be qualitatively changed by increasing the interval between drug injections due to what the authors referred to as a “postdrug dysphoric rebound.” As a result, the pharmacological properties of THC that may be contributing to this effect may help to explain why THC and synthetic cannabinoids often produce conditioned place aversions, rather than place preferences, in rats and mice using similar dose ranges and standard place-preference conditioning procedures (Lepore et al. 1995; McGregor et al. 1996; Sanudo-Pena et al. 1997; Mallet and Beninger 1998; Chaperon et
A study by Valjent and Maldonado (2000) also found positive place preferences with THC by using another variation in the procedure. These investigators found that when using a standard place-conditioning protocol in mice, a relatively low dose of THC (i.e., 1.0 mg/kg) produced neither place aversion nor preference while a high dose (i.e., 5.0 mg/kg) produced place aversion. On the other hand, when mice received an additional exposure to THC before the conditioning test such that a 1.0 mg/kg injection was administered in their home cage 24 h before the start of the experiment and were exposed to the drug-paired conditioning chamber for a longer period of time (45 min instead of 15–30 min), the lower dose of 1.0 mg/kg THC produced a place preference while the higher dose of 5.0 mg/kg produced neither place preference nor aversion. This finding has also been replicated by a separate group of investigators (Ghozland et al. 2002). Overall, these results suggested that prior exposure to the drug may facilitate the development of positive place preferences, which may be otherwise overridden by certain aversive effects of THC that are apparent upon initial exposure. These findings may be particularly informative for cannabinoid self-administration experiments because they imply that certain parametric manipulations may need to be designed into studies in order to account for the effects of THC may inhibit the expression of its reinforcing effects.

**Drug self-administration**

The drug self-administration procedure is the primary methodology for demonstrating the reinforcing effects of drugs that are abused by humans. The procedure is built on principles of operant conditioning such that laboratory animals are trained to respond on a manipulandum to receive an injection of a drug. Like any other operant behavior, the drug self-administration procedure can be broken down into
specific components using Skinner’s 3-term contingency (Skinner, 1938), which is described as follows:

\[ S^D \rightarrow R \rightarrow S^C, \]

where \( S^D \) designates the discriminative stimulus, \( R \) designates an operant response, and \( S^C \) designates a consequent stimulus. The arrows specify the contingency (i.e. performance of the response during the \( S^D \)) that will result in delivery of the consequent stimulus \( S^C \). Thus, a typical drug self-administration experiment would involve the illumination of a stimulus light inside an operant chamber (the \( S^D \)), signaling to an animal that depression of a response lever (\( R \)) will result in delivery of a drug injection via an indwelling intravenous catheter (\( S^C \)). Consequent injections of a drug that increase the probability of responding leading to their delivery are operationally defined as reinforcers, while the discriminative stimulus, responses, and consequent stimuli are defined by the schedule of reinforcement.

The first demonstrations of drug self-administration came in the 1960s where rats and monkeys previously made physically dependent on morphine were trained to press a lever when those responses produced intravenous injections of morphine (Weeks, 1962; Thompson and Schuster 1964). Shortly following, the reinforcing effects of opioids in subjects that had not previously been made dependent prior to the start of the self-administration studies were demonstrated (Yanagita et al., 1965a, b; Deneau et al., 1969). Since then, the reinforcing effects of practically every drug abused by humans have been demonstrated in experimental animals by the self-administration procedure under a variety of routes, schedules of reinforcement, and species. These studies have been paramount in advancing our understanding of drug abuse on both a conceptual and scientific level. For example, prior to the demonstrations that laboratory animals will self-administer a variety of drugs, drug abuse was widely considered to be a result of an “addictive personality” or weakness in self-control. Moreover, self-administration studies
have shed light on many of the pharmacological, environmental, and biological determinants of drug-taking behavior as a model of addiction. However, the one drug class that has remained a “false negative” in the self-administration procedure has been the cannabinoids including THC. Successful efforts have been limited to one laboratory using squirrel monkeys and a unique set of parameters that have not been previously employed (Tanda et al., 2000; Justinova et al., 2003). Every other THC self-administration attempt to date using Old World nonhuman primate species (rhesus monkeys) or rodents has been unsuccessful (Kaymakcalan 1972, 1973; Pickens et al., 1973; Harris et al., 1974; Leite and Carlini 1974; Carney et al., 1977; Van Ree et al., 1978; Mansbach et al., 1994; Li et al., 2012; Lefever et al., 2014). Therefore, the lack of a widespread, suitable method to establish robust levels of THC SA in animals remains an impediment for directly assessing the abuse-liability of novel cannabinoid compounds and for developing treatments for human marijuana abuse. Thus, the first aim of this dissertation was to establish the experimental conditions necessary for THC SA to be maintained by Old World monkeys, a species more closely related to humans than the New World squirrel monkeys. Previous studies on the reinforcing effects of cannabinoids in human subjects and preclinical animals will be presented. This will be followed by a discussion on potential factors that may be limiting the expression of cannabinoid reinforcement in animal models and how the studies within this dissertation sought to address those factors in order to demonstrate the reinforcing effects of THC.

THC self-administration in humans

Several studies in humans have provided experimental data supporting THC’s integral role in the reinforcing effects of smoked marijuana. For instance, when subjects are given a choice between THC-containing cigarettes and a non-drug reinforcer (e.g., snack food) or between marijuana cigarettes with different THC contents, the THC
cigarettes are consistently chosen over non-drug reinforcers and cigarettes with a greater THC content have been shown to be consistently preferred to cigarettes with a lower THC content, even when the choice between the two was not mutually exclusive (Haney et al., 1997, Kelly et al., 1997 and Ward et al., 1997). Other studies in humans have also attempted to better define THC's role as the primary psychoactive ingredient in marijuana by investigating whether the its reinforcing effects are dose-dependent. In order to do this, the THC content in a cigarette is systematically manipulated during self-administration sessions and the behavioral endpoints associated with reinforcement are compared to placebo self-administration or self-administration of a cigarette containing a low amount of THC. For example, Herning et al. (1986) investigated whether marijuana smoking behavior was dose-dependent in experienced users by examining whether the subjects would titrate their intake according to differing THC concentrations in order to achieve a defined subjective state. It was observed that when cigarettes containing a high concentration of THC (3.9% THC) was available, the subjects took significantly more puffs with longer intervals between puffs compared to when cigarettes containing low amounts of THC (1.2% THC) were available. It was also found that the subjects inhaled substantially larger (46%) volumes of air when self-administering the high THC-content cigarettes. Thus, it was concluded that perhaps the subjects were demonstrating different smoking-related behavior as a mechanism to titrate the THC dose, thus indicating a dose-related effect. Another study by Nemeth-Coslett et al. (1986) also demonstrated compensatory changes in marijuana smoking as a function of THC dose where expired air carbon monoxide (CO) levels following smoking were inversely related to THC content of the marijuana (1.29%, 2.84% or 4.0% THC). Thus, it was suggested that subjects reduced their smoke intake as THC content increased thereby providing evidence for dose-dependency in THC’s reinforcing effects.
Taken together these studies demonstrate that, aside from epidemiological evidence, THC produces clear and mostly dose-related reinforcing effects in controlled experimental studies using human subjects. What is not clear, however, is that despite these demonstrations in humans, every attempt in experimental animals with the exception of one laboratory has failed to demonstrate the ability of THC to function as a reinforcer. Going forward, it will be important to “reverse translate” these findings from humans to animals in order to optimize the cannabinoid self-administration model. For instance, given the limited dose range that smoking titration occurs in humans, careful consideration of the unit dose made available for self-administration as well as the pharmacokinetic factors of cannabinoids will be vital for optimizing the cannabinoid self-administration model in experimental animals.

**Cannabinoid self-administration in experimental animals**

Numerous attempts have been made over the past 3 decades to demonstrate persistent, dose-related reinforcing effects of THC in experimental animals; however, successful efforts have been limited to one laboratory using squirrel monkeys and a set of unique experimental parameters (see Table 1). Therefore, the majority THC self-administration studies in animal models are incongruent to the numerous laboratory demonstrations of the reinforcing effects of THC in human subjects. In early preclinical studies, many procedural factors were taken into consideration including route of administration (e.g., inhalation, oral, intravenous), previous drug history, and schedule of reinforcement, however, none of these studies showed that THC could maintain rates of responding above vehicle levels. For example, in one early study, Harris et al. (1974) failed to demonstrate reinforcing effects of THC (0.025-0.3 mg/kg) under an FR1 schedule (12 h access per day, 20 days per dose) in drug-naïve rhesus monkeys or monkeys that had histories of cocaine, phenobarbital, PCP, ethanol, or amphetamine
self-administration. THC also failed to function as a reinforcer in this study following 30 days of programmed infusions of THC (2.0 mg/kg/day) or in monkeys that had been trained to self-administer a cocaine-THC mixture. In another early study using rhesus monkeys trained to self-administer PCP, THC was substituted and maintained low rates of behavior but did not persist above vehicle levels over repeated sessions (Pickens, 1973). Kaymakcalan (1973) provided the most positive demonstration of THC reinforcement in rhesus monkeys where two of six monkeys acquired THC self-administration under an FR1 schedule of reinforcement, but only during withdrawal following the development of physical dependence via 36 days of forced automatic THC

Table 1. Cannabinoid self-administration studies in experimental animals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose and vehicle of drug</th>
<th>Species</th>
<th>Procedure/schedule</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deneau and Kaymakcalan (1971), Harris et al., (1974)</td>
<td>100–400 μg/kg Tween 20/saline or 25–300 μg/kg PVP/saline</td>
<td>Rhesus monkeys</td>
<td>IVSA, FR1, drug naïve</td>
<td>Negative</td>
</tr>
<tr>
<td>Pickens et al. (1973)</td>
<td>0.025-0.1 mg/kg PVP/saline</td>
<td>Rhesus monkeys</td>
<td>IVSA, FR1, substitution for phencyclidine</td>
<td>Negative</td>
</tr>
<tr>
<td>Pickens et al. (1973)</td>
<td>0.025-0.1 mg/kg and 25 mg burned hashish</td>
<td>Rhesus monkeys</td>
<td>Inhalation and food presentation/FR3 and 4 s mouth contact to smoke tube, 24 h per day, 4 days each week</td>
<td>Negative</td>
</tr>
<tr>
<td>Carney et al. (1977), Harris et al. (1974), Kaymakcalan (1973)</td>
<td>3–300 μg/kg EL620; 25–200 μg/kg PVP/saline; 100–400 μg/kg Tween 20/saline</td>
<td></td>
<td>IVSA, substitution for cocaine</td>
<td>Negative</td>
</tr>
<tr>
<td>Kaymakcalan (1973)</td>
<td>100–400 μg/kg Tween 20/saline</td>
<td>Rhesus monkeys</td>
<td>IVSA, monkeys made dependent on THC</td>
<td>33% positive</td>
</tr>
<tr>
<td>Mansbach et al. (1994)</td>
<td>17–100 μg/kg in EL620/EtOH/saline</td>
<td>Rhesus monkeys</td>
<td>IVSA, FI 10 min (2 hr TO), substitution for phencyclidine</td>
<td>Negative</td>
</tr>
<tr>
<td>Li et al. (2012)</td>
<td>3.2–32 μg/kg EL620/EtOH/saline</td>
<td>Rhesus monkeys</td>
<td>IVSA, history of heroin SA, or naïve</td>
<td>Negative</td>
</tr>
<tr>
<td>Corcoran and Amit</td>
<td>0.25 mg</td>
<td>Rats</td>
<td>Forced drinking</td>
<td>Negative</td>
</tr>
<tr>
<td>(1974)</td>
<td>(hashish)/ml</td>
<td>Rats</td>
<td>IVSA, naïve</td>
<td>40 % positive</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>Van Ree et al. (1978)</td>
<td>7.5–300 μg/kg Tween 20/saline</td>
<td>Rats</td>
<td>IVSA, naïve, food deprived, with automatic food pellet delivery</td>
<td>Positive only with deprivation conditions</td>
</tr>
<tr>
<td>Takahashi and Singer (1979, 1980)</td>
<td>6.25–50 μg/kg Tween 80/saline</td>
<td>Rats</td>
<td>IVSA, naïve, food deprived, with automatic food pellet delivery</td>
<td>Positive only with deprivation conditions</td>
</tr>
<tr>
<td>Braida et al. (2004)</td>
<td>0.01–1 μg/inf cerebrospinal fluid, EtOH, cremophor</td>
<td>Rats</td>
<td>ICVSA, trained with water</td>
<td>Positive, max responding at 0.02 μg/inf</td>
</tr>
<tr>
<td>Lefever et al., (2014)</td>
<td>3-10 μg/kg in polysorbate 80/saline</td>
<td>LongEVans Rats</td>
<td>IVSA, FR 3, substitution for WIN 55,212-2</td>
<td>Negative</td>
</tr>
<tr>
<td>Tanda et al. (2000), Justinova et al. (2008), Justinova et al. (2003)</td>
<td>1–16 μg/kg EtOH/Tween 80/saline</td>
<td>Squirrel monkeys</td>
<td>IVSA, naïve, or cocaine trained with saline extinction/FR or second order</td>
<td>Positive, max responding at 4 μg/kg</td>
</tr>
</tbody>
</table>

**2-AG**

<table>
<thead>
<tr>
<th>(2014)</th>
<th>(hashish)/ml</th>
<th>Rats</th>
<th>IVSA, naïve</th>
<th>90 % positive, max responding at 25 μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Luca et al. (2014)</td>
<td>12.5–50 μg/kg Tween 80/saline/eth</td>
<td>Sprague-Dawley rats</td>
<td>IVSA, naïve</td>
<td>90 % positive, max responding at 25 μg/kg</td>
</tr>
<tr>
<td>Justinova et al. (2011)</td>
<td>0.1–100 μg/kg EtOH/Tween 80/saline</td>
<td>Squirrel monkeys</td>
<td>IVSA, nicotine substitution or history IVSA of anandamide, anandamide reuptake inhibitors or nicotine</td>
<td>100 % Positive, max responding at 3 μg/kg</td>
</tr>
</tbody>
</table>

**CP 55,940**

<table>
<thead>
<tr>
<th>(2014)</th>
<th>(hashish)/ml</th>
<th>Rats</th>
<th>IVSA, naïve</th>
<th>90 % positive, max responding at 25 μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mansbach et al. (1994)</td>
<td>0.3–3 μg/kg emulphor/EtOH/saline</td>
<td>Rhesus monkey</td>
<td>IVSA, FI 10 min (2 hr TO), PCP substitution</td>
<td>Negative</td>
</tr>
<tr>
<td>Braida et al. (2001a, b)</td>
<td>0.1–1.6 μg cerebrospinal fluid, EtOH, cremophor</td>
<td>Wistar rats</td>
<td>IVSA</td>
<td>Positive, max responding at 0.4 μg/kg</td>
</tr>
</tbody>
</table>

**WIN 55,212-2**

<table>
<thead>
<tr>
<th>(2007a, b)</th>
<th>(hashish)/ml</th>
<th>Rats</th>
<th>IVSA, naïve</th>
<th>90 % positive, max responding at 25 μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martellotta et al. (1998)</td>
<td>10–500 μg/kg</td>
<td>CD1 mice</td>
<td>IVSA, naïve</td>
<td>Positive, max responding at 100 μg/kg</td>
</tr>
<tr>
<td>Fattore et al. (2001)</td>
<td>6.25–50 μg/kg Tween 80/heparin/saline</td>
<td>Wistar rats</td>
<td>IVSA, naïve</td>
<td>87 % positive, max responding at 12.5 μg/kg</td>
</tr>
<tr>
<td>Solinas et al. (2007a, b)</td>
<td>12.5–25 μg/kg Tween 80/saline</td>
<td>Long-Evans rats</td>
<td>IVSA, naïve</td>
<td>Positive, max responding at 12.5 μg/kg</td>
</tr>
<tr>
<td>Justinova et al. (2013)</td>
<td>12.5 μg/kg Tween 80/heparin/saline</td>
<td>Long-Evans rats/Sprague-Dawley</td>
<td>IVSA, naïve</td>
<td>Positive</td>
</tr>
<tr>
<td>Lecca et al. (2006)</td>
<td>12.5 μg/kg Tween 80/saline</td>
<td>Sprague-Dawley rats</td>
<td>IVSA, naïve</td>
<td>Positive</td>
</tr>
<tr>
<td>Lefever et al., (2014)</td>
<td>5.6-30 μg/kg in polysorbate 80/saline</td>
<td>Rats</td>
<td>IVSA, FR 3, naïve</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Injections (0.4 mg/kg/injection, i.v.). Surprisingly, only two other studies in rhesus monkeys involving cannabinoid self-administration have been published since the 1970s. One of these by Mansbach et al. (1994) used a schedule of reinforcement designed to compensate for the biodispositional factors of THC (i.e. slow-onset of behavioral effects and extended elimination phase) that may limit the expression of reinforcing effects. Here, individual drug injections were spaced by 2-hour intervals and paired with a distinct conditioned stimulus; however, THC still did not maintain rates of responding above vehicle levels although this factor of temporal contiguity was taken into consideration. The other study, Li et al., 2012, substituted THC for heroin SA under an FR 50, 180-s timeout schedule of reinforcement in male rhesus monkeys. Although some evidence indicates a shared mechanism between cannabinoid and opioid receptor compounds, these conditions were also unsuccessful in demonstrating the reinforcing effects of THC.

Studies in rodents have also been largely unsuccessful in demonstrating the reinforcing effects of THC by the self-administration paradigm. One exception was a study by Takahashi and Singer (1979) where 6.25 and 12.5 μg/kg/injection THC maintained responding above vehicle in food-restricted rats at 80% of normal body weight and by using a concurrent fixed-time (FT) 1-min schedule of food-delivery and FR1 schedule THC self-administration. THC self-administration above vehicle levels was not obtained in rats that were only food-restricted (without the FT 1-min schedule of food

<table>
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<tr>
<th>JWH 018</th>
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<tbody>
<tr>
<td>De Luca et al. (2015)</td>
<td>10–20 μg/kg EtOH/Tween 80/saline</td>
<td>Sprague-Dawley rats</td>
<td>IVSA, naïve</td>
<td>90% positive, max responding at 20 μg/kg</td>
</tr>
<tr>
<td>De Luca et al. (2015)</td>
<td>15–30 μg/kg EtOH/Tween 80/saline</td>
<td>C57BL/6 mice</td>
<td>IVSA, naïve</td>
<td>90% positive, at 30 μg/kg</td>
</tr>
</tbody>
</table>
delivery) or in rats with the FT1-min schedule at free-feeding body weight. Therefore, these results indicated a role of adjunctive behavior, that is the environmental contingencies introduced by a combination of the FT 1-min schedule and food deprivation were the critical variables in demonstrating THC reinforcing effects in this study. On the other hand, several studies in rodents have demonstrated reinforcing effects of synthetic CB receptor agonists by intravenous self-administration. The first report by Martellotta et al. (1998) showed reinforcing effects of the synthetic CB1 receptor agonist WIN 55,212-2 in drug-naïve mice. In that study, self-administration was only studied during one experimental session in which WIN 55,212-2 was made available under an FR 1 schedule for one mouse while another served as a yoked control. The ratio between the number of responses by the active-lever mice and yoked controls indicated that WIN 55,212-2 reinforcing effects were obtained in a dose-related manner. Despite the fact that “reinforcing effects” were determined by using a grouped experimental design, these findings were replicated by another laboratory using the same procedure (Ledent et al., 1999).

The early studies of synthetic cannabinoid self-administration in rodents by Martellotta et al. (1998) and Ledent et al. (1999) were important advancements in cannabinoid self-administration methodology; however, these studies also contained several caveats that limited the translational nature of the procedure, such as studying each dose during a single self-administration session and the use of a grouped design to measure reinforcing effects. Since then, other studies have addressed these issues by showing that synthetic cannabinoids can function as reinforcers within-subject and over consecutive experimental sessions. For example, Fattore et al. (2001) showed that stable and robust rates of i.v. WIN 55,212-2 self-administration was maintained in rats responding under an FR 1 schedule (10-s timeout) in 3-h sessions. Stable responding was acquired in approximately 16 sessions at a dose of 12.5 μg/kg/injection where
maximal rates of responding occurred at approximately 25 injections per session. It was also shown that WIN 55,212-2 self-administration was subject to saline extinction and antagonism by the CB₁ antagonist/inverse agonist SR 141716A. It is also important to note that rats were maintained at 80% of free-feeding weights in these studies. Furthermore, reinforcing effects of the bicyclic cannabinoid analog, CP 55,940 have been demonstrated in rodents via intracerebroventricular (ICV) self-administration (Braida et al., 2001). This study also used a form of diet restriction as an antecedent variable in which the animals only had access to water during the experimental session. During each session, two levers were made available and water delivery was contingent on responding on either lever. In addition to response contingent water delivery, responding on one lever also delivered a paired ICV injection of CP 55,940 while responding on the other delivered an ICV injection of vehicle paired with water delivery. The lever producing water delivery paired with ICV CP 55,940 dose-dependently maintained greater rates of responding than the lever paired with vehicle. One limitation to these studies, however, is that diet restriction was required for the acquisition and maintenance of self-administration. Food restriction is a well-established procedural factor in drug self-administration studies that has been shown to enhance both the acquisition (Carroll et al., 1979; de la Garza and Johanson, 1987; Cabeza de Vaca and Carr, 1998) and maintenance (Carroll and Meisch, 1984) of drug-maintained behavior. More recently, Lefever et al. (2014) also demonstrated the reinforcing effects of WIN 55,212-2 in rodents responding under an FR 3 schedule of reinforcement and maintained at 90% free-feeding weight, as opposed to 80% in the initial successful demonstration of WIN 55,212-2 self-administration by Fattore et al. (2001). However, self-administration was not consistent among all subjects in this study with considerable within-group variability in the number of infusions earned per session.
The initial demonstration of robust THC-maintained responding in experimental animals by Tanda et al. (2000) was achieved by using a set of experimental parameters not previously employed. First, squirrel monkeys (Saimiri sciureus), a species of New-World primates, served as subjects. In contrast, previous unsuccessful attempts of THC self-administration in nonhuman primates exclusively used Old-World primates (i.e. rhesus monkeys). Furthermore, the squirrel monkeys used were not food restricted in contrast to studies in rodents that demonstrated reinforcing effects of synthetic cannabinoids. The initial demonstration of THC self-administration in squirrel monkeys was performed in monkeys that had a prior history of cocaine self-administration and at the time, was considered by others to be an antecedent variable that contributed to its effectiveness as a reinforcer in these studies (Maldonado, 2002). However, subsequent work showed that drug-naïve squirrel monkeys readily acquired and maintained robust rates of THC self-administration (Justinova et al., 2003) thereby demonstrating that a prior cocaine self-administration history was not a necessary condition among the other procedural variations.

The successful demonstrations of THC reinforcement in squirrel monkeys employed an FR 10 schedule of drug injection with a 60-s timeout after each injection. In the initial study, saline was substituted for cocaine until behavior was reliably extinguished. Subsequently, THC was made available for self-administration by substituting the drug for saline with periods of vehicle substitution between different doses of THC. This substitution procedure is in contrast to earlier studies in rhesus monkeys where THC was substituted directly for cocaine or other drugs like PCP or heroin (Mansbach et al., 1994, Pickens et al., 1973; Carney et al., 1977; Harris et al., 1974; Kaymakcalan, 1973; Li et al., 2012). In fact, this substitution procedure for studying self-administration is in contrast to what has been used historically to assess the reinforcing effects of a stimulus where a test drug is substituted for a stimulus (e.g.
food pellets, another drug) that previously maintained responding. The significance of substituting THC for saline vs. a stimulus with reinforcing effects in order facilitate the acquisition of THC-maintained behavior remains to be determined. This procedural variation could suggest that THC is more likely to maintain high levels of behavior only when it is substituted for a stimulus that is either not reinforcing or is of lower reinforcing efficacy than THC.

Perhaps the most critical procedural factor other than species differences for initially demonstrating the reinforcing effects of THC in squirrel monkeys was the dose range used. Specifically, the dose range of THC (2.0 – 8.0 μg/kg/injection) that maintained behavior above vehicle levels in the squirrel monkeys used by Tanda et al. (2000) was relatively lower than what was used in previously unsuccessful attempts in rhesus monkeys (Fig. 1).

![Fig. 1. Dose comparisons for preclinical monkey THC self-administration studies. Note: there is no ordinate.](image)
Peak rates of responding and maximal numbers of injections were maintained by 2.0 – 4.0 μg/kg/injection doses of THC in squirrel monkeys whereas many of the early unsuccessful studies with rhesus monkeys did not test doses lower than 100 μg/kg/injection. It is interesting to note that squirrel monkeys have been reported to show no visible signs of intoxication after reinforcing doses of THC are made available for self-administration. In contrast, studies in rhesus monkeys have reported that monkeys did appear intoxicated after the session when relatively higher doses of THC were used, although few injections were received. These observations suggest that the dose range necessary for engendering THC-maintained behavior might be significantly lower what appears to be behaviorally active by visual inspection. Furthermore, the dose range of THC that maintains peak levels of behavior in squirrel monkeys has been reported to be analogous to the dose range self-administered by humans smoking a single marijuana cigarette. For instance, the average marijuana cigarette contains approximately 15 mg of THC but the actual THC intake is only 3 mg considering the bioavailability of THC from smoking is about 20% (Agurell et al., 1986). Distributed over 10 to 15 puffs per cigarette, this equates to approximately 2.9 – 4.3 μg/kg of THC delivered per puff, which corresponds to the unit doses used to maintain responding in squirrel monkeys. The total intake of THC obtained in squirrel monkeys when 2 - 4 μg/kg/injection of THC was available for self-administration is 50 and 120 μg/kg, respectively, over a 1-hour session. This is also analogous to a report in humans that showed an i.v. injection of 5 mg of THC (approximately 70 μg/kg) produced the same degree of euphoria compared to smoking a marijuana cigarette (Ohlsson et al., 1980).

Another methodological difference between successful THC self-administration studies in squirrel monkeys compared to others involves the vehicle used to dissolve THC in solution for intravenous injection. Many early studies that assessed i.v. THC self-administration were conducted with solutions of THC in suspension due to its lipophilic
nature, low solubility in water, and high doses used. In contrast, THC was dissolved in a Tween 80/ethanol/saline vehicle to produce a clear solution for the studies in squirrel monkeys. This vehicle has been shown to provide rapid distribution of THC to the brain compared to when the drug is in suspension (Mantilla-Plata and Harbison, 1975) and significantly elevates dopamine levels in the nucleus accumbens shell of rodents (Tanda et al., 1997). The bioavailability of a drug is certainly a factor that affects its behavioral effects. Therefore, vehicle preparation as a means for increasing the bioavailability of THC likely represents an important methodological factor that should be taken into consideration for enhancing the likelihood of THC to function as a reinforcer in experimental animals.

**Potential factors limiting the reinforcing effects of cannabinoids in experimental animals**

It is currently unknown whether previous unsuccessful attempts at establishing the reinforcing effects of cannabinoids in rhesus monkeys were due to methodology or a direct species difference between squirrel (New World) and rhesus (Old World) monkeys. To address this, in Chapter II, THC was made available to rhesus monkeys under similar SA methodological conditions as used in the successful demonstrations in squirrel monkeys, including schedule of reinforcement, dose range, and vehicle preparation. *It is important that the THC SA procedure be extended to include rhesus monkeys because this species is phylogenetically closer to humans than any other currently available preclinical laboratory species including squirrel monkeys, which would enhance the ability to accurately generalize results to human drug abuse.* The reinforcing effects of the synthetic bicyclic cannabinoid analog, CP 55,940, a potent CB1/CB2 agonist, were also evaluated in Chapter II using the same monkeys, which allowed for a better understanding of the role of pharmacological efficacy at the CB
receptor in SA, such as differences in potency and magnitude of response. It was hypothesized that by using conditions that promoted THC self-administration in squirrel monkeys, successful self-administration in rhesus monkeys would be obtained.

Besides methodological factors, one hypothesis for the failure of THC to function as a reinforcer in most studies using experimental animals is that the initial aversive (e.g., rate-decreasing) effects of THC counteract the reinforcing effects. Multiple stimulus effects of drugs of abuse have been increasingly recognized, not all of which are rewarding. Thus, drug-taking behavior has been conceptualized as a balance between a drug’s reinforcing effects and the rate-suppressing effects (e.g., aversion) that limit responding leading to a drug’s presentation (Verendeev and Riley, 2013). The impact of these two opposing systems is demonstrated through the inverted U-shaped nature of a drug self-administration dose-response curve. For example, on the ascending limb, the reinforcing effect of a drug increases in proportion to the size of the drug’s unit dose and thus leads to an increase in rate of responding. However, on the descending limb, response rate and reinforcer magnitude are inversely proportional due to the direct rate-suppressing effects of high drug doses (e.g., sedation, induction of stereotypies, aversion, etc.) that serve to counteract the drug’s reinforcing (i.e., rate-increasing effects). This concept has been experimentally validated by cocaine self-administration studies showing that the development of tolerance to the rate-decreasing effects leads to a vertical shift of the dose-response curve, which primarily occurs on the descending limb (Ahmed and Koob, 1998; Deroch et al., 1999; for review see Zernig et al., 2004).

The notion of THC-maintained behavior being limited by the drug’s initial rate-decreasing effects is an empirical question and one not previously tested in animal models of self-administration. Supporting evidence for this hypothesis, however, has been demonstrated in parallel studies involving conditioned place preference in rodents as well as epidemiological evidence in humans. For instance, as described previously,
cannabinoids have been shown to generally induce conditioned place aversion rather than place preference in rodents (e.g. Lepore et al., 1995; McGregor et al., 1996; Sanudo-Pena et al., 1997; Mallet and Beninger, 1998; Chaperon et al., 1998; Cheer et al., 2000; Zimmer et al., 2001) suggesting that there may be an initial dysphoric/anxiogenic effect of THC upon first exposure which may correspond to negative results in self-administration studies. Moreover, retrospective studies in humans show that individuals who are more sensitive to the aversive effects of cannabis were less likely to be current users (Haertzen et al., 1983; Thomas, 1996).

In order to test the hypothesis that sensitivity to the rate-decreasing effects would influence measures of cannabinoid reinforcement, the potency of both THC and CP 55,940 to decrease food-maintained responding was correlated with rates of SA in each subject. It was hypothesized that individual subjects less sensitive to the rate-decreasing effects of CP 55,940 and THC will have greater rates of SA of both drugs. Furthermore, the rate-decreasing effects of cannabinoids that may be counteracting initial reinforcing effects may also represent a variable that could be modified to facilitate acquisition of SA. Preclinical data show that tolerance to the effects of repeated THC administration can differentially develop depending on the behaviors studied (Tanda and Goldberg, 2003). For instance, tolerance to repeated THC administration has been demonstrated for discriminative stimulus effects, decreases in operant responding, hypothermia, locomotor activity, nociception, and ingestion (McMillan et al., 1970; Miczek, 1979; Wiley et al., 1993a; Fan et al., 1994; McKinney et al., 2008; McMahon, 2011). However, it has been reported that tolerance does not develop to the subjective “high” of marijuana after chronic consumption in humans (Lindgren et al., 1981; Perez-Reyes et al., 1991; Kirk and de Wit, 1999). These effects are consistent with the lack of tolerance development to THC-induced increases in ventral tegmental area dopamine neuron firing rates both in vivo (Wu and French, 2000) and in vitro (Cheer et al., 2000). Overall, these data suggest
that tolerance may preferentially develop to the rate-decreasing effects and not the reinforcing effects of THC. Experiments within Chapter II extended the assessment of tolerance to the behavioral effects of CB agonists to include THC-induced decreases in operant responding that may impact measures of THC reinforcement using a drug SA paradigm. We hypothesized that tolerance would develop to the rate-decreasing effects of THC and this would result in increases in rates of THC SA compared to the initial assessment.

The pharmacokinetic profiles of drugs are crucial for their reinforcing effects. Thus, another reason for the relativity ineffectiveness of cannabinoids to function as reinforcers in preclinical animal models is because the appropriate conditions have not been implemented to control for the pharmacokinetic and metabolic factors that may limit drug maintained responding. Cannabinoids have a relatively delayed onset of their behavioral effects and an extended elimination phase (Ohlson et al., 1980; Hollister et al., 1981). For instance, experiments in humans have shown that maximal intoxication produced by THC can occur 30 min or more after intravenous injection. In drug discrimination studies in rodents, maximal THC-appropriate responding was not observed until 60 min after i.m. injection and substantial THC-appropriate responding was observed for as long as 6 h after the initial administration (Prescott et al., 1992). In rhesus monkeys, it has been reported that the discriminative stimulus effects of THC can be detected within 20 min but remain present for up to 4 h after intravenous administration (McMahon, 2006). Therefore, under schedules of self-administration where response rates and reinforcement is directly related (i.e., fixed ratio schedules), these pharmacokinetic variables likely limit subsequent self-administration either by reducing the temporal contiguity between the drug effect and the response that produces it or through rate-decreasing effects (e.g., satiation, decreased discriminability of additional drug injections) resulting from slow offset of drug effects. In Chapter II, these
possibilities were examined by studying THC self-administration under a second-order schedule where behavior was maintained across long intervals between drug injections by conditioned stimuli. Mansbach et al. (1994) attempted to also take these factors into consideration in order to demonstrate the reinforcing effects of THC in rhesus monkeys. That study used a procedure where individual THC injections were spaced by 2 h intervals and paired with a distinct visual stimulus; however, THC-maintained responding above vehicle levels was not obtained. Notably, the dose range tested in this study was 0.017 – 0.1 mg/kg/injection, which was considerably higher than the dose range that was eventually found to maintain the highest amount of behavior leading to THC injections in squirrel monkeys (0.001 – 0.004 mg/kg/injection). Therefore, we hypothesized that by using a lower range of doses coupled with second-order contingencies that would help to mitigate the biodispositional factors of THC that may inhibit the expression of its reinforcing effects, THC-maintained behavior would be obtained.

The role of cognitive function in substance use disorders

Despite advances in pharmacotherapies over the last few decades, behavioral treatments are often the most efficacious option for the treatment of various substance use disorders. Among those with the strongest level of empirical support from randomized clinical trials are 1) contingency management, where abstinence or other targeted outcomes are reinforced with incentives (Higgins et al., 1991; Petry, 2006), 2) cognitive behavioral therapy, which teaches specific strategies and skills to reduce substance use (Carroll et al., 1994), and 3) motivational interviewing, where a specific, nonjudgmental interviewing style is used to enhance motivation and harness the patient’s capacity for change (Hettema et al., 2005; Miller, 1985). However, despite empirical support, the established efficacy of many of these approaches remain at low levels. For instance, a meta-analysis of randomized controlled trials for cognitive-
behavioral therapy approaches to treat adult alcohol and illicit drug users reported only a small, but statistically significant treatment effect (g = 0.154), with effect sizes depending on the substance of abuse (Magill and Ray, 2009). Moreover, the successful implementation and broad dissemination of behavioral treatments are hindered by considerable costs and resources needed. Thus, many areas for improvement are present and more efficient ways to implement behavioral treatments are needed. In an effort to do so, it is important to better understand how and when the treatments work. This information can then be used to maximize the effects and develop a more targeted approach.

One strategy is through understanding the cognitive mechanisms underlying the effectiveness of behavioral treatments, which can ultimately be used to develop cognitive enhancement-based interventions to supplement standard behavioral therapies. Indeed, the idea of cognitive control is central to many contemporary models of addiction (Jentch and Pennington, 2014) and is a factor that can affect treatment outcome (Sofuoglu et al., 2010). For example, cognitive impairments to executive control in drug-dependent populations have been proposed as one mechanism to explain the maintenance of drug use and the difficulty many individuals have in resisting habitual drug use once established (Everitt et al., 2007; Goldstein and Volkow, 2011; Li and Sinha, 2008; Porrino et al., 2007). In particular, aspects of executive function such as decision-making, response inhibition (failure to abstain from substance use), error processing (failure to adapt/learn with respect to prior harmful behavior), planning, working memory, set-shifting, and attention appear to be associated with substance use and substance use disorders when studied in adults (Sofuoglu et al., 2013). Further, cognitive impairments in substance abusers at treatment initiation are generally associated with poorer treatment retention and treatment outcomes. For instance, research with polydrug users in a residential treatment program found that participants
who scored low on a neuropsychological test battery had had shorter lengths of stay in
treatment and were viewed less favorably by the treatment staff (Fals-Stewart, 1993).
Therefore, evidence indicates that attenuating cognitive impairments may serve as
potential treatment targets for substance use disorders.

The effects of THC and marijuana use on cognitive function have been studied extensively in humans and animal models given the increasing prevalence of cannabis use and the number of treatment-seeking patients with cannabis use disorder. Although much progress has been made in understanding the acute (i.e., in the presence of intoxication) cognitive consequences of cannabis use (Crean et al., 2011; Crane et al., 2013), the residual effects of cannabis on cognitive function (i.e., after the intoxicating effects have subsided) and cannabis-induced impairments to cognitive function during abstinence are much more equivocal. It is particularly important to better characterize these cognitive effects of THC because growing evidence suggests that cannabis-induced cognitive deficits are not only apparent after smoking, but persist for a period of time after the drug has been used (Bolla et al., 2005), which may hinder an individual's ability to make the best use of behavioral therapies. This issue, however, lies in better characterizing the time course of effects and determining which specific cognitive domains affected are most strongly related to treatment outcome. The remainder of this chapter will present an overview of the effects of cannabis on neurocognitive function and how these studies will inform the research within this dissertation focused on elucidating the exact nature, and persistence of THC-induced cognitive impairment that can ultimately be used to develop cognitive enhancement approaches to treat cannabis use disorder.

Residual effects of cannabis on cognition in humans

For the following studies reviewed in this section, the residual effects of cannabis
on cognition refer to a period of time after which the direct intoxicating effects of cannabis have subsided. Pharmacokinetic studies in humans showed that the peak level of subjective “high” of smoked cannabis was observed after 20-30 minutes and decreased to baseline levels by 4 hours after smoking. Thus, the current operational definition of residual effects only includes assessments occurring after this time frame but within 24 hours of last use. Furthermore, the cognitive domains discussed only pertain to the domains studied in the present dissertation. These cognitive domains have been selected from a therapeutic standpoint because they have been shown to have prognostic significance or relevance in mediating the skills necessary for successful behavioral modification for cannabis or other substance use disorders.

One of the domains of cognitive function most extensively studied as it relates to the residual effects of cannabis in humans is inhibition. Inhibition is a significant feature of many drug dependent individuals, which is defined by an inability to override a prepotent response (i.e. drug taking) in response to drug cues. As a result, improving behavioral inhibition by shifting focus away from drug use and toward non-drug reinforcement (e.g., improved health, stronger interpersonal relationships, stable employment, etc.) represents a viable cognitive domain to be targeted by cognitive rehabilitation strategies. One of the first groups (Pope et al., 1996) to study inhibition in cannabis users examined heavy and light users after a minimum of 19 hours of abstinence. Heavy cannabis users demonstrated significantly more errors of inhibition and perseveration compared with light users. Solowij et al. (2002) replicated these findings in cannabis users after at least 12 hours of abstinence. The severity of these deficits was correlated with years of use. Several other researchers have found a similar pattern of impairment within inhibitory functioning (Aharonovich et al., 2008; Cunha et al., 2010). In contrast, a number of researchers found no residual effects of cannabis use on inhibition (Whitlow et al., 2004; Gruber et al., 2005; Hermann et al., 2007); however,
these studies had small sample sizes (e.g. N=10) and the length of abstinence was unspecified or was highly variable, ranging from 12 hours to 18 years. Thus, the residual effects of cannabis use within this cognitive domain are equivocal, although clear indication exists of impairment after acute cannabis intoxication (McDonald et al., 2003; Ramaekers et al., 2006). As a result, studies are needed that more strictly control for amount of time of THC intake.

Another cognitive domain with strong implications in substance use disorders and treatment response is attentional control. Measures of attention have been closely linked with inhibitory control and are also largely mediated by the prefrontal cortex. Several studies have examined the residual effects chronic cannabis use on measures of attention and as with inhibition, the findings have been inconsistent. In one study for example, Pope et al. (2002) tested current, heavy cannabis users (i.e., users that had smoked cannabis at least 5000 times and were smoking daily at the time of study entry), former heavy cannabis users (i.e., users that had also smoked at least 5000 times but had smoked fewer than 12 times in the past 3 months), and control subjects (who had only tried cannabis < 50 times during their lifetime) on various measures of selective and divided attention during experimentally controlled days of abstinence (i.e. 0, 1, 7, and 28 days since last drug use). At all four time points, no significant differences were found on attentional abilities. Similarly, Jager et al., (2006) also found no differences in attentional abilities as a result of the residual effects of long-term cannabis use. However, in contrast, Solowij et al. (1995, 2002) found significantly impaired attentional abilities in short- and long-term cannabis users compared to control subjects when assessed 24 hours since last use. It was also found that cannabis users demonstrated longer reaction times to complete the tasks and impairments involved in information processing abilities associated with the task. Furthermore, Wadsworth et al. (2006) examined attentional capacities under a choice reaction time task within the context of a “real world” setting,
such that performance was assessed before and immediately after work, at both the beginning and end of the work week. They found that, cannabis use was associated with significantly impaired performance on the attention task both at the beginning of the work week and at the end, which was significantly correlated with duration of cannabis use. However, cannabis use was not found to be associated with an increase in workplace errors.

Working memory, the ability to hold and manipulate information and remember it after a short delay, is a major component of executive function and is a cognitive domain associated with well documents in chronic drug abusers. Working memory has consistently been shown to be impaired by cannabis shortly after use (e.g. Hart et al., 2001, 2010); however, the residual effects of cannabis use on measures of working are a lot more inconsistent. For example, some studies show residual impairments in heavy cannabis using young adults on immediate recall (Fried et al., 2005), verbal reasoning (Wadsworth et al., 2006), and verbal n-back tasks (Herzig et al., 2014). In contrast, several studies have found no residual impairments in working memory abilities between heavy and light cannabis users compared with control subjects (Pope et al., 1996; Kanayama et al., 2004; Jager et al., 2006; Solowij et al., 2002; Whitlow et al., 2004; Fisk and Montgomery, 2008); however, it is unclear whether these results are due to the duration and quantity of cannabis use or a lack of temporal specificity to detect deficits or small sample size.

Another cognitive domain associated with substance abuse and treatment outcome is associative learning. For instance, impairments in associative learning have been hypothesized to play a role in drug craving and relapse through a process of “overlearning” drug-stimulus reinforcement-outcome pairings at the expense of nondrug reinforcers (Gould, 2010). However, intact associative learning systems are crucial for acquiring new skills and coping strategies during behavioral therapy sessions and
applying those skills outside of the treatment setting to facilitate abstinence (Rezapour et al., 2016). In humans, associative learning is typically measured by word-list learning tasks and much like the previously discussed cognitive domains, findings for the residual effects of chronic cannabis use are equivocal. For instance, residual impairments (i.e. > 12 hours since last use) have been reported in adolescents (Harvey et al., 2007; Solowij et al., 2011; Dougherty et al., 2013) and young adults (Cuttler et al., 2012; Gonzalez et al., 2012; Becker et al., 2014) as well as in occasional users (Hanson et al., 2010).

Several of these studies report significant associations between impairment and frequency, quantity, and duration of use and age of onset (e.g., Solowij et al., 2011; Becker et al., 2014). On the other hand, several studies report no difference in associative learning performance in recently abstinent cannabis users compared to nonusers (Fisk and Montgomery, 2008; Gruber et al., 2012).

**Long-term effects of cannabis on cognition in humans**

While the residual effects of cannabis use on cognition have been operationalized to refer to the period after the intoxicating effects have subsided but within 24 hours of last use, evidence indicates that certain cognitive impairments may persist for several weeks into abstinence. As a result, the human literature has designated the term “long-term effects” to indicate a period of 3 weeks or longer since last use (Crean et al., 2011). This cutoff ensures that the acute and residual effects of cannabis in the brain were eliminated by the time of assessment and thus provides a reflection of any long-lasting impairments in basal functioning. The long-term effects of cannabis use on cognition have received considerable research attention; however, much like the literature on the residual effects, the findings for most cognitive domains are equivocal. The one exception is episodic memory, which has been shown by a number of investigators to be impaired for up to 28 days following abstinence (Gonzalez
2007; Grant et al. 2003; Solowij and Battisti, 2008).

For the long-term effects of cannabis on behavioral inhibition, studies using the Stroop Test have consistently found no significant differences between chronic cannabis users and control groups at 28 days of abstinence (Pope et al., 2001; 2002; 2003) or up to a year of abstinence (Lyons et al., 2004; Verdejo-Garcia et al., 2005). In contrast, studies using the Wisconsin Card Sort Test have all found significant differences in performance up to 28 days of abstinence (Bolla et al., 2002, Pope et al., 2001; 2002; 2003), with the exception of Lyons et al. (2004), who compared cannabis use in heavy users (>1 time per week for a minimum of 1 year) vs. non using (< 5 times in their life time) male monozygotic twins. These findings may indicate that certain aspects of inhibition as assessed by different tasks are more vulnerable to persistent impairment during abstinence. Other measures of inhibition in abstinent cannabis users derived from tasks such as the go/no-go or stop-signal reaction time tasks have not been assessed.

Much like the residual effects, the long-term effects of cannabis on attention are also mixed. In adolescent cannabis users, measures of attentional abilities have been found to be impaired after at least 21 days of abstinence (Hanson et al. 2010; Jacobsen et al. 2004; Medina et al. 2007; Tapert et al. 2007), with evidence of a dose-dependent relationship with amount of lifetime use (Medina et al. 2007). Conversely, others studies in adolescent cannabis users show that performance on measures of selective and divided attention were not different compared to controls after 45 days of abstinence (Jacobsen et al. 2004). In samples of heavy, chronic adult cannabis users, attentional deficits have been reported after 28 days of abstinence (Bolla et al., 2002) as well as after more variable periods of abstinence (i.e., 6 weeks to 2 years) (Solowij, 1995). Moreover, several studies have shown no attentional deficits in heavy cannabis users after periods of abstinence ranging from 28 days to one year (Lyons et al., 2004; Pope et al., 2001; 2002; 2003; Verdejo-Garcia et al., 2005), with some indication that disparate
findings are the result of task complexity.

In studies examining working performance during long-term abstinence from chronic cannabis use, there appears to be a relationship between the age of the user and the cognitive load. For instance, adolescent regular cannabis users that met DSM criteria for cannabis abuse or dependence demonstrated poorer working memory compared to controls after 3 and 13 days of abstinence during the Letter-Number Sequencing task, which required the active manipulation of multidimensional items (Hanson et al. 2010). However, working memory performance in adolescent users with similar use characteristics was not different from controls after 8 days of abstinence during the simpler Sternberg's item recognition matching task (Jager et al. 2010). After longer periods of abstinence, the evidence is mixed regarding the persistence of working memory deficits among adolescents. For instance, some studies reported deficits after 28 days of monitored abstinence when performance was assessed with the n-back task (Jacobsen et al. 2004; Jacobsen et al. 2007), whereas others reported no deficits compared to control subjects after similar lengths of abstinence and on similar tasks (Hanson et al. 2010; Padula et al. 2007; Schweinsburg et al. 2010). In contrast to studies with adolescent samples, most studies using adult subjects have reported no differences in working memory performance compared to controls after long-term abstinence (Becker et al. 2010; Chang et al. 2006; Fisk and Montgomery 2008; J. Grant et al. 2011; Jager et al. 2006) indicating a potential interaction between cannabis use and neurodevelopmental processes that mediate working memory. On the other hand, working memory deficits may also be related to amount of cannabis use, as Harvey and colleagues (2007) found that the amount of cannabis used was one of the strongest predictors of poorer spatial working memory performance in regular adolescent cannabis compared to occasional users.

The long-term effects of chronic cannabis use on associative learning in humans
have also been mixed with some studies showing improvement during prolonged periods of abstinence while others do not. For instance, in one study where cognitive performance was studied during closely monitored abstinence periods, chronic marijuana users showed impaired performance on a verbal list learning task at 3 days since last use compared to non-using controls, although performance improved to control levels by two weeks of abstinence (Hanson et al., 2010). In a study with lower temporal resolution, it was also found that performance on a verbal list learning task in former heavy cannabis users (> 12 months since last use) had improved to similar performance levels of non-users (Tait et al., 2011). In contrast, Medina et al. (2007) found impaired associative learning performance in adolescent marijuana users compared to controls after > 23 days of monitored abstinence suggesting persistent impairment in some populations of users.

Overall, the non-acute studies of cannabis on cognition in humans seem to raise more questions than answers with regard to the specific cognitive domain that is affected. In particular, some of the inconsistencies among these studies arise from the heterogeneity of the participants. For instance, factors such as length of abstinence, age at which cannabis users discontinue their use, amount and duration of cannabis use, concurrent use of other substances, and whether prospective study designs were used all complicate the interpretation of many of these studies. In addition, another factor that seems to be overlooked when considering the effects of cannabis on cognition is the cognitive load for each task. For example, it is unknown for many studies whether a lack of effect is merely due to the task not containing a high enough complexity in order to detect more subtle brain abnormalities among cannabis users. Moreover, studies in humans cannot distinguish whether marijuana abusers exhibit innate cognitive differences prior to initiating drug taking. Therefore, it is difficult to clearly establish a causal role of long-term THC exposure in producing cognitive deficits.
Cognitive effects of THC in preclinical animal models

The utilization of preclinical animal models to study the long-term effects of THC on cognition is crucial because many of the variables that contribute to the equivocal findings in studies with human subjects can be controlled for in a systematic manner. The acute effects of THC and synthetic cannabinoids on learning and memory processes have been extensively studied in rodents and nonhuman primates and have been shown to produce impairments in a variety of tasks including the eight-arm radial maze (Han and Robinson, 2001; Mallet and Beninger, 1998; Winsauer et al. 1999), two-component instrumental discrimination task (Han & Robinson, 2001; Mallet and Beninger, 1998; Nava et al., 2000; Winsauer et al., 1999); conditional discriminations (Han & Robinson, 2001; Mallet & Beninger, 1998; Nava et al., 2000; Winsauer et al., 1999; Zimmerberg et al., 1971), recognition memory tasks (Aigner, 1988; Schulze et al., 1989; Kangas et al., 2016), spatial delayed response tasks (Rupniak et al., 1991; Taffe, 2012), and time estimation (Peiper, 1976; Schulze et al., 1988, 1989).

In addition to examining the acute effects of THC on cognition, it is critical to employ longitudinal studies in preclinical animal models in order to disentangle the temporal ordering of cannabis-related cognitive deficits involving the exact nature, persistence, and reversibility of these deficits. The use of nonhuman primates is especially well-suited for these types of studies due to their long lifespan, capability to learn complex cognitive tasks, and similarity to humans in regards to neuroanatomy and neurochemistry (Berger et al., 1991; Joel and Weiner, 2000). However, to date, only one preclinical study in nonhuman primates has examined the residual effects of chronic THC exposure (Verrico et al., 2014). In that study, adolescent rhesus monkeys were treated with THC (120 or 240 µg/kg, i.v.), 5 days per week for approximately 6 months during which working memory performance was assessed 23 hours after drug
administration throughout the study. It was found that chronic THC exposure impaired spatial-, but not object-working memory; tolerance or sensitization did not develop to these effects.

Studies in Chapter 3 expanded on these findings by examining both the residual effects of THC administration (i.e., 22 hours after administration) and the effects of several weeks of abstinence (i.e., 4-6 weeks since discontinuation of chronic THC administration) on four cognitive domains that assessed 1) associative learning through simple and multidimensional discrimination learning, 2) attention and working memory through delayed-match to sample performance, 3) inhibition though reversal learning requiring the ability to inhibit a previously established response based on an acquired stimulus-reinforcement association, and 4) behavioral flexibility through set-shifting performance requiring the formation of an attentional set based on relevant stimuli for reinforcement and the disregard to irrelevant stimuli, all by using a within-subjects design in monkeys. The development of tolerance to the acute effects of THC during chronic exposure was also examined for each of these cognitive domains. These particular domains of cognition were assessed because of their relevance in a cognitive rehabilitation context, in that they underlie the skills necessary to assimilate and integrate new information into a plan for behavior change that can lead to sobriety and the ability to maintain focus on long-term goals (Rezapour et al., 2016). Moreover, as described earlier, the residual effects of THC within these domains are equivocal in studies with human subjects, most likely due to the numerous variables that are difficult to control for in human research including differences in time since last use, amount and duration of cannabis use, previous drug histories, social variables, and concurrent use of other substances. In addition, studies in humans cannot distinguish whether marijuana abusers exhibit innate cognitive differences prior to initiating drug taking so it is difficult to clearly establish a causal role of long-term THC exposure in producing cognitive
deficits. By controlling for these factors in a systematic manner, the results of the studies within this dissertation can be used to improve the efficacy of behavioral treatments for cannabis use disorder by identifying the specific domains of cognitive functioning and a temporal window that can be targeted for successful treatment outcome.
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Chapter II

BEHAVIORAL DETERMINANTS OF CANNABINOID SELF-ADMINISTRATION IN OLD-WORLD MONKEYS

William S. John¹, Thomas J. Martin², and Michael A. Nader¹

¹Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC
²Department of Anesthesiology, Wake Forest School of Medicine, Winston-Salem, NC

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Abstract

Reinforcing effects of Δ⁹-tetrahydrocannabinol (THC), the primary active ingredient in marijuana, as assessed with self-administration (SA), has only been established in New World primates (squirrel monkeys). The objective of this study was to investigate some experimental factors that may enhance intravenous SA of THC and the cannabinoid receptor (CBR) agonist CP55,940 in Old World monkeys (rhesus and cynomolgus), a species more closely related to humans. In one experiment, male rhesus monkeys (N=9) were trained to respond under a fixed-ratio 10 schedule of food presentation. The effects of CP55,940 and THC on food-maintained responding and body temperature were determined in these subjects prior to giving them access to SA each drug. Both drugs dose-dependently decreased food-maintained responding. CP55,940 (0.001-3.0 μg/kg) functioned as a reinforcer in three monkeys, while THC (0.01-10 μg/kg) did not have reinforcing effects in any subject. CP55,940 was least potent to decrease food-maintained responding in the monkeys in whom CP55,940 functioned as a reinforcer. Next, monkeys were administered THC daily until tolerance developed to rate-decreasing effects. When THC SA was reexamined, it functioned as a reinforcer in three monkeys. In a group of cocaine-experienced male cynomolgus monkeys (N=4), THC SA was examined under a second-order schedule of reinforcement in which reinforcement frequency is relatively independent of response rates; THC functioned as reinforcer in two monkeys. These data suggest that sensitivity to rate-decreasing effects may not be a critical determinant for the lack of CBR agonist SA by Old World monkeys. Understanding individual differences in vulnerability to THC SA may lead to novel treatment strategies for marijuana abuse.

Key Words: THC; CP55,940; Marijuana abuse; Self-administration; Rhesus monkey; cynomolgus monkey
INTRODUCTION

Marijuana (Cannabis sativa) is the most commonly abused illicit drug in the United States, with an estimated 4.2 million people that meet DSM criteria for dependence (SAMHSA, 2013). Moreover, marijuana use has been steadily increasing among 12th graders and the current watershed in cannabis legalization suggests that rates of use will increase further (Johnston et al, 2014; Cerdá et al, 2012). As a result, there is a need for more preclinical research to better understand the pharmacological, environmental, and biological determinants of cannabis use in order to develop more effective treatment strategies for cannabis use disorder. A major obstacle preventing such progress has been the difficulty in demonstrating the reinforcing effects of Δ9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana, using self-administration (SA) procedures in animals. Successful efforts have been limited to one laboratory using squirrel monkeys (Tanda et al, 2000; Justinova et al, 2003). Every other THC SA attempt using other nonhuman primate (NHP) species (i.e. rhesus monkeys) or rodents has been unsuccessful (Kaymakcalan 1972, 1973; Pickens et al, 1973; Harris et al, 1974; Leite and Carlini 1974; Carney et al, 1977; Van Ree et al, 1978; Mansbach et al, 1994; Li et al, 2012; Lefever et al, 2014).

It is currently unknown whether previous unsuccessful attempts in rhesus monkeys were due to methodology or a direct species difference between squirrel (New World) and rhesus (Old World) monkeys. Thus, one goal of the present study was to make THC available to rhesus monkeys under similar SA conditions as used in squirrel monkeys, schedule of reinforcement and vehicle preparation. With THC being a low efficacy, partial agonist at the CB1/CB2 receptors, the present study also evaluated the reinforcing effects of the synthetic bicyclic cannabinoid receptor (CBR) analog, CP55,940, a high efficacy, full CB1/CB2 agonist, in the same monkeys to allow for a better understanding of the role of pharmacological efficacy at the CBR in SA.
Furthermore, the characterization of individual qualitative and quantitative differences in CBR agonist SA was examined. One hypothesis to explain the failure of THC to function as a reinforcer among experimental animals involves the initial rate-decreasing effects of THC, which may mask the reinforcing effects, such that subjects less sensitive to the rate-decreasing effects of CP55,940 and THC would have greater rates of SA of both drugs. To test this hypothesis, the potency of both drugs to decrease food-maintained responding was correlated with rates of SA.

The rate-decreasing effects of cannabinoids represent a variable that could be modified to facilitate acquisition of SA. For instance, data suggest that through repeated drug exposure, tolerance may develop to the aversive/rate-decreasing effects but not the reinforcing effects of THC (Lepore et al, 1995; Valjent and Maldonado, 2000; Ghozland et al, 2002; Lindgren et al, 1981; Perez-Reyes et al, 1991; Kirk and de Wit, 1999; Sherma et al, 2016). Whether tolerance to THC-induced rate-decreasing effects will enhance THC SA is an empirical question that has not been documented in animal models; this was examined in the present study. We hypothesized that tolerance would develop to the rate-decreasing effects of THC and this would result in increases in rates of THC SA compared to the initial assessment.

Another behavioral variable that can influence drug-self-administration is the schedule of reinforcement, especially for drugs that are not considered highly efficacious reinforcers (Nader et al, 2015). As it relates to THC self-administration, schedules of reinforcement where the frequency of reinforcement is relatively rate independent may be better suited to uncover the reinforcing effects. Thus, the present study also examined THC SA in another group of monkeys in whom responding was maintained under a second-order schedule of reinforcement. It was hypothesized that responding under a second-order schedule, in which behavior was maintained across long intervals between drug injections by conditioned stimuli, would lead to THC reinforcement.
MATERIALS AND METHODS

Subjects. Nine individually housed adult male rhesus (Macaca mulatta) and four pair housed adult male cynomolgus (M. fascicularis) monkeys served as subjects. Six rhesus monkeys had histories of methamphetamine or cocaine SA (John et al, 2015a,b) but had been abstinent for approximately four months before these experiments began. Three rhesus monkeys did not have a SA history prior to this study (Table 1). All four cynomolgus monkeys had extensive cocaine histories (~ 8 years) at the initiation of this study. Each monkey was fitted with an aluminum collar (Model B008, Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). Monkeys were fed sufficient standard laboratory chow (Purina LabDiet 5045, St Louis, MO) to maintain healthy body weights (determined by veterinary staff). Animal housing and all experimental procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Animal Care and Use Committee of Wake Forest University.
Table 1. Ages, weights, and self-administration histories for rhesus monkeys

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Drug SA history (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1567</td>
<td>20</td>
<td>10.0</td>
<td>134.9 (methA)</td>
</tr>
<tr>
<td>R-1690</td>
<td>12</td>
<td>12.2</td>
<td>373.9 (methA)</td>
</tr>
<tr>
<td>R-1691</td>
<td>10</td>
<td>8.7</td>
<td>366.8 (methA)</td>
</tr>
<tr>
<td>R-1693</td>
<td>11</td>
<td>11.7</td>
<td>283.7 (methA)</td>
</tr>
<tr>
<td>R-1710</td>
<td>9</td>
<td>9.8</td>
<td>68.7 (cocaine)</td>
</tr>
<tr>
<td>R-1711</td>
<td>9</td>
<td>11.2</td>
<td>10.3 (cocaine)</td>
</tr>
<tr>
<td>R-1682</td>
<td>15</td>
<td>13.8</td>
<td>0</td>
</tr>
<tr>
<td>R-1713</td>
<td>9</td>
<td>14.4</td>
<td>0</td>
</tr>
<tr>
<td>R-1756</td>
<td>19</td>
<td>13.3</td>
<td>0</td>
</tr>
</tbody>
</table>

Surgery. Subjects were prepared with chronic indwelling venous catheters under aseptic conditions as previously described (John et al, 2015a,b). All monkeys were implanted subcutaneously with a transponder (Model IPTT-300; Bio Medic Data Systems, Inc., Seaford, DE) to non-invasively measure body temperature.

Apparatus. Behavioral sessions were carried out in ventilated, sound-attenuating chambers (1.5x0.74x0.76m; Med Associates, East Fairfield, VT) designed to accommodate a primate chair. Two photo-optic switches (5 cm wide; Model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) were located on one side of the chamber with a horizontal row of three stimulus lights positioned 14 cm above each switch. A food receptacle was located between the switches and connected with a Tygon tube to a pellet dispenser (Med Associates) located on the top of the chamber. A peristaltic infusion pump (7531-10, Cole-Parmer Co., Chicago, IL) for delivering drug injections at a
rate of approximately 1.5 ml/10 sec was also located on the top of the chamber. White
noise was continuously present to mask extraneous noise.

**Experiment 1: Self-administration of THC and CP55,940 and potential behavioral
phenotypes related to reinforcement in rhesus monkeys.**

Behavioral phenotype assessments: Rhesus monkeys were trained to respond
under a fixed-ratio (FR) 10 schedule of food presentation, followed by a 60-sec time-out
(TO). Sessions began with illumination of a white stimulus light above one of two photo-
optic switches in the chamber, counterbalanced between monkeys. Ten consecutive
responses emitted on the appropriate switch delivered a 1.0-g banana-flavored food
pellet (Bio-Serv, Frenchtown, NJ) accompanied by extinction of the white light and
illumination of a red light above the food receptacle for 3 sec. Responses emitted on the
alternate switch had no programmed consequence. Sessions lasted 60 min or until 30
reinforcers were obtained, whichever occurred first. Once responding was stable (3
consecutive sessions with response rates within ±20% of the mean rate for those
sessions, with no trends), non-contingent injections of CP55,940 (1.0-10 μg/kg, i.v.) and
THC (3.0-300 μg/kg, i.v.) were administered one minute prior to operant sessions. All
doses were tested 2-3 times for each monkey; CP55,940 was tested first in all monkeys.
Body temperature was taken non-invasively using the implanted telemetry device (Bio
Medic Data Systems, Inc.) immediately prior to CP55,940 or THC administration and
then again 60-min following the start of the session.

Substitution studies: After completion of the non-contingent dose-response
curves and when stable food-maintained responding was re-established, i.v. injections of
vehicle (0.4-1.0% Tween 80 and 0.4-1.0% ethanol in sterile water) and different doses of
CP55,940 (0.001-3.0 μg/kg) and THC (0.01-10 μg/kg) were substituted for food pellets
for at least 5 sessions and until responding was stable. CP55,940 SA dose-response
curves were determined first in all monkeys followed by THC SA dose-response curves with the exception of one monkey that only completed the CP55,940 dose-response curve and another monkey that only completed the THC dose-response curve.
Completion of each FR delivered an i.v. infusion (~ 0.5 ml in 3-s), followed by a 60-s TO; sessions ended after a maximum of 30 injections or 60 min elapsed, whichever occurred first. There was a return to food-maintained responding for at least 3 sessions between substitutions of different drug doses. Doses were tested in quasi-random order for each monkey, such that the highest dose was not tested first in any monkey.

**Experiment 2. Effects of chronic THC treatment on THC SA**

Following determination of SA dose-response curves for both drugs, responding was maintained by food presentation for at least 5 sessions and until stable. Next, the ED$_{50}$ for THC to decrease food-maintained responding, individually determined for each monkey, was administered non-contingently (i.v.) one min prior to each session for at least 3 sessions and until tolerance developed to the rate-decreasing effects. Tolerance was said to occur when average rates of responding were within 20% of baseline rates for three consecutive sessions. At that point, the dose for non-contingent administration was increased between an eighth and one-half log-units, depending on the monkey, and administered for at least 5 sessions and until tolerance developed to those rate-decreasing effects on food-maintained responding. Once responding was stable, THC SA dose-response curves were redetermined. During THC SA, non-contingent THC injections (10-100 μg/kg, i.v.) were administered after SA sessions in order to avoid a direct interference with SA but to maintain consistent daily levels of THC. Between THC SA dose substitutions, there was a return to food-maintained responding for at least 3 sessions with THC administered non-contingently, using a dose that reduced food-maintained responding to below 20% of baseline rates, prior to those sessions.
Experiment 3. THC self-administration under a second-order schedule of reinforcement

Four cynomolgus monkeys had been trained to self-administer cocaine under a second-order fixed-interval (FI) 600-s [FR 30:S] schedule of i.v. cocaine injection. At the beginning of the session, a white light was illuminated over one of the photo optic switches (counterbalanced between monkeys), which served as a discriminative stimulus. Following the completion of every 30th response (FR 30) during the 600-s FI, the white light was extinguished and a red light was illuminated for 2-s. Once the interval elapsed, the first FR 30 completed produced an i.v. injection of cocaine delivered over 10 s paired with the red light. Thus, the red light served as the conditioned stimulus (CS). A 60-s TO followed each injection, during which all lights were extinguished and responses had no scheduled consequences. Daily sessions ended after the completion of five cycles of the schedule or 90 min elapsed, whichever occurred first.

Following the determination of a cocaine dose-response curve (3.0 – 560 μg/kg/injection), saline was substituted for the training dose of cocaine (100 μg/kg/injection) and the CS was turned off until responding was reduced to 20% of baseline rates for 3 consecutive sessions. Next, the CS was reintroduced for at least 3 consecutive sessions during which saline was still available for SA. When responding was stable, 3.0 μg/kg THC was substituted for at least 7 consecutive sessions and until the overall rate of responding was stable (3 consecutive sessions with response rates within ±20% of the mean rate for those sessions, with no trends). A dose response curve was determined by substituting vehicle, which included presentation of the CS, and a range of doses (1.0 – 30 μg/kg/injection) in random order separated by five consecutive sessions of substitution of either 3.0 μg/kg THC or the dose of THC that maintained the highest rate of responding.
**Data analysis.** For the effects of CP55,940 and THC on food-maintained responding, the primary dependent variable was response rate (responses/second). Drug effects were expressed as a percentage of baseline responding, which was designated as 3 consecutive sessions of stable responding prior to the start of the study. Change in body temperature (°C) following administration of either CP55,940 or THC was determined by subtracting the temperature recorded prior to the start of the session from the temperature recorded at the end of the 60-min session. The potency of CP55,940 and THC to decrease food-maintained responding was estimated by calculating the ED$_{50}$ using the linear portion of the curve that crossed 50% reduction in baseline response rate. To calculate the relative potency of both drugs to decrease body temperature, the dose decreasing temperature by 0.5 °C was estimated by interpolation of the data.

For SA, the primary dependent variables were the number of reinforcers earned per session and mean rate of responding. Data were expressed as the mean (± S.D.) over the last three sessions for each drug dose. Dose-response curves for THC and CP55,940 were analyzed using one-way repeated-measures ANOVA; significant main effect was followed by Dunnett’s multiple comparisons post-hoc tests. The criterion for significance was set a priori at $p < 0.05$. For Experiment 1, Pearson’s correlation analysis was used to examine the relationship between reinforcing effects and sensitivity to rate-decreasing and hypothermic effects. For this analysis, reinforcing effects were quantified by calculating the area under the curve; sensitivity to rate-decreasing effects and hypothermic effects were quantified by the CP55940 and THC ED$_{50}$ values to decrease food-maintained responding and the slope of the regression lines of temperature change relative to vehicle, respectively.

**Drugs.** Δ$^9$-tetrahydrocannabinol (THC) and CP55,940, both obtained from the National Institute on Drug Abuse (NIDA), Bethesda, MD, were dissolved in a vehicle containing
0.001–4% Tween 80 and 0.001–4.0 % ethanol in sterile water. (−)-Cocaine HCl (NIDA) was dissolved in sterile 0.9% saline. Different SA doses were studied by varying the concentration of drug available per injection. Drug doses are expressed in terms of the salt form of cocaine.

RESULTS

Experiment 1. The mean (± S.E.M.) rate of FR food-maintained responding was 1.16±0.2 responses/second in which the maximum number of reinforcers (30) was always earned. Although two monkeys showed increases at low THC doses, mean response rates were dose-dependently decreased by non-contingent administration of CP55,940 [F(5,36)=11.5; p<0.0001; Fig. 1, closed circles] and THC [F(5,33)=3.37; p<0.05; Fig. 1, closed triangles]. The mean ED$_{50}$ values (95% confidence limits) for CP55,940 and THC to decrease food-maintained responding were 3.8 (2.1–6.8) and 59.8 (28.6–125.1) μg/kg, respectively. CP55,940 was ~16-fold more potent at decreasing food-maintained responding than THC. Non-contingent administration of CP55,940 and THC also produced dose-dependent reductions in body temperature, which corresponded to the rate-decreasing effects on food-maintained responding, although individual differences were noted (Fig. 1, open symbols). The dose of CP 55,940 and THC that produced a 0.5 °C decrease in body temperature (ED$_{-0.5°C}$ value) was estimated to be 17 and 180 μg/kg, respectively; CP55,940 was ~11-fold more potent than THC.
Figure 1. Effects of CP55,940 (circles) and THC (triangles) on food-maintained responding (filled symbols) and body temperature (open symbols) in individual rhesus monkeys ($n = 9$). CP55,940 and THC were administered non-contingently 1 min prior to the start of the session and body temperature was recorded prior to each injection and then again 60 min following the start of the session. Tolerance to the rate-decreasing effects of THC on food-maintained responding was developed by repeated administration across consecutive sessions (filled squares). Abscissae: Dose of CP55,940 and THC in $\mu$g/kg. Left ordinate: Mean ($\pm$ S.D.) response rate expressed as a percentage of baseline. Right ordinate: Mean ($\pm$ S.D.) change in body temperature expressed in $^\circ$C.
When CP55,940 was substituted for food pellets, reinforcing effects were achieved in 3 of 8 rhesus monkeys (Fig. 2, closed circles, Fig. 3) as determined by repeated-measures one-way ANOVA (R-1567: F(5,10)=21.76, p<0.0001; R-1690: F(5,10)=13.48, p<0.001; R-1691: F(5,10)=9.07, p<0.01). The peak injections of CP55,940 occurred at 0.04, 0.02 and 0.00125 μg/kg/injection for R-1567, R-1690 and R-1691, respectively. CP55,940 was least potent at decreasing food-maintained responding in the 3 monkeys in whom the drug functioned as a reinforcer (Table 2). No relationship was found between CP55,940 reinforcing effects and CP55,940-induced changes in body temperature.

In contrast to CP55,940, THC over a broad range of doses (0.03-10 μg/kg) did not maintain responding above vehicle levels, even in the 3 monkeys in which CP55,940 functioned as a reinforcer (Fig. 2, closed triangles). In 4 of 8 monkeys, however, THC produced transient reinforcing effects in which the number of injections earned was greater than vehicle levels for 2 or more consecutive sessions (Fig. 4).

Table 2. Relationship between CP55,940 potency to decrease food-maintained responding and CP55,940 self-administration

<table>
<thead>
<tr>
<th>Subject</th>
<th>ED$_{50}$ (95% confidence interval)</th>
<th>Max injections (SD)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1567</td>
<td>0.007 (0.006-0.008)</td>
<td>20.67 (1.16)</td>
<td>68.67</td>
</tr>
<tr>
<td>R-1691</td>
<td>0.006 (0.004-0.009)</td>
<td>11.67 (1.16)</td>
<td>39.33</td>
</tr>
<tr>
<td>R-1690</td>
<td>0.006 (0.004-0.009)</td>
<td>6.67 (0.58)</td>
<td>13.83</td>
</tr>
<tr>
<td>R-1693</td>
<td>0.0009 (NA)</td>
<td>1.0 (0)</td>
<td>4.5</td>
</tr>
<tr>
<td>R-1682</td>
<td>0.003 (0.001-0.01)</td>
<td>2.0 (1.73)</td>
<td>4.83</td>
</tr>
<tr>
<td>R-1711</td>
<td>0.001 (0.0006-0.002)</td>
<td>1.67 (0.58)</td>
<td>6.17</td>
</tr>
<tr>
<td>R-1713</td>
<td>0.003 (0.002-0.006)</td>
<td>1.33 (0.58)</td>
<td>4.33</td>
</tr>
<tr>
<td>R-1756</td>
<td>0.003 (0.001-0.008)</td>
<td>2.33 (1.53)</td>
<td>7.33</td>
</tr>
</tbody>
</table>
Figure 2. Self-administration dose-response curves for CP55,940 (filled circles), THC (closed triangles) and THC after the development of tolerance to rate-decreasing effects on food-maintained responding (open triangles). Abscissae: Unit dose (µg/kg/injection) available for self-administration. Ordinate: Number of injections earned in the 60-min session. Data represent mean ± S.D. of last three sessions. *p < 0.05, **p < 0.01 compared to vehicle.
Figure 3. Acquisition of CP 55,940 self-administration in individual rhesus monkeys. Data are expressed as number of reinforcers earned across consecutive sessions. Open squares represent number of food reinforcers earned prior to substituting CP 55,940 (filled symbols) or vehicle (open circles) for self-administration.
Figure 4. Transient reinforcing effects of THC in individual rhesus monkeys. Data are expressed as number of reinforcers earned across consecutive sessions. Squares represent number of food reinforcers earned prior to substituting THC (filled symbols) or vehicle (open circles) for self-administration.
**Experiment 2.** Daily administration of the THC ED$_{50}$ resulted in tolerance to the rate-decreasing effects within 5.25 (±0.75 S.E.M.) sessions. This dose was increased by an eighth to one-half log units and tolerance developed to those effects within 6.17 (±1.14 S.E.M.) sessions (closed squares, Fig. 1). Following the development of tolerance to the rate-decreasing effects of THC, SA was reexamined and THC functioned as a reinforcer in 3 monkeys (Fig. 2 open triangles; Fig. 5), as determined by repeated measures one-way ANOVA (R-1710: $F(3,6)=65.69; p<0.001$; R-1567: $F(3,6)=95.43; p<0.001$; R-1711: $F(4,8)=19.47; p<0.001$).
Figure 5. THC functioned as a reinforcer in three monkeys after the development of tolerance to rate-decreasing effects on food-maintained responding. Open squares represent food-maintained responding following increasing doses of daily THC treatment. The vertical line designates the development of tolerance after which THC (open triangles) and vehicle (closed triangles) were substituted for self-administration. There was a return to food-maintained responding between doses of THC and vehicle. Left ordinate: Percentage of baseline rate of food-maintained responding. Right ordinate: Number of THC or vehicle injections earned per session. Abscissae: Consecutive sessions.
Experiment 3. Prior to the start of THC SA, monkeys responded under a second-order FI 600-s (FR 30:S) schedule of cocaine reinforcement. Cocaine dose-response curves, determined for each monkey, were represented as an inverted U-shaped function of cocaine dose (Fig. 6, open squares). After saline substitution, with removal of the CS, responding declined; response-contingent presentation of the CS did not increase responding. Substituting different THC doses resulted in THC maintaining responding at significantly higher rates than vehicle in two of four subjects (Fig. 6, closed triangles) as determined by repeated measures one-way ANOVA (C-7427: $F(4,8)=14.94$, $p<0.001$; C-6526: $F(4,8)=41.72$, $p<0.001$), with maximal rates maintained when 10 and 3.0 $\mu$g/kg/injection were available, respectively. All monkeys responded for the maximal number of injections available for each dose of THC and vehicle except C-6526, who responded for no vehicle injections and 0.67 ($\pm 0.58$ S.D.) and 2.67 ($\pm 1.53$ S.D.) THC injections at 1.0 and 30 $\mu$g/kg/injection, respectively.
**Figure 6.** THC self-administration under a second-order [FI 600-s (FR 30:S)] schedule of reinforcement. Each dose was studied for a minimum of 7 sessions and until responding was stable. Data represent mean (±S.D.) response rates for THC (filled triangles) and cocaine (open squares) during last 3 sessions of substitution. Abscissae: Unit dose (µg/kg/injection) available for self-administration. Ordinate: Mean overall rate of responding (responses/sec).
DISCUSSION

One goal of the present study was to investigate cannabinoid SA in rhesus monkeys under similar parameters successfully used to demonstrate the reinforcing effects of cannabinoids in squirrel monkeys. We also examined behavioral phenotypes that may mediate individual differences in SA. The CBR agonist CP55,940 functioned as a reinforcer in 3 of 8 monkeys; reinforcing effects were inversely related to CP55,940-induced behavioral disruption. In those same monkeys, THC did not have reinforcing effects in any animal. Daily THC treatment with a dose that initially produced significant rate-decreasing effects resulted in THC functioning as a reinforcer in three monkeys. In another group of monkeys, THC functioned as a reinforcer in two of four animals responding under a second-order schedule. Across all conditions, of the 13 monkeys studied, CBR agonists functioned as reinforcers in 7 animals. Understanding these individual differences may lead to novel treatments for marijuana abuse.

Studies in humans show that individuals who are more sensitive to the aversive effects of cannabis were less likely to be current users (Haertzen et al, 1983; Thomas, 1996). The prominent aversive effects of cannabinoids have been demonstrated in conditioned place preference studies in rodents where several studies report that THC and synthetic CBR agonists generally induce conditioned place avoidance rather than place preference, except following a period of extended drug exposure (Lepore et al, 1995; Valjent and Maldonado, 2000; Ghozland et al, 2002). Thus, one hypothesis for the lack of cannabinoid SA in experimental animals is that the aversive (and rate-decreasing) effects of THC and other CBR agonists are inhibiting measures of reinforcement in macaques (and probably rodents) to a greater degree than is apparent in squirrel monkeys. Results with CP55,940 supported this hypothesis; CP55,940 functioned as a reinforcer in the three monkeys least sensitive to the drug’s rate-disruptive effects. However, in those same three monkeys, THC was also the least
potent to decrease rates of food-maintained responding, but THC did not function as a reinforcer in any monkey following the initial determination.

These results suggest that there may be a relationship between CB₁ receptor agonist efficacy and reinforcing effects considering the low efficacy CB₁ partial agonist THC did not function as reinforcer in the same monkeys that the high efficacy CB₁ full agonist CP55,940 did. However, due to the large number of CB₁ receptors in the central nervous system (Gifford et al., 1999), THC often produces the same maximum effect as that obtained with high efficacy CB₁ agonists for a range of behaviors including drug discrimination, antinociception, and locomotor activity (Fan et al., 1994; McMahon, 2011); however, exceptions have been noted for hypothermic effects (Paronis et al., 2012). The other exception may also include reinforcing effects. Several CBR agonist SA studies in rodents support this notion in which the high efficacy CBR agonist WIN55,212, but not THC, maintained behavior above vehicle levels (Martellotta et al., 1998; Fattore et al., 2001; Lefever et al., 2014).

These data also suggest a direct inter-species difference between rhesus and squirrel monkeys as it relates to the initial reinforcing effects of THC. Consistent with our hypothesis, the ED₅₀ for intravenous THC to decrease food-maintained responding in squirrel monkeys (Justinova et al., 2013) is ~1.0 log-units higher (i.e., less potent) compared to the rhesus monkeys used in the present study. These data indicate that the reinforcing effects of THC may only be unmasked when there is a certain degree of insensitivity to the rate-decreasing effects, similar to what was shown in the present study involving CP55,940 SA.

The precise mechanism underlying the rate-decreasing effects of CBR agonists, including individual-subject variability, is uncertain. Previous studies in rats and monkeys indicate that CB₁ receptors do not exclusively mediate the rate-decreasing effects of THC as evidenced by the inability of the prototypic CB₁ antagonist SR141716A to
attenuate these effects (McMahon et al, 2005; Järbe et al, 2003; DeVry and Jentzsch, 2004). On the other hand, SR141716A has been reported to attenuate many of the physiological and unconditioned behavioral effects of THC such as the hypothermic, antinociceptive, respiratory depressant, and cardiovascular effects (McMahon et al, 2005; Vivian et al, 1998). As a result, the factors mediating individual differences in the ability of CP55,940 to function as a reinforcer may not be related to CB1 receptor function considering no relationship was found between reinforcing effects and CP55,940-induced changes in body temperature. Moreover, the doses of CP55,940 that had reinforcing effects in these subjects were approximately 1.0-log units lower than what was previously tested in an unsuccessful attempt to obtain CP55,940 reinforcement in rhesus monkeys (Mansbach et al, 1994). These results highlight the importance of using low doses to demonstrate the reinforcing effects of CBR agonists, as emphasized by Goldberg colleagues (Tanda et al, 2000).

To further evaluate the relationship between sensitivity to the rate-decreasing effects of THC and SA, we conducted two additional experiments. In one study, THC was administered daily in order for tolerance to develop to the rate-decreasing effects of THC. After reassessing SA, THC only functioned as a reinforcer in three animals. Interestingly, one of these subjects (R-1567) did not completely develop tolerance to the rate-decreasing effects prior to reassessing SA. This suggests that a period of pre-exposure to THC, rather than the development of tolerance to rate-decreasing effects, could be the more crucial factor for acquiring THC SA. Future studies could determine if other experimental manipulations (e.g., lengthen TO or change the schedule to progressive-ratio) could yield more robust THC SA. Furthermore, no observational signs of physical withdrawal were noted during daily THC administration, which is consistent with previous studies showing that much higher doses (e.g., 1.0 mg/kg/12 hr or 0.05-0.17 mg/kg/hr) were required to produce physical withdrawal symptoms in rhesus
monkeys (Beardsley et al., 1986; Stewart and McMahon, 2010) than what was used for
the present study. It remains to be determined if physical dependence would enhance
the likelihood of THC SA.

A second method to address the role of sensitivity to rate-disruptive effects and
SA was to use a schedule of reinforcement in which response rates and reinforcement
frequency were not directly related. Two of four monkeys self-administering THC under a
second-order FI 600-s (FR30:S) schedule showed reinforcing effects at a doses similar
to those that resulted in reinforcing effects in squirrel monkeys responding under a
second order schedule (Justinova et al., 2008); there was no apparent relationship
between rates of responding maintained by cocaine and the ability for THC to have
reinforcing effects. Mansbach et al. (1994) also used an FI 600-s schedule but did not
report any evidence that THC maintained responding higher than vehicle, suggesting
that perhaps the use of conditioned stimuli may enhance the likelihood that future
investigators can obtain robust THC self-administration under similar schedule
conditions.

The failure of THC to function as a reinforcer in the majority of subjects following
the development of tolerance to rate-decreasing effects suggests other factors may be
involved. One possible explanation is that THC reinforcement was related to individual
differences in THC pharmacokinetics and/or metabolism following tolerance. However,
studies in animals and humans have shown that chronic THC exposure does not
significantly change THC metabolism or disposition (Dewey et al, 1973; McMillan et al,
1973; Siemens and Kalant, 1974; Martin et al, 1976; Hunt and Jones, 1980; Ginsburg et
al, 2014). Even so, this possibility cannot be rejected and should be investigated with
particular attention to individual differences in correspondence with other behavioral
endpoints related to reinforcement.
It is important to note that there was a relationship between drug history and CBR agonist SA in that three of the four monkeys that had an extensive methamphetamine SA history were the subjects in whom CP55,940 functioned as a reinforcer. Although previous studies in squirrel monkeys have demonstrated that a drug SA history is not necessary for acquiring robust levels of THC SA (Justinova et al, 2003), the present results suggest a potential relationship as it relates to the acquisition of CP55,940 SA. A better understanding of the importance of drug history, schedule of reinforcement, behavioral, and physiological phenotypes related to pharmacological sensitivity to cannabinoid SA are needed to establish a reliable preclinical model of CBR abuse.
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CONFLICT OF INTEREST. The authors declare no conflicts of interest.

CONTRIBUTIONS. WSJ, TJM, and MAN designed the studies. WSJ conducted the experiments and analyzed the data. WSJ, TJM, and MAN wrote the manuscript.
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CHAPTER III

CHRONIC Δ⁹-THC IN RHESUS MONKEYS: EFFECTS ON COGNITIVE PERFORMANCE AND DOPAMINE D₂/D₃ RECEPTOR AVAILABILITY

William S. John¹, Thomas J. Martin², Kiran Kumar Solingapuram Sai³, Susan H. Nader¹,
H. Donald Gage³, Akiva Mintz³, and Michael A. Nader¹,³

¹Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC
²Department of Anesthesiology, Wake Forest School of Medicine, Winston-Salem, NC
³Department of Radiology, Wake Forest School of Medicine, Winston-Salem, NC

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ABSTRACT

**Background:** Cannabis-related impairments to cognitive function may represent novel therapeutic targets for cannabis-use disorder; however, the specificity, persistence, and reversibility of those deficits remain unclear.

**Methods:** Each day, adult male rhesus monkeys (N=6) performed cognitive tasks and responded under a fixed-ratio (FR)10 schedule of food presentation afterwards. After the acute effects of Δ⁹-tetrahydrocannabinol (THC; 0.01-0.56 mg/kg, i.v.) on cognitive performance and food-maintained responding were assessed, 1.0–2.0 mg/kg THC (s.c.) was administered after FR10 sessions for 12 weeks during which the effects on cognition, 22 hours after administration, were assessed daily. Prior to discontinuing treatment, the acute THC dose-response curve was redetermined. Dopamine D₂/D₃ receptor availability was assessed after 4 weeks of chronic THC exposure and compared to drug-naïve controls (N=4/group) using positron emission tomography and [¹¹C]-raclopride.

**Results:** Acute administration of THC dose-dependently deceased cognitive performance and food-maintained responding. During chronic treatment, THC produced persistent impairment 22 hrs after administration to working memory but not to discrimination and reversal learning nor attentional set-shifting performance. Chronic THC administration resulted in tolerance to acute rate-decreasing effects on food-maintained responding and the acute impairments on cognitive performance. During abstinence, there was recovery of impairments within 2 weeks. D₂/D₃ receptor availability was not different in chronic THC-treated monkeys compared to control subjects.

**Conclusions:** Chronic THC-induced disruptions in cognitive performance depended on the cognitive domain, cognitive load, and time of assessment following THC administration. These cognitive-domain specific effects of THC were not accompanied
by changes in dopamine D₂/D₃ receptor availability, which suggest that long-term marijuana use may not produce comparable neuropharmacological adaptations as other drugs of abuse.

**Key Words:** THC; CANTAB; Marijuana; Rhesus monkey; delayed-match-to sample, PET imaging, set-shifting
INTRODUCTION

The disruption in cognitive function is a major consequence of drug abuse (Jentsch and Pennington, 2014) and a factor that has been shown to predict relapse and is associated with poorer treatment retention and outcome (Aharonovich et al., 2006, 2008; Carroll et al., 2011). As a result, cognitive enhancement may hold promise as an adjunct treatment strategy for substance use disorders (Sofuoglu et al., 2013; Rezapour et al., 2016).

Marijuana is the most widely abused illicit substance in the United States (SAMHSA, 2013) and the neurocognitive effects of Δ⁹-tetrahydrocannabinol (THC), the major psychoactive constituent of marijuana, have been extensively studied (Batalla et al., 2013). Although studies in humans have shown that the acute effects of cannabis use consistently impair executive cognitive functions such as attention (Hart et al., 2001; D’Souza et al., 2008; Anderson et al., 2010), working memory (Heishman et al., 1997; Hart et al., 2001, 2010;), and inhibition/impulsivity (McDonald et al., 2003; Ramaekers et al., 2006), the residual effects (i.e. after the intoxicating effects have subsided) and effects during abstinence, which are relevant for treatments that target cognitive functioning, are equivocal for most cognitive domains (Crean et al., 2011; Crane et al., 2013). Some of these disparate findings may be the result of experimental factors that are difficult to control in studies with human subjects such as differences in time since last use, amount and duration of cannabis use, previous drug histories, social variables, as well as concurrent use of other substances. In addition, studies in humans cannot distinguish whether marijuana abusers exhibit innate cognitive differences prior to initiating drug taking so it is difficult to clearly establish a causal role of long-term THC exposure in producing cognitive deficits. Therefore, it is critical to employ longitudinal, within-subject studies in preclinical animal models that can control for these variables in a systematic manner.
The present study used a within-subjects study design in monkeys to examine the acute effects of THC 30-min prior to cognitive sessions and during chronic treatment, and the effects of THC 22 hours after administration in an effort to model residual drug effects, on multiple cognitive domains. Long-term cannabis use has also been shown to produce differential tolerance to the acute effects of THC on cognitive function compared to infrequent users (Kirk and de Wit, 1999; D’Souza et al., 2008; Ramaekers et al., 2009). In the present study, the cognitive domains were 1) attention and working memory using the delayed match to sample task, 2) associative learning using the simple/compound discrimination task, 3) behavioral inhibition using the reversal learning task, and 4) behavioral flexibility using dimensional set-shifting tasks.

Intact functioning within these particular cognitive domains is important for positive treatment response as they underlie the skills necessary for behavioral change and for reducing the likelihood of relapse during treatment (Rezapour et al., 2016); however, studies in chronic cannabis users show differential effects within these cognitive domains regarding the time course of impairments (Crane et al., 2013; Crean et al., 2011). For instance, some studies report deficits in attentional abilities, learning and memory, inhibition, and cognitive flexibility in recently abstinent (12 hr–3 d) cannabis users (Solowij et al., 1995, 2002, 2011; Pope and Yurgelun-Todd, 1996; Harvey et al., 2007; Hanson et al., 2010) while others do not (Whitlow et al., 2004; Kanayama et al., 2004; Fisk and Montgomery, 2008; Grant et al., 2011). Moreover, other studies show cognitive impairments after 28 days of abstinence or longer (Solowij et al., 1995; Bolla et al., 2002; Jacobson et al., 2004, 2007; Medina et al., 2007). As a baseline for comparison, the effects of chronic THC treatment was also studied on food-maintained responding, which has been shown to result in tolerance to THC-induced decreases in operant responding (McMahon, 2011). It was hypothesized that THC-induced impairments to cognitive function would be independent of nonselective disruption to
operant responding and be most sensitive to working memory performance based on previous research in nonhuman primates (Taffe, 2012; Wright Jr. et al., 2013; Kangas et al., 2016).

As it relates to neurobiological substrates that mediate cognition and executive function, dopamine (DA) D2-like receptors, consisting of the DA D2 and D3 receptor subtypes, are particularly relevant (Tomasi and Volkow, 2013). For instance, D2/D3 receptors in the dorsal striatum have been shown to be associated with activation of prefrontal brain regions implicated in executive function (Volkow et al., 1993, 1998) and with frontostriatal circuitry implicated in inhibitory control (Ghahremani et al., 2012). Moreover, chronic drug abuse in humans has been associated with a reduction in D2/D3 receptor availability compared to healthy control subjects for a variety of substances (Volkow et al., 2012). Taken together, cognitive impairment resulting from chronic drug use may be related to low striatal D2/D3 availability. Indeed, cognitive impairment in long-term marijuana users has been associated with reduced activity in frontal cortical regions (Block et al., 2002; Eldreth et al., 2004; Gruber et al. 2005; Bolla et al., 2005); however, the relationship between chronic THC exposure, cognitive performance, and D2/D3 receptor availability has not been investigated. In the present study we assessed D2/D3 receptor availability using PET and [11C]-raclopride in relation to cognitive performance during chronic THC treatment.

METHODS

Subjects

Eight individually housed adult male rhesus monkeys (Macaca mulatta) served as subjects. Four monkeys were drug-naïve at the start of these studies and baseline [11C]-raclopride scans were obtained (see below). Two of these monkeys, along with four animals with extensive drug histories (see John et al., 2015a,b) were involved in the
THC-cognition studies. Each monkey was fitted with an aluminum collar (Model B008, Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). Monkeys were housed in stainless-steel cages with visual and auditory contact with each other, *ad libitum* access to water in their home cage and were fed sufficient standard laboratory chow (Purina LabDiet 5045, St Louis, MO) to maintain healthy body weights as determined by veterinary staff. Environmental enrichment was provided as outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and was approved by the Animal Care and Use Committee of Wake Forest University.

**Apparatus**

The apparatus for food-maintained responding was a ventilated, sound-attenuating chamber (1.5 x 0.74 x 0.76 m; Med Associates, East Fairfield, VT) designed to accommodate a primate chair. Two photo-optic switches (5 cm wide) were located on one side of the chamber with a horizontal row of three stimulus lights 14 cm above each switch and a food receptacle between the switches. The food receptacle was connected with tygon tubing to a pellet dispenser (Gerbrands Corp., Arlington, MA) located on the top of the chamber for delivery of 1.0-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). An infusion pump (Cole-Palmer, Inc., Chicago, IL) was located on the top of the chamber.

The apparatus for cognitive testing was a separate sound-attenuating, ventilated chamber (0.8 x 0.8 x 1.32 m) consisting of a CANTAB panel (0.38 x 0.56 x 0.31 m) that included a touch-sensitive screen (0.3 x 0.23 m), a stimulus light, a non-retractable response lever, and a pellet receptacle located on the right side. For each task, responding was maintained by 190-mg sucrose pellets.
Surgery

Monkeys included in behavioral experiments were surgically prepared with a chronic indwelling venous catheter (femoral, internal or external jugular) and subcutaneous vascular port (Access Technologies, Skokie, IL) using aseptic surgical procedures, as described in detail elsewhere (John et al., 2015a). These monkeys were also implanted subcutaneously with a transponder (Model IPTT-300; Bio Medic Data Systems, Inc., Seaford, DE) to non-invasively measure body temperature.

Experimental Timeline

Six monkeys underwent two behavioral sessions each day (Figure 1). First, cognitive performance was assessed using the CANTAB apparatus (Lafayette Instruments, Lafayette, IN). Immediately following, monkeys were transported to a different room and operant sessions were conducted in separate experimental chambers where monkeys responded under a fixed-ratio (FR) 10 schedule of food (1.0 g banana-flavored pellets) reinforcement with a 60 second timeout (TO) following each food presentation. Monkeys were returned to their cages following the completion of the second behavioral session.

Acute THC Treatments: The acute effects of THC were determined separately on food-maintained responding and cognitive performance. For FR 10 food-maintained responding, when stable (± 20% of the mean rate of responding for 3 consecutive sessions, with no trends), non-contingent injections of THC (vehicle, 0.003-0.3 mg/kg, i.v.) were administered one minute prior to operant sessions; all doses were tested 2-3 times for each monkey. Body temperature was taken immediately prior to THC administration and then again 60-min following the start of the session. Between each test session, at least two sessions were conducted where no drug or vehicle was administered. Following one week of chronic THC (1.0 mg/kg, s.c.) administration, the
effects of acutely administered THC on FR responding was redetermined during which monkeys were still treated daily with 1.0 mg/kg THC after the session. For cognitive performance, following stable performance, the acute effects of vehicle and THC (0.003 – 0.3 mg/kg, i.v.) were determined on each task prior to chronic THC treatment. Each dose was administered 30-min prior to the start of the session; all doses were tested 2-3 times for each monkey and at least two intervening sessions were conducted where no drug or vehicle was administered. Following 8 weeks of chronic THC administration, the acute effects of THC on cognitive performance were redetermined over the course of two weeks while monkeys still received 1.0 mg/kg THC at the end of FR sessions.

**Chronic THC Treatments:** After completion of all acute THC dose-response curves, THC was administered chronically for 12 weeks with daily injections (1.0 mg/kg for 10 weeks and 2.0 mg/kg, s.c., for 2 weeks) occurring immediately following the second behavioral session (i.e. FR food-maintained responding) or at similar times when sessions did not occur (i.e. weekends). Thus, during this phase of the study, cognitive performance and FR responding were measured approximately 22 and 23 hours after daily THC administration, respectively. These doses were selected based on pilot studies on FR responding. Moreover, doses of THC within the same range administered to rhesus monkeys via the same route (s.c.) have been shown to produce similar blood levels of THC as those of humans smoking cannabis in controlled laboratory studies (Ginsberg et al., 2014) The daily dose was not raised to 2.0 mg/kg in R-1710 due to marked behavioral disruption during 1.0 mg/kg THC, to which tolerance did not develop. Following completion of the chronic dosing phase, THC administration was discontinued and performance during 4-6 weeks of abstinence was examined.
Experiment 1: Effects of acute and chronic THC on food-maintained responding

Sessions began with illumination of a white stimulus light above one of two photo-optic switches in the chamber, counterbalanced between monkeys. Ten consecutive responses emitted on the appropriate switch produced the delivery of a banana-flavored food pellet accompanied by extinction of the white light and illumination of a red stimulus light (conditioned stimulus) above the food receptacle for 3 sec. Responses emitted on the alternate photo-optic switch had no programmed consequence. Sessions lasted 60 min or until 30 reinforcers were obtained, whichever occurred first. The effects of acute (1 min pretreatment) and chronic (23 hrs after daily administration) THC were assessed on FR 10 responding, as described above.

Figure 1. Experimental timeline. Monkeys performed two operant behavioral sessions each day, which included cognitive testing immediately followed by responding for food pellets under an FR 10 schedule. Following stable performance, the acute effects of THC (0.003–0.3 mg/kg, i.v.) were determined on FR responding (Experiment 1) and cognition (Experiment 2). THC (1.0–2.0 mg/kg, s.c.) was then administered chronically for 12 weeks during which the effects 22 hrs after administration were assessed daily on cognitive performance and FR responding (Experiment 2). During chronic treatment, dopamine D2 receptor availability was assessed using PET and $^{11}$C-raclopride at four weeks (Experiment 3) and the acute effects of THC were redetermined on FR responding (Experiment 1) and cognition (Experiment 2) at one week and 8 weeks, respectively (Experiment 1). Behavioral performance continued to be assessed for 4 weeks following the discontinuation of THC treatment.

Exp., Experiment; + THC #2, redetermination of acute effects of THC.
Experiment 2: Effects of acute and chronic THC on cognitive performance

Each week, DMS performance was assessed Monday-Wednesday and stimulus discrimination, reversal learning, and set-shifting performance were assessed on Thursday and Friday.

Delayed Match-to-Sample (DMS): The DMS task is a measure of attention and working memory. Each trial began with the appearance of a “target” stimulus in the center of the screen (sample phase). A response on this stimulus was followed by a delay and then the presentation of a stimulus matching the previous image and at least two distractor stimuli that do not match. Responding on the match resulted in delivery of a sucrose pellet followed by a 10-s TO, whereas responding on the non-match resulted in trial termination and the 10-s TO. Three delays were randomly distributed throughout a total of 60 trials per session (20 trials/delay). Delays and number of distractor stimuli were individually determined (Table 1) in order to generate delay-effect curves that met the following criteria: short delay (80-100% accuracy); middle delay (60-79% accuracy); long delay (<60% accuracy). The stimuli used were unique abstract patterns preprogrammed in CANTAB software, which consisted of four rectangular quadrants of different colors and shapes.
Table 1. Individual parameters used throughout study that produced delay-dependent effects on DMS performance; dist., distractor stimuli.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Delays (sec)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Short</td>
<td>Mid</td>
<td>Long</td>
</tr>
<tr>
<td>R-1567</td>
<td>1</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>R-1682</td>
<td>0</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>R-1710</td>
<td>0</td>
<td>10</td>
<td>30</td>
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<tr>
<td>R-1711</td>
<td>0</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>R-1713</td>
<td>0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>R-1756</td>
<td>1</td>
<td>30</td>
<td>60</td>
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**Stimulus discrimination, reversal learning and intradimensional/extradimensional set-shifting (ID/ED):** This task involved 5 distinct stages that tested attention, rule learning and reversal, and executive function. Stages advanced within-session following acquisition of performance criteria, defined as 16 of 20 consecutive trials correct. Stage 1, simple discrimination (SD): Two stimuli (e.g. shapes) were presented, one of which was reinforced upon selecting (S+) while the other was not reinforced (S-) and resulted in trial termination. Stage 2, compound discrimination (CD): The same stimuli as in the previous stage remained present, however, additional stimuli (e.g. lines) were superimposed to construct compound stimuli that consisted of shape and line dimensions. The same stimulus dimension that was associated with reinforcement in the first stage (e.g. shapes) remained the relevant stimuli in this stage, while the other dimension (e.g., lines) was irrelevant. Stage 3, compound reversal (CR): Stage 2 rules were reversed such that the previously non-reinforced stimulus within the dimension was reinforced. Stage 4, intra-dimensional shift (ID): A new pair of compound stimuli consisting of shape and line dimensions was presented; however, despite new stimuli,
the same dimension of the stimuli as in stages 1 - 3 (e.g. shapes) remained relevant for reinforcement. Stage 5, extra-dimensional shift (ED): another novel set of compound stimuli was introduced; however, in this stage the previous irrelevant stimulus dimension (e.g. lines) became relevant for reinforcement. Each session lasted for 100 min or after criterion was met for each stage, whichever occurred first.

Experiment 3: Effects of chronic THC on D$_2$/D$_3$ receptors

Two groups of monkeys underwent $[^{11}C]$-raclopride PET scans. One group (N=4) was studied after 4 weeks of daily THC administration while another group (N=4) was drug-naïve and served as control subjects. Two of the control subjects became subjects in Experiments 1 and 2 and were also included in the group of monkeys scanned after THC administration. Magnetic resonance imaging (MRI) scans were acquired for each monkey. For those images, subjects were anesthetized with ketamine (10 mg/kg, i.m.) and transported to the MRI facility, where anesthesia was maintained during the scanning procedure with supplemental ketamine (up to 15 mg/kg, i.m.). T1-weighted images of the brain were acquired with a 3.0-T Siemens SKYRA scanner. Images were used to anatomically define regions of interest (ROI), including the caudate nucleus, putamen, ventral striatum, and cerebellum, for later registration with PET images.

For the $[^{11}C]$-raclopride studies, approximately 30 min prior to the PET scan, the monkey was anesthetized with ketamine (10 mg/kg, i.m.) and transported to the PET Center. Anesthesia was maintained during the scan by inhaled isoflurane (1.5 %). The monkey was placed in the scanner (GE 64 slice PET/CT Discover VCT scanner, GE Medical Systems, Milwaukee, WI) and a catheter was inserted into an external vein for tracer injection and fluid replacement throughout the study. Body temperature was maintained at 40°C and vital signs (heart rate, blood pressure, respiration rate, and temperature) were monitored throughout the scanning procedure. A 5-min transmission
scan was acquired in 2D mode. Next, the monkey received a bolus dose of $[^{11}\text{C}]$-raclopride (6.5–8.0 mCi) and a 90-min dynamic acquisition scan was acquired consisting of 18 frames over 90 min (18 × 5 min) in 3D mode (i.e., septa retracted). Image reconstruction of 3D data was done using the 3D-reprojection method (Kinahan and Rogers, 1989) with full quantitative corrections. Once scanning was complete, the transmission scan data were smoothed transaxially using a 4-mm Gaussian filter and segmented (Bettinardi, 1999). Emission data were corrected for attenuation and reconstructed into $128 \times 128$ matrices using a Hanning filter with a 4-mm cutoff transaxially and a ramp filter with an 8.5-mm cutoff axially.

**Data Analysis**

**Experiment 1:** The primary dependent variable for food-maintained responding was response rate (responses/second). Drug effects were expressed as a percentage of baseline responding, which was designated as 3 consecutive sessions of stable responding prior to the start of the study. Change in body temperature ($\degree$C) following administration of either vehicle or THC was determined by subtracting the temperature recorded prior to the start of the session from the temperature recorded at the end of the 60-min session. The potency of THC to decrease food-maintained responding was estimated by calculating the ED$_{50}$ using the linear portion of the curve that crossed 50% reduction in baseline response rate.

**Experiment 2:** The primary dependent variable for DMS performance was percent accuracy, which was determined by dividing the number of correct responses by the total number of trials completed for each delay (short, mid, long) multiplied by 100. Omitted trials during either the sample or match phase were not counted towards the total trials. During each condition (THC treatment and abstinence), changes in percent accuracy from baseline, defined as 7 consecutive sessions with percent accuracy for
each delay ±25% of the mean rate for those sessions with no trends, were examined by repeated-measures two-way repeated measures ANOVA using delay (short, middle, long) and treatment condition (baseline, chronic, abstinence) as factors. Additional dependent variables for DMS performance measured were number of omissions for sample and match phases, response latencies for sample and match phases, and pellet retrieval latencies. One-way repeated measures ANOVA was used to compare test conditions with baseline conditions. Sessions during the chronic THC treatment phase in which the total number of omissions exceeded 50% of the total trials were excluded from analyses. The primary dependent variable for the ID/ED task was percent accuracy (number of correct trials divided by number of total trials X 100) from baseline (four consecutive sessions over two weeks with percent accuracy for each stage ±25% of the mean rate for those sessions, with no trends). Repeated measures two-way ANOVA was conducted using stage (SD, CD, CDR, ID, ED) and treatment condition (baseline, chronic, abstinence) as factors. Omitted trials were excluded from all analyses. Significant main effects were followed by post-hoc Fisher LSD tests. For all analyses, p < 0.05 was considered statistically significant.

**Experiment 3:** PET data were co-registered to individual T1-weighted MRIs, which were used to anatomically define regions of interest (ROI) including the caudate nucleus, putamen, ventral striatum, and cerebellum. PMOD Biomedical Image Quantification Software (version 3.1; PMOD Technologies, Zurich, Switzerland) was used for image registration and was used to calculate distribution volume ratios (DVRs) for the caudate nucleus, putamen, and ventral striatum by implementing the “Logan method” of analysis (Logan et al. 1990) using the cerebellum as the reference region. DVRs for each region were not different between left and right sides therefore data from each monkey was expressed as a mean of both sides. To compare DVRs between
control and THC-treated subjects, a two-way ANOVA was conducted using region and group as factors.

RESULTS

Experiment 1: Effects of acute and chronic THC on food-maintained responding

Mean (±SEM) rate of responding under the FR 10 (TO 60-s) schedule of food presentation, before THC administration was 1.18 (0.19) responses/sec and mean body temperature was 35.97 (0.54) °C. Acute THC administration resulted in dose-dependent decreases in food-maintained responding (Figure 2A, open circles) and biphasic effects on body temperature (Figure 2A, open triangles). The ED$_{50}$ (± 95% confidence limits) for THC on reducing response rates was 0.034 (0.012-0.099) mg/kg and for hypothermia was 0.068 (0.024-0.19) mg/kg. In general, daily post-session administration with 1.0 mg/kg THC for 10 weeks and 2.0 mg/kg THC for 2 weeks, did not affect food-maintained responding (Figure 2B). However, after one week of daily THC treatment (1.0 mg/kg, s.c.), the ED$_{50}$ for acutely administered THC to decrease food-maintained responding was 0.34 (0.16-0.51) mg/kg - a 9.7–fold ($p < 0.05$) reduction in potency (Figure 2A, closed circles). The ED$_{50}$ for THC to decrease body temperature after treatment was 0.46 (0.23-0.9) mg/kg, which was increased by 6.8-fold (Figure 2A, closed triangles).
Experiment 2: Effects of THC on cognitive performance

DMS performance

For each monkey, three delays were then chosen (short, mid, long) that resulted in similar delay-effect curves between monkeys (Table 2). For the acute effects of THC on DMS performance prior to chronic treatment, there was a main effect of dose [F(4, 63) = 13.55, p < 0.0001]; post-hoc analyses revealed that 0.03 mg/kg THC significantly reduced percent accuracy from baseline at the short delay and 0.03 and 0.056 mg/kg THC dose-dependently decreased percent accuracy from baseline at the middle and long delays (Fig. 3A, open symbols). These effects occurred at doses that did not produce omissions in more than 30% of total trials (Fig. 3B, open symbols). When the
THC dose-response curve was redetermined during chronic THC treatment, there was a main effect of delay \[F(2, 54) = 37.36, \ p < 0.0001\], dose \[F(5, 54) = 5.38, \ p < 0.0001\], and a significant interaction \[F(10, 54) = 4.52; \ p = 0.0001\). Post-hoc analyses indicated that tolerance developed to the performance-disrupting effects of THC such that 0.03 and 0.056 mg/kg THC did not alter percent accuracy from baseline at the short and middle delays. However, a larger dose (0.3 mg/kg) of THC produced comparable decreases to other doses in the initial assessment at the middle but not short delay.

The effects of THC (1.0-2.0 mg/kg, s.c.) 22 hours after treatment on DMS performance over the course of 12 weeks of treatment and during 4 weeks of abstinence (Figure 4A) showed a significant main effect of condition (THC treatment regimen) on percent change in accuracy from baseline \[F(14, 42) = 2.29; \ p < 0.05\). Significant impairments were detected at the middle delay during week four of treatment and at the long delay during the first four weeks of treatment and again during weeks 6 and 7. By week 8, tolerance developed to THC-induced impairments such that decreases in percent accuracy at the long delay were not different from baseline. At week 11, the dose of daily administered THC was increased to 2.0 mg/kg (s.c.) and percent accuracy from baseline at the longest delay was significantly decreased again at week 12, which persisted until 2 weeks of abstinence (Figure 4A).
Table 2. Baseline percent accuracy (± SD) at each delay for individual monkeys responding under the DMS task.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Short</th>
<th>Mid</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1567</td>
<td>78.8 (7.2)</td>
<td>46.5 (17.2)</td>
<td>38.3 (9.9)</td>
</tr>
<tr>
<td>R-1682</td>
<td>81.7 (5.9)</td>
<td>70.9 (9.8)</td>
<td>58.7 (9.8)</td>
</tr>
<tr>
<td>R-1710</td>
<td>75.8 (4.0)</td>
<td>52.9 (11.4)</td>
<td>41.7 (13.6)</td>
</tr>
<tr>
<td>R-1711</td>
<td>82.2 (10.2)</td>
<td>72.0 (15.5)</td>
<td>58.1 (8.7)</td>
</tr>
<tr>
<td>R-1713</td>
<td>87.1 (5.7)</td>
<td>56.5 (10.2)</td>
<td>49.3 (14.0)</td>
</tr>
<tr>
<td>R-1756</td>
<td>72.7 (11.2)</td>
<td>52.0 (11.0)</td>
<td>48.5 (5.1)</td>
</tr>
</tbody>
</table>

Stimulus discrimination, reversal learning, and set-shifting performance

For the acute effects of THC on set-shifting performance prior to chronic THC treatment, two-way ANOVA demonstrated a main effect of stage \([F(16,98) = 10.69, p < 0.001]\) and dose \([F(4, 98) = 3.49, p < 0.05]\). Post-hoc analysis revealed a significant decrease in percent accuracy for CD at 0.1 mg/kg THC \((p < 0.05)\) and for ED at 0.056 and 0.1 mg/kg THC \((p < 0.05)\) (Figure 3C). During chronic treatment, the acute effects of only 0.1 mg/kg THC were reexamined because it was the highest dose that produced impairments without producing a significant number of omissions in the initial assessment prior to treatment. Two-way ANOVA demonstrated a main effect of stage \([F(4, 68) = 4.35, p < 0.05]\) and condition \([F(3, 68) = 3.55, p < 0.05]\); post-hoc analysis revealed that percent accuracy was significantly lower for vehicle administration during chronic treatment compared to vehicle prior to treatment. Percent accuracy following 0.1 mg/kg THC was not significantly different compared to vehicle administration prior to chronic treatment (Fig. 3D).
The effects of THC 22 hours after administration on SD, CD, CDR, ID and ED set-shifting performance (Figure 4B) during chronic treatment and abstinence showed a significant main effect of stage \(F(4, 296) = 12.53; \ p < 0.0001\) although post-hoc analysis revealed no significant decreases in percent accuracy from baseline at any stage during the course of treatment and throughout abstinence.
A

Short Delay

Mid Delay

Long Delay

Percent Correct (% of baseline)

Δ⁹-THC (mg/kg)

B

Phase 1

Phase 2

Number of Omissions

Δ⁹-THC (mg/kg)

C

D

Δ⁹-THC (mg/kg)

Percent Correct

+ Vehicle

+ Vehicle (During)

+ 0.1 mg/kg THC

+ 0.1 mg/kg THC (During)

SD

CD

CDR

ID

ED

Percent Correct

50

60

70

80

90

100

SD

CD

CDR

ID

ED

Δ⁹-THC (mg/kg)
Figure 3. Effects of acute THC on cognitive performance before and during chronic THC (1.0 mg/kg, s.c.) treatment. Doses of THC were administered 30 min before experimental sessions. Panel A: Delayed-match-to-sample performance at short (left panel), mid (middle panel), and long (right panel) delays. Abscissae: dose of THC in mg/kg that was administered 30 min prior to assessment before and during chronic THC treatment. Ordinate: Percent accuracy as a percent of baseline performance. Panel B: Number of omissions during sample (left panel) and target phase (right panel) of delayed-match-to-sample task. Panel C: Acute effects of THC on SD, CD, CDR, ID, and ED set-shifting performance prior to chronic THC treatment. Panel D: Effects of 0.1 mg/kg THC and vehicle on SD, CD, CDR, ID, and ED set-shifting performance before and during chronic THC treatment. Asterisks indicate significant difference from baseline. *p < 0.05, **p < 0.01, ****p < 0.0001. #, significant difference from vehicle administration during chronic THC treatment. Data represent mean (SEM) of n = 6. SD, stimulus discrimination, CD, compound discrimination, CDR, compound reversal, ID, intradimensional set-shifting, and ED, extradimensional set-shifting.
Figure 4. Effects of THC 22 hrs after administration during chronic treatment and effects of abstinence on delayed match-to-sample performance (panel A) and discrimination and reversal learning and attentional set-shifting performance (panel B). Data are expressed as percent of baseline performance. Filled symbols indicate a significant difference from baseline (p < 0.05). Break in x-axis indicates two-week period where acute effects of THC on cognitive performance were reassessed during THC treatment. Vertical line indicates discontinuation of chronic THC treatment after which effects during abstinence were assessed. SD, stimulus discrimination, CD, compound discrimination, CDR, compound reversal, ID, intradimensional set-shifting, and ED, extradimensional set-shifting.
Experiment 3: Effects of chronic THC on D$_2$/D$_3$ receptors

In control monkeys, the distribution volume ratio (DVR) for [$^{11}$C]-raclopride was highest in the putamen, followed by the caudate nucleus and ventral striatum (Table 3). When D$_2$/D$_3$ receptor availability was compared between control monkeys and monkeys receiving chronic THC administration, there were no significant differences in the caudate nucleus, putamen and ventral striatum (Table 2, Fig. 5; p<0.05). For the two monkeys that participated in scans both before and during THC treatment, DVRs were decreased in the caudate nucleus (R-1710: -19.6%; R-1711: -1.51%) and increased for both monkeys in the ventral striatum (R-1710: +10.2%; R-1711: +3.9%). However, DVRs in the putamen were decreased by 14.2 percent during THC treatment in one monkey (R-1710) and increased by 15.3 percent in the other (R-1711).

Table 3. Individual and mean ($\pm$SEM) [$^{11}$C]-raclopride DVRs in control and THC treated monkeys.

<table>
<thead>
<tr>
<th></th>
<th>Caudate</th>
<th>Putamen</th>
<th>Ventral Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1710</td>
<td>2.36</td>
<td>2.55</td>
<td>1.51</td>
</tr>
<tr>
<td>R-1712</td>
<td>2.14</td>
<td>2.15</td>
<td>1.89</td>
</tr>
<tr>
<td>R-1711</td>
<td>2.39</td>
<td>2.15</td>
<td>1.82</td>
</tr>
<tr>
<td>R-1681</td>
<td>1.68</td>
<td>2.53</td>
<td>1.99</td>
</tr>
<tr>
<td>Mean</td>
<td>2.14</td>
<td>2.35</td>
<td>1.80</td>
</tr>
<tr>
<td>$\pm$SEM</td>
<td>0.16</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>$\Delta^9$-THC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1710</td>
<td>1.90</td>
<td>2.18</td>
<td>1.67</td>
</tr>
<tr>
<td>R-1711</td>
<td>2.35</td>
<td>2.48</td>
<td>1.89</td>
</tr>
<tr>
<td>R-1713</td>
<td>2.07</td>
<td>2.62</td>
<td>2.57</td>
</tr>
<tr>
<td>R-1682</td>
<td>2.04</td>
<td>2.32</td>
<td>1.54</td>
</tr>
<tr>
<td>Mean</td>
<td>2.09</td>
<td>2.40</td>
<td>1.92</td>
</tr>
<tr>
<td>$\pm$SEM</td>
<td>0.09</td>
<td>0.10</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Difference  -2.3%  +2.1%  +6.7%
Figure 5. Effects of chronic THC treatment on DA D₂/D₃ receptor availability as measured with [¹¹C]-raclopride. PET scans occurred after four weeks of chronic THC treatment. Different shaped symbols represent individual subjects while open symbols represent control subjects and filled symbols represent THC-treated subjects. Two monkeys (R-1710, R-1711) served as both control and THC-treated subjects.
DISCUSSION

The main goal of the present study was to examine the effects of THC on multiple cognitive domains in nonhuman primates. Two sets of studies were conducted: examination of the acute effects of THC before and during chronic THC treatment and examination of the effects of THC 22 hrs after daily, 12 week, chronic treatment as a model of residual drug effects, which reflect the period after direct intoxicating effects have subsided. Indeed, studies in humans and nonhuman primates have confirmed the direct effects of THC are absent at the time of behavioral assessment used presently (Hollister et al., 1981; Ginsberg et al., 2014). Acutely, THC dose-dependently decreased working memory (DMS performance) and attentional set-shifting (ID/ED performance). During chronic treatment, differential tolerance developed to impairments in discrimination-based learning but not working memory when the cognitive demand was high. When cognitive performance was assessed 22 hours after THC administration during daily treatment, impairments were specific to working memory. Tolerance developed to the deficits in working memory, but an increase in THC dose resulted the reemergence of working memory impairments. Working memory deficits recovered within 2 weeks of abstinence. In an effort to examine potential mechanisms of action mediating the differential effects of THC on cognition, dopamine D2/D3 receptor availability was examined and found not to be different compared to control monkeys.

Tolerance to the acute effects of cannabinoids following a period of chronic cannabinoid exposure can differ depending on the behavioral outcome measured (Lichtman and Martin, 2005). As such, studies in humans suggest that chronic THC exposure can produce differential tolerance to the acute effects of THC on cognition by comparing groups of frequent vs. infrequent cannabis users. For instance, compared to infrequent users, studies showed that frequent users were less impaired to the acute effects of THC on measures of critical thinking, divided attention, reaction time, verbal
learning, and memory (D'Souza et al., 2008; Ramaekers et al, 2009). However, these studies depended on the use of a group design, therefore, the extent to which the drug history was actually a factor is unclear without knowing the effects of acute THC on cognitive performance prior to the initiation of frequent marijuana use. By using a within-subjects design, the present study systematically showed that tolerance developed to THC-induced cognitive impairments in working memory and discrimination learning. These data may be particularly relevant for determining levels of THC that reflect impairment for driving and other tasks and suggest that cannabis use history is a corresponding factor that should be considered.

With regard to the residual effects of chronic THC exposure on cognition, results from studies in humans that assessed cognitive performance within a similar time frame are inconsistent with the present results. For instance, several studies in recently abstinent (6-36 hr) adult cannabis users showed no differences in working memory performance compared to infrequent or non-users (Pope and Yurgelun-Todd, 1996; Solowij et al., 2002; Kanayama et al., 2004; Whitlow et al., 2004; Fisk and Montgomery, 2008). Moreover, studies assessing the residual effects of cannabis use on inhibition and cognitive flexibility are more mixed with some studies showing impairments (Pope and Yurgelun-Todd, 1996; Solowig et al., 2002) and others that do not (Whitlow et al., 2004; Gruber and Yurgelun-Todd, 2005; Herman et al., 2007); associative learning in recently abstinent adolescent and adult users has been shown to be unaffected (Harvey et al., 2007; Fisk and Montgomery, 2008; Jager et al., 2010), which is consistent to the present findings.

One possibility for discrepant findings may be due to the daily amount of THC consumed across studies. Although participants in the previously mentioned studies used marijuana on a near-daily basis per month, the number of marijuana cigarettes smoked per day is often not reported, which makes it difficult to discern THC-induced
effects across studies. It is also possible that tolerance or certain deficits emerge after longer periods of chronic THC exposure than what was presently studied. For instance, Solowij (2002) found that the severity of cognitive deficits after at least 12 hrs of abstinence in frequent cannabis users was correlated with years of use. Furthermore, inconsistent results between studies could be a reflection of differential cognitive demand across the tasks employed. This notion is supported by several studies in humans that have examined the effects of chronic cannabis use on working memory. For example, in one study, adolescent regular cannabis users demonstrated worse working memory after 3 and 13 days of abstinence compared to control subjects during a challenging task that required active manipulation of items (Hanson et al., 2010), but not after 8 days during a simpler matching task (Jager et al., 2010). Similarly, Jacobsen et al. (2007) reported worse verbal working memory in the n-back test in abstinent adolescent cannabis users relative to control subjects but only during high memory load. Thus, it may be reasoned that the DMS task had the highest cognitive demand compared to the other tasks used in the present study, thus making it the most sensitive to impairment during chronic THC treatment. Future studies examining the effects of THC on cognition should incorporate varying degrees of complexity into tasks that assess different cognitive domains, which would eliminate potential ceiling effects and make assessments more task-specific and not just related to general behavioral complexity.

Another explanation for the specificity of chronic THC to impair working memory in the present study may be the result of regional differences in brain CB1 receptor function related to this domain of executive function. Electrophysiological and lesion studies demonstrate that working memory is preferentially activated by the ventrolateral prefrontal cortex (Jones and Miskin, 1978; Miskin and Manning, 1978; Wilson et al., 1993), while discrimination reversal learning and set-shifting are preferentially
associated with activation of the orbitofrontal cortex and dorsolateral prefrontal cortex, respectively (Dias et al., 1996a, b). Thus, it is likely that working memory impairments during chronic THC treatment were a reflection of greater dysregulation of CB1 receptor dynamics (e.g. downregulation/densensitization) or signaling in regions that mediate working memory such as the ventrolateral prefrontal cortex.

Also related to the residual effects of chronic THC treatment on cognition in the present study, previous studies in rhesus monkeys showed that indications of withdrawal (e.g., yawning, anorexia, irritability, headshaking, onset of cannabinoid receptor antagonist discriminative stimulus effects) can be observed within 24-48 hr of discontinuation of chronic THC treatment (Deneau and Kaymakcalan, 1971; Beardsley et al., 1986; Stewart and McMahon et al., 2011), thus raising the question of whether cognitive deficits were related to withdrawal as opposed to pharmacological-induced changes to brain function that mediate such behavior. Although cognitive assessment in the present study occurred at a similar time frame as withdrawal symptoms detected in previous studies, no overt physical signs of withdrawal were noted throughout the study. This was mostly likely due to the once-a-day administration of a lower daily dose of THC compared to continuous infusion of higher daily doses used in previous studies (e.g. 1.0 mg/kg/12hr by Stewart and McMahon, 2010 or 0.05-0.17 mg/kg/hr by Beardsley et al., 1986) shown to produce physical withdrawal symptoms. Nevertheless, it cannot be completely discounted that cognitive impairment was the result of THC withdrawal considering this may have been the only measure sensitive enough to detect such effects under the present treatment regimen. Regardless of whether impairments were the result of physical withdrawal or pharmacokinetics, rates of food-maintained responding and numbers of omissions in five of six monkeys were not altered during the course or after discontinuation of chronic treatment, which suggests that cognitive impairment was not the result of a non-selective disruption in operant responding.
The present study did not find any differences in striatal D$_2$/D$_3$ receptor availability measured by PET and [${}^{11}$C]-raclopride in monkeys chronically treated with THC compared to control subjects, even though deficits in working memory performance at the same time point were apparent. These findings are consistent with studies in humans also showing no differences in baseline D$_2$/D$_3$ receptor availability between marijuana abusers and healthy controls (Stokes et al., 2012; Urban et al., 2012; Albrect et al., 2013; Volkow et al., 2014) and suggest that THC-induced cognitive impairment was not directly related to striatal DA D$_2$/D$_3$ receptor function, despite the fact that the DA D$_2$/D$_3$ system plays a prominent role in modulating prefrontal cortical function (Groman and Jentsch, 2012).

One of the two monkeys (R-1710) scanned both before and during chronic THC treatment showed a large reduction in D$_2$/D$_3$ receptor availability in the dorsal striatum (Cd: -19.6%, Pt: -14.2%) during chronic THC treatment. Interestingly, this monkey was also the most sensitive to the residual rate-decreasing effects of chronic THC treatment on food-reinforced responding in which reduced rates of responding did not fully recover to baseline levels until 6 weeks after discontinuation of daily THC treatment. These findings suggest that there may be an inverse relationship between behavioral sensitivity to THC and THC-induced changes to D$_2$/D$_3$ receptor availability. These findings could also imply that higher daily doses of THC may be required to produce reductions in D$_2$/D$_3$ receptor availability. Despite showing no changes in D$_2$/D$_3$ receptor availability in the striatum, recent PET studies have shown reduced amphetamine-elicited striatal dopamine release in cannabis abusers (Volkow et al., 2014; Van Giessen et al., 2016). These results suggest that striatal dysfunction is a component of cannabis abuse, albeit to a lesser extent perhaps than other drugs of abuse, and may have implications in THC-induced cognitive impairment.
In summary, the present study demonstrated that chronic THC exposure produced persistent and selective impairment to aspects of working memory by using a longitudinal, within-subject study design in nonhuman primates. These data suggest that cognitive impairments in cannabis dependent patients, particularly those involving working memory, may not be the result of innate differences in cognitive function, but rather a vulnerable, pharmacological-induced target that can be harnessed for therapeutic value.
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CHAPTER IV
DISCUSSION

Recent epidemiological data report that nearly 3 of every 10 marijuana users, approximately 6.8 million Americans, have a diagnosis of marijuana use disorder (Hasin et al., 2015); however, there is currently no FDA-approved pharmacotherapy to treat cannabis dependence and abuse and behavioral modification strategies are only modestly effective (Nordstrom and Levin, 2007). Thus, more basic research is needed in order to improve our understanding of the neurobiological and behavioral mechanisms underlying the effects of THC, the main psychoactive component of marijuana. A better understanding of these variables will aid in the development of new treatments for maintaining abstinence in patients with cannabis use disorder. Preclinical studies with other drugs of abuse demonstrate that a multitude of factors govern their contingent delivery such as dose, behavioral history, drug history, and schedule of reinforcement. These findings have been instrumental in shaping the current understanding of drug abuse as a disease process and for guiding more effective treatment strategies. In this regard, a major obstacle preventing such progress for cannabis abuse has been the difficulty in demonstrating the reinforcing effects of THC in self-administration (SA) animal models. Thus, the work described in this dissertation contributed to furthering our understanding of the behavioral mechanisms involved in the reinforcing effects of THC that has implications for 1) potential risk factors that may predict vulnerability to cannabis abuse, 2) the variables that control cannabis use in humans that could be amenable to therapeutic intervention and 3) parametric manipulations that can be used to advance the cannabinoid self-administration methodology in preclinical animal models.

In addition to the reinforcing effects, a better understanding of the neurocognitive effects of THC will also aid in the improvement of treatment strategies for cannabis dependent patients. For instance, long-term marijuana use has been associated with
deficits in executive function across multiple domains integral in facilitating behavioral modification including behavioral flexibility and memory (Bolla et al., 2002; Pope and Yurgelun-Todd, 1995, 1996; Solowij et al., 1995, 2002). Moreover, cognitive function at the time of treatment initiation has correlated with treatment success (Aharonovich et al., 2003, 2006, 2008; Carroll et al., 2011). As a result, cognitive enhancement has been proposed as a strategy to improve behavioral modification practices to treat substance abuse, including cannabis use disorder (Sofuoglu et al., 2013). However, the exact nature and time course of THC-induced deficits to executive function are unclear (Crean et al., 2011; Crane et al., 2013). Through the use of a longitudinal, within-subjects study design in nonhuman primates, the second major contribution of the work described in this dissertation provided insight on the specificity and temporal nature of chronic THC exposure on cognitive function. This information can be used to direct more targeted therapies aimed at enhancing cognitive function in patients with cannabis use disorder.

These results, taken together, provide a better understanding of not only how THC exerts its control over behavior, but also the resulting consequences of chronic exposure, namely on cognitive functioning.

**Cannabinoid self-administration in nonhuman primates**

Reliable and persistent SA in experimental animals has been demonstrated for virtually every drug that is abused by humans including psychostimulants, opiates, alcohol, and nicotine. Thus, the SA procedure has strong face validity to the human condition. However, the one drug class that has functioned as a false negative in the drug self-administration procedure by a majority of laboratories is the cannabinoids. It is important to establish the conditions for demonstrating the reinforcing effects of cannabinoids through the SA procedure for multiple reasons. First, the SA procedure provides a baseline of drug-reinforced behavior that can be used to better understand
the environmental, biological, and pharmacological determinants of the reinforcing effects of drugs. This information can be useful for understanding both the factors that render one more susceptible to the abuse-related effects of THC as well as those that can be used to mitigate the reinforcing effects of THC in order to facilitate abstinence.

Secondly, development of cannabinoid SA will be important because of its utility to screen novel cannabinoid compounds for abuse liability and their potential scheduling as controlled substances by the DEA (see Ator and Griffiths, 2003; Carter and Griffiths, 2009). As such, there is an increasing interest for the use of cannabis and other cannabinoids for medicinal purposes to relieve symptoms associated with glaucoma, nausea in patients undergoing chemotherapy, AIDS-associated anorexia, chronic pain, inflammation, multiple sclerosis, and epilepsy (Hill, 2015). However, the abuse liability of THC and other cannabinoids raises particular concerns for long-term use by vulnerable populations. In this regard, the preclinical SA model as an abuse liability assessment will be an important component for their future development and ultimate utility of novel cannabinoid compounds in the clinical context.

As described in Chapter 1, currently, successful efforts at demonstrating the reinforcing effects of THC and other cannabinoids by the SA procedure is limited to one laboratory in the world using squirrel monkeys and parameters not previously employed in rhesus monkeys or rodents including schedule of reinforcement, a broader range of doses, faster injection speed, and a clear drug solution rather than a drug in suspension (Tanda et al., 2000). However, the extension of cannabinoid SA procedures to rhesus monkeys is highly desirable because this species is phylogenetically closer to humans than squirrel monkeys, and would aid in the most accurate translation of results. It is also important to extend the characterization of cannabinoid SA to other species in addition to squirrel monkeys and the use of rhesus monkeys will allow for the systematic manipulation of environmental (e.g., schedule of reinforcement) and pharmacological
(e.g., chronic treatment) variables using a within-subjects, longitudinal design over years. Such information will allow for a better understanding of the critical variables necessary to establish cannabinoid SA that may extend to other species, including rodent models.

However, it is currently unknown whether previous unsuccessful attempts in rhesus monkeys were due to methodology or a direct species difference between squirrel and rhesus monkeys. To address this, in Chapter 2, THC was made available to rhesus monkeys under similar SA methodological conditions as used in the successful demonstrations in squirrel monkeys, including dose range, schedule of reinforcement, and vehicle preparation. Moreover, the reinforcing effects of THC were compared to reinforcing effects of the synthetic bicyclic cannabinoid analog, CP 55,940, a high efficacy CB$_1$/CB$_2$ agonist, which allowed for a better understanding of the role of pharmacological efficacy at the CB receptor in SA, such as differences in potency and magnitude of response. We hypothesized that the reinforcing effects of the synthetic cannabinoid receptor agonist CP55,940 and THC will be established by utilizing methods that have shown success for demonstrating the reinforcing effects of cannabinoids in squirrel monkeys.

The results from these studies indicated that CP55,940, but not THC, functioned as a reinforcer in a subset (3 of 8) of rhesus monkeys when studied under similar experimental conditions that have been used to demonstrate cannabinoid reinforcement in squirrel monkeys. Notably, the doses of CP55,940 that had reinforcing effects were considerably lower than the doses tested in the only other study assessing the reinforcing effects of this compound in rhesus monkeys, which was unsuccessful (Mansbach et al., 1994). As described below, one goal from this initial assessment was to determine differences in biomarkers related to the three monkeys that showed CP55,940 reinforcement versus the five that did not.
The results from this part of the dissertation have several important implications. First, these experiments demonstrated, for the first time, reinforcing effects of a cannabinoid receptor agonist (i.e. CP55,940) without the use of induction procedures such as food deprivation, forced automatic injections, or the development of physical dependence. Although reinforcing effects were not achieved in all subjects, a surprising discovery was the dose range that was required for the subjects that did demonstrate reinforcing effects. These data can be especially useful for comparing the potency of CP55,940 reinforcing effects to other behavioral effects of CP55,940 in nonhuman primates. For example, the discriminative stimulus effects of i.v. CP55,940 in rhesus monkeys occurred at a dose of 0.003 mg/kg (McMahon, 2006), which is 75 - 2,400-fold higher than the unit doses of CP55,940 that had reinforcing effects in the 3 monkeys in the present study. The enormous differences in potency between the reinforcing and discriminative stimulus effects of CP55,940 remain an intriguing area for future investigation.

Moreover, the SA data generated with CP55,940 provide an important foundation that future studies can build on, especially with regard to the range of doses that may be used as a starting point to demonstrate reinforcing effects of this compound. Although CP55,940 SA may have limited utility as a screening procedure for the assessment of cannabinoid abuse liability (since the reinforcing effects of THC, as a positive control, were not also achieved in the same subjects that had reinforcing effects of CP55,940), it would still provide valuable information on the reinforcing effects that may be extended to other synthetic cannabinoids, which are becoming increasingly abused (Rosenbaum et al., 2012; Johnson et al., 2013; Nelson et al., 2014).

Furthermore, these studies have important implications because they allow for a direct comparison of THC SA between squirrel monkeys and rhesus monkeys. The fact that THC reinforcement was not obtained in rhesus monkeys using the same schedule
of reinforcement, dose range, and vehicle used in THC SA studies with squirrel monkeys suggest that inherent species differences may be the biggest determinant regarding the expression of the reinforcing effects of THC under these conditions. Interestingly, other atypical reinforcers such as nicotine and response-contingent shock presentation also seem to be more effective reinforcers in squirrel monkeys compared to other species of nonhuman primates (e.g., rhesus monkeys); however, the potency and efficacy of drugs such as cocaine, methamphetamine, and heroin to maintain behavior are similar between Old- (e.g., rhesus monkey) and New- (e.g., squirrel monkey) World primate species. Therefore, to address the factors underlying the qualitative differences in the reinforcing effects of cannabinoids across species, future studies should systematically investigate the behavioral, physiological, and pharmacokinetic effects of THC between species, using squirrel monkeys as a positive control. These data will provide valuable information regarding the factors that may correlate with the reinforcing effects of cannabinoids that can then be accounted for by future cannabinoid SA studies in order to optimize the model for more widespread use.

**Behavioral and Physiological phenotypes underlying the reinforcing effects of cannabinoids**

Another important contribution of the work presented in this dissertation was the characterization of behavioral and physiological phenotypes underlying the individual differences in cannabinoid SA. The characterization of such factors are important for predicting drug effects and for understanding what renders certain individuals more vulnerable to the abuse-related effects of marijuana. Ultimately, this information can be used to generate more targeted treatment and prevention strategies. In addition, a better understanding of the individual differences in cannabinoid SA will be helpful for optimizing the SA model for more widespread use in laboratory animals. One such
variable that was investigated in Chapter 2 was the relationship between behavioral sensitivity to CP55,940 and THC and the ability of those drugs to function as reinforcers. It was hypothesized that individual subjects less sensitive to the rate-decreasing effects of CP55,940 and THC will have greater rates of SA of both drugs. To test this hypothesis, the potency of both drugs to decrease food-maintained responding was correlated with rates of SA in each subject.

It was found that there was an inverse relationship between the reinforcing effects of CP55,940 and its behavioral-disruptive effects (Chapter 2, figure 4). Such an analysis was precluded for THC considering it did not function as a reinforcer in any subject. When administered non-contingently, CP55,940 was ~ 15-times more potent than THC; when studied in SA, doses of THC overlapped this potency relationship in order to assure a broad range of doses that encompassed no behavioral effects to doses that non-contingently decreased operant behavior. Nevertheless, these data indicate a novel variable (i.e., sensitivity to rate-decreasing effects) involved in the reinforcing effects of cannabinoids that has never been empirically validated. The significance of this finding indicates that 1) this is a factor that may predispose certain cannabis users to subsequent abuse and dependence and 2) this is a factor could be accounted for in animal models in order to obtain cannabinoid SA at greater levels and in more subjects.

Studies in humans have indicated other behavioral and biological phenotypes that may predict vulnerability to cannabis abuse and dependence involving personality, subjective effects, and genetic factors (Hurd et al., 2014). For instance, genes encoding the cannabinoid type 1 receptor CB1R (CNR1) and fatty acid amide hydrolase (FAAH), an enzyme responsible for the hydrolysis of the endogenous cannabinoid anandamide, have been shown to modulate risk for developing cannabis dependence (Agrawal et al., 2009; Hopfer et al., 2006; Tyndale et al., 2007). Other studies indicate that personality
traits such as neuroticism, anxiety, and depression are risk factors associated with cannabis dependence. Moreover, in population based cohorts, increased risk of cannabis dependence has been associated with self-reported subjective effects of cannabis use such that positive effects (e.g., feeling energetic, creative, euphoric, and sociable) were associated with cannabis abuse and dependence and negative effects (e.g., feeling confused, paranoid, and anxious) were associated with shorter duration and lower frequency of cannabis use (Lyons et al., 1997; Grant et al., 2005; Scherrer et al., 2009). Although informative, the interpretation of these findings are complicated due to retrospective design, reliance on self-report, polysubstance use, and uncontrolled exposure to THC. Thus, the present results represent the first empirical evidence in animal models indicating a relationship between behavioral sensitivity and subsequent SA of cannabinoids. These findings emphasize the role of individual differences involved in the reinforcing effects of THC and suggest that the behavioral sensitivity to THC, during the early stages of drug use, may form part of a liability for future cannabis use and abuse.

Another phenotype that was found to predict the cannabinoid reinforcing effects, specifically CP55,940, was the individual subject’s drug history. In particular, it was found that three of the four monkeys that had an extensive methamphetamine SA history were the subjects in whom CP55,940 functioned as a reinforcer. Previous studies in squirrel monkeys have demonstrated that a drug SA history was not necessary for acquiring robust levels of THC self-administration in that drug-naïve subjects with no drug SA experience acquired and maintained THC SA at similar, if not higher, levels than subjects with an extensive history of self-administering cocaine (Justinova et al., 2003). The studies in Chapter 2 also found no relationship between previous SA history and the acquisition of THC SA (only one subject with a history of methamphetamine SA acquired THC SA but only after daily treatment), therefore, the relationship between
methamphetamine SA history and the acquisition of cannabinoid SA appears to be specific to CP 55,940. The mechanisms underlying this relationship and why it is specific to the synthetic full cannabinoid receptor agonist (i.e., CP55,940), however, remains to be determined. One possibility may involve specific neurobiological adaptations induced by a chronic methamphetamine SA history and the involvement of those changes in enhancing the reinforcing effects of a full agonist at the cannabinoid receptor. For instance, we have previously reported that monkeys with a methamphetamine SA history demonstrated increased sensitivity to the unconditioned behavioral effects of the dopamine D₃ receptor preferring agonist quinpirole relative to drug-naïve monkeys or those that had a cocaine SA history (Martelle et al., 2014). It has also been shown that THC and synthetic CB₁ agonists potentiate some behavioral effects (e.g. hyperlocomotion) of quinpirole (Gorriti et al., 2005; Moreno et al., 2005). Taken together, one might hypothesize that sensitivity at the D₃ receptor is positively related to cannabinoid SA. However, the present study found no relationship between the unconditioned behavioral effects of quinpirole and CP55,940 SA, which suggests another methamphetamine-induced neuroadaptation could be involved. A better understanding of the relationship between previous drug history and qualitative and quantitative differences in cannabinoid SA may provide novel insight into the mechanisms involved in mediating the reinforcing effects of cannabinoids.

**Effects of attenuating the rate-decreasing effects of THC on its reinforcing effects**

In order to extend this observation to factors that may be inhibiting the expression of THC’s reinforcing effects in experimental animals, monkeys were treated daily with THC in order to develop tolerance to the rate-decreasing effects of non-contingent THC on food-maintained responding. After daily treatment, THC SA was reassessed and was found to function as a reinforcer in 3 subjects. Thus, despite daily
THC treatment in all eight monkeys, our hypothesis was confirmed in only three monkeys. Interestingly, in one monkey in whom THC functioned as a reinforcer, tolerance did not develop to the rate-decreasing effects prior to reassessment suggesting that another factor resulting from the daily treatment was responsible for uncovering the reinforcing effects of THC in these subjects. At present, there are no substantial observational differences in those three monkeys compared to the other five, but identification of potential biomarkers, including perhaps changes in CB1 receptor density and function (as measured by PET) may provide further insight into vulnerable phenotypes.

Furthermore, THC self-administration was also assessed in a separate group of monkeys responding under a second-order schedule of reinforcement that used response-contingent conditioned stimuli to maintain behavior by relatively few drug injections spaced between long intervals of time. It was hypothesized that reducing the frequency of drug injection would allow time for rate-decreasing effects to dissipate while the presence of conditioned stimuli within those intervals would help to maintain high rates of behavior leading to subsequent injections. Under these schedule contingencies, THC functioned as a reinforcer in two of four monkeys.

The failure of THC to function as a reinforcer in the majority of subjects following the development of tolerance to rate-decreasing effects and by accounting for those effects through the use of a second-order schedule does not support our hypothesis and suggest other factors may be involved, as described in Chapter 2. This is not to say, however, that rate-decreasing effects have no role in influencing the reinforcing effects of cannabinoids. The fact that reinforcing effects were achieved in a subset of subjects suggest that attenuating the rate-decreasing effects is a feasible strategy but the methods used in Chapter 2 may not have been the most appropriate. The following
sections will discuss other strategies to further investigate the reinforcing effects of cannabinoids in the context of accounting for their simultaneous rate-decreasing effects.

**Other schedules of reinforcement**

Similar to animal models, the reinforcing effects of THC were also surprisingly not reliably demonstrated in early laboratory studies in human subjects. For instance, early studies showed that cigarettes containing THC were not self-administered more than placebo under free-access conditions (Zacny and de Wit, 1991; Kelly et al., 1994a, b). Early studies also could not demonstrate a systematic relationship between THC dose and the amount self-administered (Chait, 1989), despite dose-related differences in ratings of intoxication or “high.” However, one approach that was later implemented to detect dose-related reinforcing effects of THC in human subjects was the use of a discrete-trials choice procedure in which subjects were offered a choice between obtaining a particular dose of THC and obtaining a non-drug alternative (Mendelson and Mello, 1984; Chait and Zacny, 1992; Chait and Burke, 1994; Kelley et al., 1994; Haney et al., 1997; Hart et al., 2005). Under these conditions, a subject first sampled a dose of THC and its matching placebo, and on a subsequent occasion the subject chose which of the two to self-administer. The frequency with which the active drug was chosen over placebo provided an index of the reinforcing efficacy of the drug.

Chait and Zacny (1992) provided some of the first evidence of THC’s reinforcing effects using a choice procedure. Here, a two-trial choice procedure was used in which experienced marijuana smokers sampled active drug and placebo during separate sessions and then on two subsequent sessions were required to choose between these two options. They found that ten of ten participants chose to self-administer smoked THC (2.3%) on both trials and ten of eleven participants chose to self-administer oral THC (10-15 mg based their subjective responses during practice sessions) on both
trials, i.e., they chose active drug on ∼90% of choice opportunities. Further, Haney et al. (1997) compared choice between marijuana cigarettes (0, 2.2, 3.9% THC w/w) and snack food where multiple choices were available each day, i.e., each concentration of THC was made available on 4 days with each day consisting of one sample trial and six choice trials. It was found that the participants selected cigarettes containing THC on more than 75% of choice opportunities compared to only about 40% when placebo cigarettes were available. Other studies have also shown that marijuana cigarettes with a greater THC content are consistently preferred to cigarettes containing lower amount of THC, even when the choice between them was not mutually exclusive (Haney et al., 1997; Kelly et al., 1997; Ward et al., 1997).

Despite the success of choice procedures to detect the reinforcing effects of THC in human subjects, there are surprisingly no published reports attempting to use this method to demonstrate the reinforcing effects of THC in laboratory animals. However, many of the features inherent to choice procedures for demonstrating the reinforcing effects of drugs may be particularly well suited for cannabinoids. For instance, one of the major advantages of choice procedures is that this method permits the dissociation of reinforcing effects from reinforcement-independent rate-decreasing effects because the primary dependent variable is behavioral allocation (e.g., percent drug choice vs. percent choice of a non-drug alternative) as opposed to rate of responding. As a result, the dose-response curve under a choice schedule is represented as a monotonic increase in frequency of drug choice with increases in dose because the high doses that typically constitute the descending limb of dose-response function under single response procedures are still chosen relative to a non-drug reinforcer (e.g. food pellets).

Therefore, this measure reinforcement (allocation of behavior away from a non-drug
reinforcer) may be more applicable for demonstrating the reinforcing effects of cannabinoids because it would not be sensitive to the rate-decreasing effects of cannabinoids that are hypothesized to inhibit rates of responding under single response procedures.

Figure 1 shows a hypothetical dose-response curve for THC self-administration under a concurrent THC-food choice procedure. Similar to what was demonstrated in Chapter 2 when THC was initially made available for self-administration under a FR 10 schedule, it is likely that the number of injections of THC will not be very high relative to vehicle across a similar dose range. However, at a certain dose of THC (e.g. 3.0 µg/kg), there may be a reallocation of behavior where THC is now exclusively chosen over food reinforcement, despite low rates of responding for those injections. If this were the case, the reinforcing effects of THC would have been effectively demonstrated due to its relative reinforcing efficacy compared to a non-drug alternative. Such an outcome would suggest that perhaps we need to shift our perspective of viewing the reinforcing effects of THC in terms of numbers of injections and rates of responding but instead to THC choice in the presence of an alternative reinforcer.
Using other drugs that are “atypical reinforcers” in laboratory animals as models for developing cannabinoid self-administration

Going forward, it will be of utmost importance to also study the methodology used for demonstrating the reinforcing effects of other drugs that are relatively less efficacious reinforcers in preclinical models in order to better understand potential pharmacological and behavioral factors that may also apply to demonstrating the reinforcing effects of cannabinoids. The reinforcing effects of nicotine in nonhuman primates, for example, have been historically difficult to demonstrate and are largely
dependent on several specific factors including the use intermittent schedules of
reinforcement, drug history, and repeated presentation of associated stimuli (Goldberg
and Henningfield, 1988). Similar to the hypothesis tested in Chapter 2 for THC, it has
been suggested that one of the reasons behind the difficulty in demonstrating the
reinforcing effects of nicotine in laboratory animals is because nicotine also has
prominent aversive effects that may be masking its reinforcing effects. For example,
studies in squirrel monkeys have shown that response-produced injections of the same
doses of nicotine (0.01 – 0.03 mg/kg/injection) that maintain behavior under a fixed-
interval (FI) or second-order schedules of reinforcement can also function effectively to
suppress food-maintained responding in squirrel monkeys under FR reinforcement
schedules (Goldberg and Spealman, 1982). Further, it has been shown that squirrel
monkeys will respond at high rates to postpone the scheduled delivery of the same
doses of nicotine (0.03 and 0.056 mg/kg/injection) that function as a positive reinforcer in
other studies with squirrel monkeys (Spealman and Goldberg, 1983). These examples
demonstrate that nicotine can function as both a reinforcer or punisher depending on the
conditions governing its delivery. Therefore, the experimental manipulations that have
been successful in producing the reinforcing effects of nicotine may have also been
successful in limiting its aversive effects to a degree that the reinforcing effects could be
unmasked. In this regard, studies of nicotine self-administration should serve as an
excellent resource for studying the reinforcing effects of cannabinoids as similar
behavioral mechanisms between these drug classes could be determining their ability to
maintain behavior. The following section will describe the conditions that are most
effective for demonstrating the reinforcing effects of nicotine and will also include a
discussion on how these principles could be applied to uncovering the reinforcing effects
of cannabinoids.
Early nicotine self-administration studies using FR 1 reinforcement schedules resulted in very low rates of responding (0.008 – 0.005 responses/second) maintained by nicotine injections (Deneau and Inoki, 1967; Hanson et al., 1979; Lang et al., 1977). Although these rates of nicotine-maintained responding were higher than rates of responding maintained by saline, they were relatively insensitive to changes in dose and pretreatments to the nicotine antagonist mecamylamine. Thus, it could not be discounted that nicotine-maintained responding in these studies were due to nonspecific rate-increasing effects. In contrast, Goldberg and Spealman (1978) and Spealman and Goldberg (1982) reported much higher rates of nicotine-maintained responding under an FI 5-min schedule of reinforcement with a one-minute timeout. During acquisition, 0.03 mg/kg nicotine was made available for self-administration and the FI value was gradually increased to 5 minutes. Another contingency during acquisition was also added in which nicotine was automatically injected if a response did not occur within 2 minutes after the interval elapsed. Once rates of responding were stable, the automatic injections were discontinued and nicotine subsequently maintained stable rates of responding at approximately 0.1 responses/second. Ator and Griffiths (1983) also studied nicotine self-administration under an FI 5-minute, one-minute timeout schedule of reinforcement in baboons. However, in contrast to the studies in squirrel monkeys by Goldberg and Spealman, much lower rates (0.007 – 0.02 responses/second) of nicotine-maintained responding were achieved, which were only marginally distinguishable from rates of responding maintained by saline. The major methodological differences between these two studies, despite species differences, was that Ator and Griffiths (1983) made available a lower dose (0.01 mg/kg/injection) of nicotine during acquisition and the use of non-contingent nicotine injections during the acquisition period were not employed.

Taken together, these studies demonstrated that interval-based schedules by themselves were no more effective than low FR schedules of reinforcement for
demonstrating the reinforcing effects of nicotine in nonhuman primates. However, the combination of a fixed-interval schedule with the implementation of forced, automatic nicotine injections and a relatively higher training dose initially made available for self-administration may have been the optimal conditions for demonstrating high levels of nicotine-maintained responding. Moreover, several other sets of studies in squirrel monkeys have demonstrated that nicotine can maintain even higher rates of responding when studied under a second-order schedule of reinforcement (Goldberg et al., 1981; Spealman and Goldberg, 1982). Under these conditions, completion of an FR 10 during a 1-, 2-, or 5-minute interval produced a brief visual stimulus that had been paired with a nicotine injection; the first ratio completed after the interval timed out then produced both the visual stimulus and an i.v. injection of nicotine, which was followed by a 1- or 3-minute timeout. Peak rates of responding maintained by nicotine ranged from 0.8 – 1.7 responses/second at a dose of 0.03 mg/kg/injection. Thus, the peak rates of nicotine-maintained responding under the second-order contingencies were 8 - 17-fold higher compared to what was produced under the fixed-interval schedule. Considering the interval between consecutive nicotine injections was similar between the two schedules, the higher overall rates produced by the second-order schedule were most likely attributable to the presence of contingent conditioned stimuli.

Overall, these findings with nicotine SA have several important implications for studying the reinforcing effects of cannabinoids in preclinical animal models. First of all, a critical determinant of robust levels of nicotine-maintained responding was due to the use of intermittent schedules of reinforcement where the frequency of reinforcement was independent of the rate of responding. This is in contrast to ratio-based schedules of reinforcement where the frequency of reinforcement is contingent on the rate of responding. Importantly, nicotine SA under a second-order schedule, which employed the use of conditioned stimuli, produced the most robust level of nicotine-maintained
behavior. These findings with nicotine parallel the findings in Chapter 2 (Figure 6) of this dissertation in which the reinforcing effects of THC were demonstrated in some subjects under a second-order schedule but in no subjects under an FR reinforcement schedule (prior to chronic treatment). Considering both nicotine and THC have well documented negative effects, the effectiveness of interval-based schedules of reinforcement, particularly second-order schedules, may be due to their ability to limit these negative effects that are probably more apparent from fast accumulation of drug intake. Moreover, as the FI nicotine SA studies by Spealman and Goldberg (1982) and Ator and Griffiths (1983) showed, the use of automatic, forced injections to facilitate acquisition of SA may be a feature that can be added to future THC SA studies to engender even greater levels of drug-maintained behavior. Although the principle behind this method is similar to what was tested in Chapter 2 (i.e., developing tolerance to the rate-decreasing effects in order to increase levels of SA), forced injections during the session could be a more effective way to test this hypothesis.

The methods used to establish ethanol as an oral reinforcer in experimental animals can also be used to recognize potential behavioral and environmental factors that may determine the reinforcing effects of cannabinoids. Similar to cannabinoids, ethanol does not readily initiate and maintain behavior in experimental animals despite its clear reinforcing effects in humans. For instance, when ethanol is initially presented to monkeys, they typically only drink low levels of ethanol and water is generally preferred over even a small concentration (5%) of ethanol (Mello, 1973; Meisch, 1977). This discrepancy has been attributed to a variety of factors including the taste of alcohol, the delay between consumption and the onset of pharmacological effects, the volume needed for a pharmacological effect, and negative effects of alcohol. As a result, initiation procedures are commonly used to establish the reinforcing effects of ethanol in laboratory animals. One of the most common ways to induce robust oral ethanol
consumption is through a schedule-induction procedure. Schedule-induced drinking involves the intermittently scheduled presentation of one reinforcer that results in an increase in the reinforcing effects of another stimulus concurrently available in the environment. This procedure was first described by Falk (1961) in which he observed in rats that the intermittent delivery of food pellets at fixed intervals of time was accompanied by the consumption of large quantities of water as a form of adjunctive behavior. Rats were maintained at 80 percent of their free-feeding weight and would consume approximately one half of their total weight in water within a few hours while food pellets were presented intermittently. Subsequent experiments showed that explanations for this phenomenon were not attributable to thirst, as pre-session water loading did not reduce polydipsic drinking (Falk, 1969), or by adventitious reinforcement (Falk, 1964).

Since then, schedule-induced polydipsia has been widely used to address the problem of demonstrating the reinforcing effects of ethanol in preclinical animal models. The application of this procedure to induce high levels of ethanol drinking typically involves food restricting an animal and then making a certain concentration of ethanol available with small quantities of food delivered intermittently either contingently under an FI or schedule or non-contingently under a fixed-time (FT) contingency. For instance, in rodents, the optimal conditions typically involve an FT 2-min schedule of pellet presentation to induce drinking of 5% v/v ethanol (Falk, 1966; Falk et al., 1972; Samson and Falk, 1975). In cynomolgus monkeys, Shelton et al. (2001) determined that 4% (w/v) ethanol and FT 5-min was the optimal schedule of food pellet delivery to induce consumption of 1.0 g/kg ethanol. Other studies in monkeys have also demonstrated that schedule-induced ethanol consumption can be produced without food restriction (Grant and Johanson, 1988; Vivian et al., 2001). Furthermore, an important feature of this method is that high concentrations of ethanol can maintain behavior following the
discontinuation of the scheduled delivery of food pellets (Vivian et al., 2001; Grant et al., 2008).

Taken together, the effectiveness of schedule-induced procedures for demonstrating the reinforcing effects of ethanol may represent an environmental factor that is important for demonstrating the reinforcing effects of cannabinoids. Indeed, schedule induction has been used for intravenous SA of several drugs in rodents (Singer et al., 1982) as well as for nicotine in nonhuman primates (Slifer, 1983; Slifer and Balster, 1985). Only one study, however, has reported the use of a schedule induction procedure to study the reinforcing effects of THC (Takahasi and Singer, 1979). In this study, THC (6.25-50 µg/kg) and vehicle were made available to rats under an FR 1 schedule of reinforcement during 1-hour sessions while food pellets were concurrently delivered under an FT 1-min reinforcement schedule. This study included four groups of rats, in which THC SA was assessed both in the presence or absence of the schedule induction procedure and at either 80% reduced body weight or at free feeding weight. It was found that reinforcing effects of THC were achieved only in the food restricted animals tested on an FT 1-min schedule. The peak number of infusions, 15.14 ± 4.31, was maintained by 125 µg/kg, which was significantly different than the number of injections earned when vehicle was available during the FT 1-min schedule (~ 4 injections).

Although the Takahasi and Singer (1979) study was successful in demonstrating the initiation of THC’s reinforcing effects through the use of a schedule induction procedure, some limitations were also present. For instance, the reinforcing effects of THC were only assessed when the inducing schedule was in effect. However, future studies should also examine the maintenance of those reinforcing effects when scheduled pellet delivery is discontinued and the necessary conditions may need to be developed in order to do so. For instance, the schedule induced ethanol self-
administration literature indicates a bitonic function that describes the relationship between the FT interval of pellet delivery and the volume of ethanol consumed following schedule removal with maximal intake occurring at FT values of 2-3 minutes for rats and monkeys (see Ford, 2014). However, the optimal inter-pellet interval may be different for inducing cannabinoid SA and subsequent maintenance of reinforcing effects. Thus, future studies should pay particular attention to the relationship between the length of the interval between pellet delivery and the amount of THC self-administered. The optimization of such a procedural variable may be the difference between maintaining cannabinoid SA after the removal of the inducing schedule.

Another aspect of ethanol SA procedures, particularly in rodent models, that could apply to producing high levels of cannabinoid SA involves the frequency of drug access. For instance, compared to ethanol SA protocols using a continuous-access-drinking paradigm (i.e., 24 hrs/day, 7 days/week), intermittent access to ethanol SA (i.e., 24 hrs/day, 3 days/week: typically Monday, Wednesday, and Friday) in rodents yields considerably higher levels of ethanol intake (Wayner et al., 1972; Wise, 1973; Simms et al., 2008). The effectiveness of this procedure may in part reflect negative reinforcement to alleviate ethanol-induced symptoms of withdrawal. The potential effectiveness of intermittent access to increase the reinforcing effects of THC may also involve negative reinforcement, although probably unlikely considering the long half-life of THC attenuates profound physical withdrawal (Hollister, 1986) and the doses required to produce physical symptoms of withdrawal in nonhuman primates, for example (2.0 mg/kg/day; Stewart and McMahon, 2010), are unlikely to be self-administered in one daily session. Instead, intermittent access to THC SA may allow time for other, more-subtle drug effects to dissipate that may be inhibiting the expression of reinforcing effects. Future studies should test this hypothesis by designing THC SA studies that
intersperse 24 hr-48 hour abstinence periods in between SA sessions, instead of making the drug available for SA across consecutive daily sessions.

**Using drug discrimination to complement THC self-administration**

Until the cannabinoid SA procedure has been further refined to produce consistent, reproducible reinforcing effects across multiple subjects and species, the drug discrimination assay represents a valid alternative in terms of cannabinoid abuse liability assessment. The discriminative stimulus effects of a drug have been suggested to be analogous to human subjective effects (Schuster and Balster, 1977; Holtzman, 1985). Thus, although the drug discrimination procedure does not measure the reinforcing effects of drugs per se, drugs that share discriminative stimulus effects usually have similar reinforcing effects as a result of similar pharmacological mechanisms of action (Schuster and Johanson, 1988).

As such, the discriminative stimulus effects of THC have been produced in multiple experimental animal species and across several different laboratories (Tanda, 2016). Drug discrimination with cannabinoids is pharmacologically selective such that naturally occurring psychoactive cannabinoids fully substitute for THC and drugs from other classes do not. Thus, THC is a well-established training drug that can provide rapid feedback on the underlying pharmacological mechanisms and abuse liability of substituted novel cannabinoid compounds. For instance, there are a number of potential therapeutic applications of cannabinoids involving their analgesic, antiemetic, antiglaucoma, and anticonvulsive effects (Hill, 2015). However, a cannabinoid compound considered for these uses would be void of any clinical utility if there also exists abuse potential of that compound since THC is Schedule I. Therefore, the evaluation of a candidate compound to produce THC-like discriminative stimulus effects is an effective way to test for this, with the goal of developing one that either does not
substitute for THC or retains its therapeutic activity at doses below those that produce THC-like discriminative stimulus effects. Moreover, cannabinoid drug discrimination procedures can be useful for evaluating potential pharmacotherapies for cannabis abuse. For instance, drugs that block or attenuate the discriminative stimulus effects of THC might be considered for achieving abstinence or preventing relapse. On the other hand, drugs that partially substitute for THC-like discriminative stimulus effects might be considered as candidate agonist medications, similar to methadone for opioid dependence, that can reduce cannabis SA or alleviate withdrawal symptoms associated with relapse without too much concern for the abuse potential of the medication itself.

This is not to say, however, that drug discrimination should serve as a replacement for cannabinoid SA. Although there is good correlation between a drug’s subjective effects and its reinforcing effects, the SA procedure measures the reinforcing effects of a drug directly, along with all the other complexities that determine its ability to maintain behavior, which offers a more detailed perspective in terms of abuse liability screening and pharmacotherapy assessment. For instance, drug-induced changes in operant behavior can vary dramatically as a function of rates and patterns of behavior independent of the reinforcer that maintains behavior (Kelleher and Morse, 1968). Therefore, one can assume that even though a drug attenuates the discriminative stimulus effects of THC, the effects of that drug to also attenuate the reinforcing effects of THC may be more ambiguous depending on factors such as the contingencies for THC reinforcement or other prevailing environmental conditions. Similarly, a drug that substitutes for the THC stimulus may not necessarily produce reinforcing effects in the self-administration procedure also depending on the schedule contingencies and other trait/state variables. The point is that both drug discrimination and self-administration should be used hand-in-hand to gain information about the abuse-related effects of drugs of abuse from two different perspectives. For example, the optimal
pharmacotherapy may be one that reduces the reinforcing effects of THC, but not the discriminative stimulus effects - this drug profile would be indicative of good therapeutic efficacy without sacrificing medication compliance. Such an assessment could not be gained from only using one procedure. However, until cannabinoid SA is more developed, THC discrimination can provide useful information on cannabinoid pharmacology, the abuse potential of novel cannabinoid therapeutic agents, and potential pharmacotherapies to treat cannabis abuse.

Going forward, cannabinoid discrimination studies should also be utilized to better understand the pharmacological variables that may influence cannabinoid SA. For instance, a strength of the drug discrimination assay lies in its capacity to investigate not only the receptor mechanisms of a drug class, but also other stimulus effects of a drug such as time course of a drug (e.g. onset of behavioral effects, duration of action, etc.), which have been shown to significantly affect the SA of other drugs of abuse (Balster and Schuster, 1973; Stretch et al., 1976; Kato et al., 1987; Beardsley and Balster, 1994). THC, in particular, has a relatively delayed onset of its behavioral effects and extended elimination phase (Ohlson et al., 1980; Hollister et al., 1981). This time course of effects is significantly different compared to other drugs that are readily self-administered (e.g., cocaine, methamphetamine, opiates, etc.) so it should not be assumed that similar self-administration parameters will be effective for demonstrating the reinforcing effects of cannabinoids. Instead, a more careful examination of the pharmacological profile of cannabinoids needs to be taken into account and drug discrimination is one tool that can elucidate many of these factors. For instance, in a study of the discriminative stimulus effects of THC in rats, Prescott et al. (1992) showed that maximal THC-appropriate responding did not occur until 60 min after intramuscular injection. Furthermore, this study also showed that THC-appropriate responding persisted until 6 hours after initial administration. These results suggest, inasmuch as discriminative stimulus effects
predict reinforcing effects, that THC’s delayed onset and long duration of action could be preventing the expression of its reinforcing effects in studies that do not account for these variables. Specifically, the delayed onset in action of THC could be functioning to un Couple the behavioral effect of the drug from the response that produced it. On the other hand, THC’s long duration of action could be preventing persistent SA within a session either through satiation or decreased discriminability of additional drug injections. In order to investigate these possibilities, future SA studies should implement even longer intervals between drug injections than 10 minutes used during the second-order schedule in Chapter 2.

Moreover, THC discrimination time course studies in the context of developing the THC SA model should also be conducted with intravenous THC considering that is the route of administration most likely to be used for contingent delivery. Future studies should also attempt to train the discrimination of lower training doses of THC. For instance, 0.1 mg/kg THC is typically used as the training dose in most drug discrimination studies using nonhuman primates (McMahon 2006, 2011), which is considerably higher than the unit dose of THC (0.003 mg/kg) that has reinforcing effects in studies with squirrel monkeys and for the subjects in Chapter 2. Thus, the examination of the discriminative stimulus effects of lower doses of THC would provide a better understanding of the stimulus effects of doses of THC that are more likely to be self-administered as opposed to studying the effects of a single, bolus dose that is probably more of a reflection of the total intake during a self-administration session. However, the relationship between doses of a drug that produce reinforcing effects compared to those that produce discriminative stimulus effects should be interpreted cautiously. Previous studies in nonhuman primates that were trained to discriminate and self-administer the same drug demonstrated that, in general, the reinforcing effects of that drug were more potent than its discriminative stimulus effects (Hoffmeister, 1988; Ator, 2002; Martelle
and Nader, 2009). For example, each of these studies showed that there were certain
doses of a drug that did not engender drug-appropriate responding during drug
discrimination but functioned as a reinforcer during self-administration. Some of these
discrepancies may be due to the training dose used for discrimination as this factor has
been shown to influence the potency of the dose-response curve (e.g. Terry et al., 1994;
Mumford and Holtzman, 1991; Young et al., 1992). Nevertheless, drug discrimination
can serve is an excellent platform to investigate many of the pharmacological factors
that may be related the reinforcing effects of cannabinoids.

**Route of administration**

One possibility for unsuccessful attempts at establishing cannabinoid SA in
preclinical animals may involve the route of administration, which is typically delivered
intravenously in preclinical animal models of SA, whereas in humans, cannabinoids are
most commonly abused via pulmonary SA (i.e., inhalation). Studies have shown that the
pharmacokinetics and metabolism of THC following intravenous injection are essentially
the same as inhalation with both routes producing rapid attainment of peak plasma
concentrations and a rapid distribution phase at similar time courses (Hollister et al.,
1981). Moreover, both routes produced a similar time course and magnitude of self-
reported ratings of feeling “high” (Hollister et al., 1981). Thus, the pharmacokinetic
parameters of THC and its positive subjective effects (e.g., subjective “high”) do not
appear to be different between both routes of administration. However, studies have also
shown that many adverse effects are reported by subjects when administered
intravenous THC, as well as smoked THC, such as dysphoria, anxiety, paranoia, and
nausea (D’Souza et al., 2008; Carbuto et al., 2012). In one study directly comparing the
psychototropic effects of both routes in humans at doses that produced similar plasma
concentrations, the adverse effects of intravenous THC were much more pronounced
compared to pulmonary administration (Naef et al., 2004). For instance, intravenous administration produced more subjective reports of feeling irritated, anxious, tenseness and aggressiveness, confusion and disorientation, nausea, headache, difficulties breathing, and vertigo (Naef et al., 2004). Significantly lower amounts of the psychoactive metabolite 11-OH-THC were formed following pulmonary administration due to the absence of first-pass metabolism, which may partly explain the differences in psychototropic side effects compared to intravenous administration. Thus, the more pronounced adverse effects of intravenous THC may be overriding its reinforcing effects when delivered through this route of administration, which could be playing a role in preventing persistent THC SA in preclinical animal models. As a result, pulmonary administration may increase the likelihood of THC functioning as a reinforcer in animal models because the adverse effects that may be overriding the reinforcing effects are more limited. Indeed, the development of inhaled delivery systems of THC have been recently validated (Manwell et al., 2014; Nguyen et al., 2016); however, future studies should continue to develop these technologies and incorporate them into operant procedures to allow the study of factors mediating THC’s reinforcing effects.

**Interactions of THC with other cannabinoids**

Moving forward, it is important to consider that the *Cannabis sativa* plant contains hundreds of known compounds and at least 70 cannabinoids in addition to THC (Elsohly and Slade 2005). Although the present study provides no evidence of interactions between THC and other marijuana constituents, it does not preclude the possibility that such interactions may influence other mechanisms that are not reflected in the behavioral endpoints studied, such as possible activity at non-CB₁ sites or at CB₁ receptors that are part of neural circuits distinct from those involved with reinforcement or rate-decreasing effects. Cannabidiol (CBD), for instance, is another major
cannabinoid found in cannabis, and has opposing effects on CB<sub>1</sub> and CB<sub>2</sub> receptors compared to THC. Although hashish may contain equal parts CBD and THC (ElSohly et al., 2003; Hardwick and King, 2008; Potter et al., 2008), CBD is typically present in low concentrations in recreational cannabis (<0.1%), based on samples seized by law enforcement. There are multiple reports of CBD and THC interacting to modify each other’s behavioral effects with some studies showing enhancement (Karniol and Carlini, 1973; Takahashi and Karniol, 1975) or antagonism (Karniol and Carlini, 1973; Borgen and Davis, 1974; Karniol et al., 1974), depending on the endpoint of interest. In particular, antagonism was previously reported in rhesus monkeys performing operant conditioning and cognitive-based tasks (Brady and Balster, 1980; Wright et al., 2013). It has also been shown that CBD significantly enhanced the potency of THC to produce discriminate stimulus effects (McMahon, 2016), albeit at a dose 100 times greater than THC, which far exceeds the amount obtained from marijuana.

Furthermore, there is evidence that CBD may reduce anxiety or transient psychosis-like side-effects of THC observed in infrequent cannabis smokers or when administered alone to patients with anxiety or psychosis (Bhattacharyya et al., 2010; Crippa et al., 2011; Bergamaschi et al., 2011; Leweke et al., 2012; Niesink and van Laar, 2013). Older studies report that CBD changes the type of psychological reaction induced by THC in infrequent cannabis smokers, reducing their anxiety and thereby rendering THC more enjoyable (e.g., Karniol et al., 1974). Taken together, the addition of CBD to THC may be a potential strategy for enhancing the reinforcing effects of THC compared to just studying the drug by itself. The key will be finding an appropriate concentration of CBD in combination with THC that attenuates THC’s rate-decreasing effects without simultaneously cancelling out its reinforcing effects.
**Relationship between THC and polysubstance use**

Finally, one additional factor that deserves mention includes interactions of cannabis with other substances. For example, co-use of marijuana and alcohol increases risk for motor vehicle crashes (Hingson, et al., 2005), short- and long-term memory impairment (Pope & Yurgelun-Todd, 1996), psychological disorders (Grech et al., 2005; Hall 2009), and lower educational performance and attainment (Brook et al., 1999; Lynskey and Hall, 2000; Brook et al., 2011; Arria et al., 2013). Tobacco and marijuana use increases the risk for adverse respiratory and cardiovascular effects (Tashkin, 1990; Polen, et al., 1993; Zhang et al., 1999; Mittleman et al., 2001; Aryana and Williams, 2007) and increased susceptibility to cancer (Hashibe et al., 2005).

Additionally, we have shown that THC, in combination with cocaine, produced an increase in the relative reinforcing strength of cocaine compared to cocaine alone as assessed in a cocaine-food choice procedure (Figure 2A). In contrast to the low-efficacy partial agonist THC, the full CB receptor agonist CP55,940 shifted the cocaine dose-response curve to the right (Figure 2B), perhaps suggesting a novel treatment for cocaine abuse involving full agonists at CB receptors. A better understanding of the behavioral and neuropharmacological interactions of marijuana and other substances will have important implications for the treatment of co-abuse.
Conclusions from studies in Chapter 2

Across all conditions, cannabinoid agonists (i.e., CP55,940 or THC) functioned as a reinforcer in 7 of 13 monkeys studied (54%). Although our hypothesis that attenuating the rate-decreasing effects of THC would enhance its reinforcing effects was not supported considering reinforcement was not obtained in every subject, it should not go unnoticed that these experiments have provided the most successful demonstration of cannabinoid reinforcement in nonhuman primates in terms of number of subjects acquiring SA and level of SA, besides only the group at NIDA-IRP using squirrel monkeys (Tanda et al., 2000). Thus, these studies provide an important foundation for which future investigations to build on and highlight the presence of individual differences involved in the abuse-related effects of cannabinoids. Future research

Figure 2. Effects of THC (A) and CP55,940 (B) pretreatment on cocaine self-administration under a food-drug choice procedure in rhesus monkeys (n = 4). Both drugs were administered intravenously one minute before the session. Abscissae: Unit dose of cocaine available for self-administration in mg/kg/injection. Left ordinate: Percent cocaine choice relative to food reinforcement. Right ordinate: Percent food choice relative to cocaine reinforcement. Data represent mean of at least two determinations per dose of THC for each monkey. *, p < 0.05. Unpublished data from John, Martin, and Nader, 2016.
should focus, rather than disregard, these individual differences in order to identify behavioral, environmental, or biological determinants of cannabinoid self-administration that can be further targeted to optimize the model. As discussed earlier, recommendations for future studies examining the behavioral mechanisms underlying THC reinforcement might include the use of intermittent or choice schedules of reinforcement coupled with an initial induction procedure such as non-contingent drug exposure and/or fixed-time schedules of food-delivery.

**Cognitive effects of THC**

The other goal of the research within this dissertation was to characterize the effects of chronic THC treatment on cognitive performance, including the residual effects (i.e., 22 hours after daily administration during chronic treatment) and during several weeks of abstinence. Cognitive-enhancement is an emerging area for the treatment of cannabis use disorder (Sofuoglu et al., 2013); however, it is not clear what specific cognitive functions are most strongly related to improved treatment outcome. Much of this discrepancy comes from the inconsistent results in human studies examining the effects of long-term marijuana use on cognitive performance. For instance, the interpretation of many studies are complicated by several variables such as the heterogeneity of the participants, differences in drug histories and social variables, as well as concurrent use of other substances, all of which are difficult to experimentally control. In addition, studies in humans cannot distinguish whether marijuana abusers exhibit innate cognitive differences prior to initiating drug taking. Therefore, it is difficult to clearly establish a causal role of long-term THC exposure in producing cognitive deficits. However, the use of preclinical animal models can control for these variables in a systematic manner but have unfortunately been underutilized as it relates to longitudinal, within-subject assessments of THC on cognition.
In Chapter 3, the effects of chronic THC exposure on four cognitive domains were examined: working memory, discrimination learning, reversal learning, and attentional set-shifting. Each of these cognitive domains are mediated by a different neurobiological substrate and are integral to facilitating the behavioral change necessary for positive treatment response (Rezapour et al., 2016). However, studies in chronic, heavy cannabis users show that impairment within these domains is equivocal depending on the time since last use (Crean et al. 2011; Crane et al., 2013). Thus, in order to better clarify the specificity and temporal ordering of effects, the studies in Chapter 3 examined the acute effects of THC on cognition before and during chronic THC treatment (1.0-2.0 mg/kg/day, s.c.) and the residual effects of daily THC treatment (i.e., cognition assessed 22 hrs after daily THC administration) as well as the persistence of effects during abstinence.

It was found that the acute effects of THC were task-specific and produced deficits to working memory in a delay-dependent manner, as well as deficits in compound discrimination learning, and extradimensional set-shifting; tolerance developed to those effects during chronic treatment. Furthermore, during chronic treatment, THC produced persistent impairments 22 hours after administration to working memory when the cognitive load was high but not to discrimination and reversal learning or attentional set-shifting performance. Impairments to working memory recovered within 2 weeks of abstinence. The implications of these findings will be discussed in the following sections.

**Task Specificity**

One of the major findings of this work involves the task specificity on which THC produced both its acute and residual effects. This finding is an important contribution to the overall theme of this dissertation involving the behavioral mechanisms of THC - the
notion that the behavioral effects of THC cannot be predicted based on its inherent pharmacological properties. Consistent with the findings in Chapter 3, other studies in nonhuman primates have also found task-specific effects of THC on cognitive performance. For instance, Winsauer et al. (1999) found that THC impaired the acquisition of a novel stimulus-response chain but not the expression of an already-learned chain of response. Furthermore, Kangas et al. (2016) found that short-term memory assessed under the DMS task was most vulnerable to impairment by acute THC pretreatment in squirrel monkeys, followed by discrimination reversal while simple discrimination learning was not affected. Moreover, in the only other study in nonhuman primates that has assessed the residual effects of THC on cognition, Verrico et al. (2014) found that adolescent rhesus monkeys were impaired by THC (0.2-0.3 mg/kg, i.v.; 23 hours after drug administration) on a spatial working memory task (i.e. location-recall) but not an object working memory task (i.e. object-recall). Overall, these findings suggest cognitive performance may be most susceptible to impairment by THC when the retention of information over time or the flexible alteration of responses based on ongoing within-trial contingencies are required.

As discussed in Chapter 3, the basis for THC to affect cognition in a task-specific manner is unclear but may be related to CB₁ receptor function in different brain regions that mediate such tasks. For example, the control of discrimination reversal performance appears to be highly localized in the orbital prefrontal cortex (Chudasama, 2011), whereas object working memory is thought to be controlled by dorsolateral prefrontal systems (Hampson et al., 2009) and hippocampal mechanisms (Eichenbaum et al., 1992) while spatial working memory likely involves more frontal-temporal circuitry (Browning and Gaffan, 2008). As such, CB₁ receptors are widely expressed throughout the brain (Freund et al., 2003); however, recent studies have indicated that CB₁ receptor dynamics (e.g., desensitization and downregulation) differ across brain regions (Lazenka
et al., 2013). For example, Sim-Selley et al. (2006) treated mice chronically with THC or the CB₁ full agonist WIN55,212-2 and showed that after cessation of treatment, cannabinoid-induced decreases in CB₁ receptor function persisted for two weeks longer in the hippocampus compared to the striatum. These findings are consistent with recent data from positron emission tomography imaging studies in human marijuana users that showed slower recovery of CB₁ receptors in hippocampus than in other brain regions (Hirvonen et al., 2012). Considering the significant hippocampal mechanism involved in working memory performance, these data support the task specificity of THC to impair DMS performance in the present study and in others.

Alternatively, it may be that differences in the relative potencies of THC across tasks most directly reflect differences in the difficulty of the tasks, i.e., more difficult tasks may be more vulnerable to cannabigeric drug action, resulting in higher potency and larger effects. For example, Branch et al. (1980) showed that squirrel monkeys trained to emit a 5-key response sequence were more sensitive to acute doses of THC than when emitting a 2-key sequence. In this regard, performances under all tasks used in the present study required discriminative behavior but differed in complexity. That is, simple discrimination trials are simple stimulus-response associations, compound discrimination adds complexity with the presentation of another stimulus dimension, and discrimination reversal, intradimensional/extradimensional shift is made even more complex due to the unsignaled shift in contingency. DMS is perhaps the most complex task among the ones used here because accuracy depends on a conditional discrimination (i.e., S+ and S- contingencies are conditional on the previously presented sample) and, moreover, a delay between the presentation of sample and comparison stimuli. Thus, although the extent to which the potency of THC depends on task complexity may be difficult to determine a priori, the present results support the view that this factor at least contributes to the behavioral mechanisms of THC cognition-related studies.
Implications for chronic THC exposure to produce tolerance to its acute cognitive effects

These findings for both the acute and persistent effects of THC on cognitive function have several important implications. First, as touched on briefly in Chapter 3, the differential acute effects of THC on cognitive performance as a function of chronic THC exposure may bear an important implication for establishing thresholds of THC that can be used to determine impairment for driving or other tasks. Ideally, a crash risk ratio of THC would be calculated similar to alcohol in which there is a direct comparison between the level of THC in the blood and impairment for driving. To this end, Colorado recently enacted a law stipulating a threshold of 5 ng/ml of THC in blood for driving under the influence, based on other published reports (Hartman and Huestis, 2013; Walker et al., 2013). Another study indicated that blood levels below 10 ng/ml were not associated with increased accident risk, and recommended a threshold of 7–10 ng/ml for driving under the influence (Grotenhermen et al., 2007).

However, based on recent research, what these thresholds don’t take into account is the level of tolerance chronic THC exposure produces to the acute intoxicating effects of THC. For instance, one study reported that in occasional (≤1 time/week) cannabis users, smoked cannabis (500 μg/kg) produced significant impairment of tracking performance, divided attention, and inhibitory control but only modestly affected inhibitory control in heavy (>4 days/week) users (Ramaekers et al., 2009). Similarly, in a sample of heavy cannabis users (>4 days/week for more than 2 years), smoked cannabis (700 μg/kg) produced no impairment on tasks measuring tracking errors or reaction time, and only produced modest effects on a divided attention task (Schwope et al., 2012). Levels of THC and its major metabolites were measured in that study, and remained above the legal threshold of 5.0 ng/ml for 2–4 h after cannabis
consumption. These findings are also consistent with a study in nonhuman primates showing that monkeys with a chronic history of THC (1.0 mg/kg/12h for several years) compared with monkeys intermittently exposed to THC (0.1 mg/kg every 3-4 days) resulted in greater tolerance to the hypothermic and rate-decreasing effects following administration of 3.2 mg/kg THC (Ginsburg et al., 2014). THC blood levels were also measured in this study, however, no differences in blood levels of THC or its metabolites were present between the groups. These findings are consistent with others showing that tolerance does not develop to the pharmacokinetics of THC despite tolerance to its behavioral effects. Interestingly, although maximal THC blood levels occurred 20 minutes after injection for both groups (457.0 and 355.2 ng/ml for the intermittent and chronic groups, respectively), maximal effects of THC to decrease response rates and to produce hypothermia in the intermittent group did not occur until 120 minutes after injection, after THC blood levels had substantially decreased. By 12 hours and 24 hours, effects on temperature and response rate was not different from control levels although blood THC levels for both groups were approximately 20 ng/ml, well above the legal threshold of 5 ng/ml. Taken together, these studies indicate that blood levels of THC do not necessarily reflect the behavioral effects of the drug when drug history is not taken into account.

The findings in Chapter 3 further add to this literature by using a within-subject study design to show that chronic THC exposure produced tolerance to the acute cognitive impairing effects of THC. Moreover, tolerance to the cognitive effects of THC such as working memory, that involves attentional processes and the active processing and manipulation of information, may more closely parallel the demanding task of driving a vehicle. Although the experiments in Chapter 3 were not designed to directly address a blood threshold that generally reflects impairment to the acute effects of THC, these findings add to the body of literature demonstrating that a chronic history of THC results
in tolerance to its acute behavioral effects, even when that behavior is complex and under strict stimulus control. It can be speculated based on previous studies in humans, however, that THC blood levels were above the legal threshold of 5 ng/ml following acute administration. For instance, i.v. administration of 5.0 mg THC (approximately 0.07 mg/kg) in a group of frequent cannabis users produced an average blood level of 20 ng/ml at 30 minutes post administration, the same time that cognitive assessment occurred in Chapter 3. Furthermore, THC levels declined rapidly to about 3 ng/ml at 4 hours post injection. These data offer a good comparison because the same route of administration was used and the dose range is within the range (0.03-0.056 mg/kg) to which tolerance developed during chronic treatment in Chapter 3. Taken together, these data suggest that the current threshold of 5 ng/ml may be too low in many cases and that drug history needs to be a critical component for determining impairment.

Pre-existing vs. drug-induced cognitive impairment

The studies in Chapter 3 also have implications regarding the extent to which cognitive impairments are caused by chronic drug use itself. For instance, while there may be a causal relationship between chronic drug use and cognitive impairment, individuals with innate cognitive deficits may also be more vulnerable to initiating drug use and/or becoming drug dependent (Wagner et al., 2012). In order to address this issue, the studies in Chapter 3 used a within-subjects study design that allowed for the assessment of THC on cognition from initial to chronic exposure and during abstinence. Thus, it was demonstrated that the residual effects of THC to impair cognitive function were a consequence of the drug exposure itself as opposed to innate differences in baseline cognitive functioning. Such an assessment is more difficult in studies with human subjects as group designs comparing marijuana users with healthy control subjects are typically used. As a result, there is always the looming question of whether
cognitive deficits associated with chronic cannabis use predated the initiation of drug use and were instead, a factor that rendered individuals more vulnerable to chronic use in the first place. Some studies, however, compare heavy cannabis users against a control group of infrequent users rather than a control group of nonusers (e.g., Pope et al., 1996; Whitlow et al., 2004). To some extent, this approach controls for some confounding factors associated with using a healthy control group; however, with each subject serving as its own control, the present results unequivocally demonstrate that THC-induced cognitive deficits were from the drug itself and not a result of baseline differences between control groups.

The implications of these findings bears particular importance in terms of the feasibility of cognitive enhancement as a treatment approach. For instance, if innate cognitive impairment contributed to the initiation of cannabis use then cognitive rehabilitation as a treatment strategy may be more challenging because of a potential ceiling effect in cognitive capabilities. However, the present study shows that cognitive deficits may be produced by the drug itself and in that case, there may be more room for cognitive enhancement to be an effective treatment option because baseline function would be the endpoint as opposed to the starting point.

Of course, in a treatment context it may be very difficult to discern whether cognitive impairments were from the drug itself or a trait variable that contributed to the initiation of compulsive use. One strategy would be to compare cognitive performance at the time of treatment to scholastic measures that have been taken prior to the initiation of use such as test scores, IQ tests, behavioral assessments etc. in order to obtain some sort of within subject comparison. However, the practicality of having access to the necessary resources for such an approach is uncertain. Nevertheless, if cognitive enhancement increases general functioning, it has the potential to influence substance use outcomes regardless of whether substance abuse arises secondary to cognitive
impairment or vice versa. In reality, cognitive impairments in most cannabis users are likely a combination of pre-existing vulnerability factors, long-term drug effects, withdrawal effects, and acute drug effects; however, a better understanding of these factors will provide clinicians with a better understanding of when a treatment works and will allow clinicians to maximize the effectiveness of cognition-based treatments.

**Working memory as a treatment target for cannabis use disorder**

Furthermore, the results in Chapter 3 show that working memory was the only cognitive domain examined that showed impairment 22 hrs after THC administration during chronic treatment with deficits persisting until two weeks of abstinence. Therefore, these findings suggest that working memory, in particular, may represent a neurocognitive domain that may be predictive of treatment outcome or one that is particularly sensitive to therapeutic interventions in cannabis dependent patients.

Several studies have suggested that working memory function is linked to inhibitory control in that high working memory demand or impairments to working memory function may facilitate drug craving or relapse during abstinence (Chambers et al., 2009). For instance, Hester and Garavan (2004) showed that under high working memory demand, cocaine users have reduced response inhibition, as measured by a Go-No/Go task, compared to healthy controls. Poorer working memory performance has also been shown to be predictive of relapse in abstinent smokers (Patterson et al., 2010). Surprisingly, the impact of cognitive function on treatment outcomes for cannabis use disorder has not been well-studied. The only such study to date evaluated cognitive functioning in 20 marijuana-dependent patients at treatment entry and its relation to retention and drug use outcome in motivational enhancement therapy and cognitive behavioral therapy (Aharanovich et al. 2008). It was found that cognitive impairments in abstract reasoning, spatial processing, and processing accuracy were predictive of poor
treatment retention but not rates of abstinence. There was no association between performance on a memory task and treatment retention or outcome, although this study was limited by small sample size and it is unclear whether the particular task was sensitive enough to detect impairments. However, given the evidence of working memory and other cognitive functions to predict the success of behavioral treatment programs in cocaine and cigarette users, future studies are likely to find cognitive functions that are predictive of treatment outcomes in marijuana users as well. The selection of validated cognitive tests that include high levels of cognitive demand that would be most sensitive to detecting impairments (i.e. in order to avoid false negatives) will be a crucial step.

In addition to the prognostic significance of working memory, the basic research in Chapter 3 also has implications for working memory to serve as a cognitive domain that could be targeted to improve the effectiveness of treatment in cannabis abusers. Working memory has been recognized as one dimension of inhibitory control so in theory, therapeutic approaches aimed at improving working memory could also help restore impairment within inhibitory control functions, which are important for overriding pre-potent responses, such as drug-taking behavior in response to drug cues (Sarter et al., 2006). A growing amount of empirical evidence also supports working memory as a treatment target for addiction (Klingberg, 2010). For instance, it has been shown that working-memory training in alcoholics reduced alcohol use for more than one month after training (Houben et al., 2011). Consistent with the association between working memory and inhibitory control, it was also found that training had an effect on alcohol use for those with strong automatic preferences for alcohol, indicating working memory training may increase control over the underlying automatic processes that drive alcohol use. Furthermore, Bickel et al. (2011) showed that working memory training improved delay discounting amongst stimulant users, suggesting that such training may lead to a
greater ability to attend to future consequences and thus reduce impulsive decision making that could contribute to relapse during abstinence. Cognitive rehabilitation approaches aimed at targeting working memory specifically have not yet been evaluated among patients with cannabis use disorder. However, the results in Chapter 3 demonstrating that chronic THC exposure produces persistent effects within this domain combined with promising evidence in alcoholics and stimulant abusers indicate that the incorporation of working memory training into behavioral treatments should be explored.

**THC-induced alteration to brain activity independent of cognitive performance**

Although working memory was the only cognitive domain impaired during chronic THC treatment, the lack of impairment during performance within the other domains (i.e., discrimination learning, behavioral flexibility, executive function) should not be completely disregarded in terms of clinical significance. First, as discussed previously, the complexity of the tasks assessing these domains of cognition may not have been sensitive enough to detect impairments during chronic THC treatment. Second, functional neuroimaging studies showed that despite similar performance on certain cognitive measures, the underlying neural mechanisms mediating such performance may still be affected (Batalla et al., 2013). These findings indicate that neuroadaptations may occur during chronic THC exposure in which additional brain regions are recruited in order to maintain normal cognitive functioning and compensate for the THC-induced deficits.

As it relates to neuroadaptations in human marijuana users, Chang et al. (2006) used fMRI to compare a visual attention task in current and abstinent cannabis users with healthy controls. Despite all groups showing normal task performance, both active and abstinent chronic cannabis users demonstrated decreased activation in the right prefrontal, medial and dorsal parietal cortices and medial cerebellar regions but greater
activation in several smaller regions throughout the frontal, posterior parietal, occipital and cerebellum. The authors suggested that their findings may reflect a compensatory role for these regions in mitigating the effects of abnormal attentional and visual processing following chronic cannabis exposure. Furthermore, in a group of abstinent cannabis users, Tapert et al. (2007) compared the activation pattern on a go/no-go task during fMRI with seventeen healthy subjects. Despite similar level of task performance, cannabis users showed greater activation during inhibitory trials in the right dorsolateral prefrontal, bilateral medial frontal, bilateral inferior and superior parietal lobules and right occipital gyrus compared to the healthy subjects. During the non-inhibitory trials, differences were located in right prefrontal, insular and parietal cortices, with cannabis users showing greater activation in these areas compared to the controls. Similar findings have also been observed in adults (Hester et al., 2009), and suggest a greater neurocognitive effort during the task in cannabis users, even after the abstinence period.

In this regard, the brain seems able to achieve some degree of reorganization during chronic THC exposure by activating brain regions not usually needed to perform the cognitive task in response to an impaired ability of the normally engaged task network. Thus, it is possible that despite control-level performance during tasks other than DMS in Chapter 3, THC-induced alterations in the primary regions controlling performance on these tasks were present but were compensated for by the recruitment of additional regions. However, it is not clear how long such compensatory mechanisms can maintain normal functioning and whether impairments over time may become apparent. The impact of these subtle brain alterations on treatment outcome remains to be determined.

**Age of onset**

It is also important to acknowledge that the cognition-related studies in Chapter 3
were conducted in adult monkeys. Cannabis use, however, is often initiated in adolescence, and recent data show that a decline in perceived risk of marijuana has been accompanied by a simultaneous increase in rates of use among adolescents (Johnston et al., 2014). Adolescence is a critical period of neurodevelopment, especially for frontal regions (Giedd et al., 1999; Gogtay et al., 2004), with synaptic pruning and refinement leading to the development of complex cognitive domains such as working memory, executive functions, decision making and emotional/motivational processes (Yurglun-Todd, 2007). Thus, chronic cannabis use during this time may disrupt normal neuromaturation (Bava and Tapert, 2010), and in turn, lead to long-lasting impairments in neurocognitive functioning more so than if use was initiated as an adult.

Indeed, studies that have examined the relationship between age of onset of regular cannabis use and cognitive function have reported long-lasting alterations on a range of tasks. Ehrenreich and colleagues (1999) reported that early onset cannabis use (prior to age 16) predicted significantly longer reaction times on a task of visual scanning and attention, while those who began use at age 16 or later performed similarly to controls. Pope and colleagues (2003) analyzed cognitive data from long-term heavy cannabis users following a 28-day period of abstinence, and compared early onset users (prior to age 17) with late onset users (use at age 17 or later). After adjusting for age, gender, ethnicity and family background, late onset cannabis users were no different from control subjects; however, early onset users demonstrated reduced performance relative to control subjects on measures of verbal learning, fluency and overall verbal intelligence quotient. Consistent with these data with human subjects, repeated THC administration in adolescent rats was shown to produce more profound effects on working memory using a novel object test than in adult rats (Cha et al., 2006; Quinn et al., 2008).

Given these data, it is possible that if the same study as in Chapter 3 were
conducted in adolescent monkeys, dramatically different results would have been
generated. In fact, the largest reduction in working memory performance during chronic
THC treatment in Chapter 3 was approximately 25% of baseline performance. Although
this was a significant decrease that persisted for several weeks, a 25% reduction in
baseline performance does not appear all that substantial. This effect also occurred at
the long delay, which was individualized to produce < 60% accuracy (chance accuracy
was 25-33% depending on the number of distractors), therefore, it is possible that a floor
effect prevented any further decreases in performance. Nevertheless, it would be
hypothesized that persistent THC-induced cognitive impairment would be even greater
in adolescent monkeys (e.g., impairments on other tasks and/or further decrease in DMS
percent accuracy at the long delay as well as at other delays) than what was seen in
adult monkeys in the present study. These results would support current evidence
showing the marijuana-induced cognitive impairments are generally more persistent
when use was initiated during adolescent compared to adulthood (Chang et al. 2006;
Jager et al. 2006; Fisk and Montgomery 2008; Becker et al. 2010; Grant et al. 2011;
Gruber et al. 2012;). Future studies should continue to characterize the specific domains
of cognitive function that are impaired from chronic THC exposure in adolescents and
the time course of effects. The persistence of cognitive impairments in adolescent
cannabis users supports the need to address these impairments early in treatment and
continually during abstinence.

**Relationships between behavioral endpoints**

One advantage of this dissertation was that the studies across both chapters
were conducted within-subject, that is, the majority of the same monkeys used for the
experiments in Chapter 2 were also used for the ones in Chapter 3. Thus, it was
possible to compare the potency and efficacy of THC across various behavioral
endpoints (i.e., food-maintained responding, hypothermia, self-administration, and cognitive performance) in order to provide some insight on the behavioral mechanisms of THC. Although differences in THC pretreatment times and the inability to calculate an ED$_{50}$ for some effects made potency comparisons across tasks difficult, examination of the smallest dose of THC that produced a significant deficit (or the highest dose that had no effect) offers some indication of relative potency. In this regard, the rank order of THC’s potency in the behavioral measures within this dissertation was: working memory < FR10 food-maintained responding = extradimensional set shifting < compound discrimination < simple discrimination = reversal learning = intradimensional set-shifting = hypothermia.

The fact that THC was more potent to decrease rates of responding under the FR 10 schedule of food presentation than to decrease body temperature is consistent with the pattern of effects seen in other studies with rodents (e.g. De Vry et al., 2004; Singh et al., 2011). It should be noted that for some subjects, no hypothermic effects were seen up to doses that abolished all operant behavior therefore higher doses were not tested because that was not the primary endpoint. However, it is likely that hypothermic effects would have become apparent if higher doses were tested, based on previous studies (McMahon, 2005; Taffe, 2012). Thus, the difference in potency for producing the two effects might reflect differential binding to multiple receptor subtypes that differentially mediate the two effects. However, the CB$_1$ receptor antagonist rimonabant antagonized the rate-decreasing and hypothermic effects of THC (Giuffrida and McMahon 2010; Singh et al., 2011), albeit antagonism of rate-decreasing effects was less orderly and of a lesser magnitude than antagonism of hypothermia. Less orderly antagonism of rate-decreasing effects could reflect actions of rimonabant at a subset of brain cannabinoid receptors that are more prominently involved in rate-
decreasing effects than hypothermic effects; however, mechanisms underlying the effects of rimonabant are not known.

Interestingly, a study in rhesus monkeys reported the opposite relationship than observed in this dissertation regarding the potency of THC to alter these behavioral effects, i.e., THC was more potent to decrease body temperature than to decrease food-maintained responding (McMahon, 2005). In that study, however, an FR10, FR10 multiple schedule of food presentation and stimulus-shock termination was used to study the effects of THC on operant behavior. Therefore, additional variables including schedule of reinforcement, environmental context and the maintaining event should also be considered with regard to the potency of THC to produce rate-decreasing effects on operant behavior. Moreover, rate dependency has also been shown to be a determinant of THC’s potency to affect operant behavior (McMillan, 1977; Brady and Balster, 1980). Supporting this notion, the overall rate of responding in the rhesus monkeys used for this dissertation under the FR10 schedule of food presentation was negatively related to the potency of THC to produce rate-decreasing effects under that schedule (Figure 3).
The higher potency of THC to disrupt working memory performance compared to hypothermia and other unconditioned behaviors is consistent with at least one other study in rodents that included these endpoints (Varvel et al., 2001). As discussed above, if both THC-induced deficits to cognition and the other unconditioned behaviors are mediated by CB$_1$ receptors, then the differences in potency might be due to a difference in the magnitude of CB$_1$ receptor stimulation (i.e., the efficacy requirement) required for the effects (e.g., hypothermia > working memory). This would imply that the receptor

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**Figure 3.** Rate-dependent effects of THC on food-maintained responding ($r = -0.85$, $p < 0.05$). Abscissa: ED$_{50}$ (mg/kg) of THC to decrease food-maintained responding under an FR10 schedule of food presentation. Ordinate: Mean overall rate of responding (responses/second) under an FR10 schedule of food presentation. *Unpublished data from John, Martin, and Nader, 2016.*
reserve level is higher for the CB\textsubscript{1} receptor population in the brain mediating the effects of cannabinoids on working memory than for the CB\textsubscript{1} receptor population mediating the hypothermic, rate-decreasing effects, and other cognitive domains assessed in this study. Collectively, these data indicate that the potency of THC is determined by multiple factors potentially involving pharmacological and behavioral mechanisms. In particular, these potency relationships across various behavioral endpoints have implications for establishing legal limits for THC in the blood when driving; however, it remains to be determined which behavioral endpoints are most predictive of driving performance. For instance, if the effects of THC on working memory performance are most predictive of its effects on driving, then a relatively lower THC blood limit might be the most suitable. On the other hand, if compound discrimination and reversal learning are more predictive, lower blood levels could result in false positives with regard to driving impairment. Moreover, as discussed previously, the potency of THC to impair behavioral performance differs as function of drug history, which should be considered along with differential levels of tolerance between behavioral endpoints.

**Conclusions**

Overall, the research conducted in this dissertation provided insight on the behavioral mechanisms underlying the reinforcing, unconditioned, and cognitive effects of THC. These data indicate that the behavioral effects of THC cannot be predicted solely on its pharmacological mechanism of action. Instead, a complex set of environmental variables must be considered including behavioral sensitivity, drug history, and the contingencies governing behavior. The adoption of a more comprehensive perspective that includes the interaction of environmental variables with neuropharmacology will be vital for the development of the most effective approaches to treat substance abuse problems.
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SCHOLASTIC VITAE

NAME: WILLIAM SCOTT JOHN

CURRENT TITLE: Ph.D. Candidate

ADDRESS: Department of Physiology & Pharmacology
Wake Forest University School of Medicine
Medical Center Boulevard, NRC 547
Winston-Salem, NC 27157-1083
Telephone: (336) 713-7166
Telefax: (336) 713-7180
E-mail: wjohn@wakehealth.edu

PERSONAL INFORMATION:

BIRTHPLACE: Mount Airy, NC, USA
DATE OF BIRTH: February 11, 1988
MARITAL STATUS: Married: Joanna Elizabeth John

EDUCATION:

2012 - Present
(July, 2016 graduation)
Wake Forest University School of Medicine
Ph.D. Integrative Physiology & Pharmacology
Dissertation: Behavioral mechanisms of Δ9-THC in nonhuman primates
Advisor: Michael A. Nader, Ph.D.

2006 - 2010
University of North Carolina at Greensboro
B.S. Biology
Graduated with Departmental Honors

RESEARCH EXPERIENCE:

Jan. 2013 – Present
Graduate Research
Michael A. Nader, Ph.D.
Department of Physiology & Pharmacology
Wake Forest University School of Medicine

Graduate Research Rotation
Christos Constantinidis, Ph.D.
Department of Neurobiology & Anatomy
Wake Forest University School of Medicine

2011 – 2012
Laboratory Technician II
Larry Liao, Ph.D.
Duke Human Vaccine Institute
Duke University Medical Center
2010 – 2011 Research Assistant
Keith Erikson, Ph.D.
Department of Nutrition
University of North Carolina at Greensboro

PROFESSIONAL MEMBERSHIPS:

2014 – Present International Study Group Investigating Drugs as Reinforcers (ISGIDAR)

2014-Present Western North Carolina Chapter of the Society for Neuroscience

2013-Present The American Society for Pharmacology and Experimental Therapeutics (ASPET)

2008-2010 TriBeta National Biological Honor Society

HONORS, AWARDS, AND FELLOWSHIPS:

2016 NIDA Director’s Travel Award to The College on Problems of Drug Dependence (CPDD)

2016 Ruth L. Kirschstein National Research Service Award (NIDA)

2016 Predoctoral Travel Award
Behavioral Pharmacology Society Meeting

2016 Graduate Student Travel Award
ASPET Annual Meeting at Experimental Biology

2015 Graduate Student Travel Award, Behavior, Biology and Chemistry: Translational Research in Addiction Annual Meeting

2014 Institutional Training Fellowship (T32) Collaborative Research on Addiction initiative at NIH (CRAN)

2014 William L. Woolverton Memorial ISGIDAR Travel Award

2014, 2015 Alumni Student Travel Award
WFU Graduate School of Arts and Sciences
2014  Graduate Student Travel Award, Behavior, Biology and Chemistry: Translational Research in Addiction Annual Meeting

2012-2013  Graduate Fellowship Department of Integrative Physiology and Pharmacology, Wake Forest University School of Medicine

2008-2010  TriBeta National Biological Honor Society

2006-2010  NCAA Div. I Scholarship Student Athlete

INSTITUTIONAL SERVICE:

2014-Present  Matching Matriculates and Return Students (MMARS) program

GUEST REVIEWER:

Addiction Biology
Biological Psychiatry
Drug and Alcohol Dependence
Human Brain Mapping
Journal of Neuroscience Methods
Neuropsychopharmacology
Pharmacology, Biochemistry and Behavior

TEACHING EXPERIENCE:

Wake Forest School of Medicine
Fall 2015  Animal Models of Human Disease (COMD 706)

BIBLIOGRAPHY:

JOURNAL ARTICLES:


John, WS and Nader, MA (2016). Effects of acute ethanol administration on cocaine self-administration under a second-order schedule of reinforcement. Under review.


BOOK CHAPTERS:


ABSTRACTS:


abuse. *Wake Forest University Neuroscience Graduate School Research Day.* Winston-Salem, NC.


**INVITED PRESENTATIONS:**


John WS (2013). Effects of dopamine D₂/D₃ receptor ligands on cocaine and methamphetamine self-administration in monkeys. Student Seminar Series, Dept. Physiology & Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC.

**CURRENT RESEARCH SUPPORT:**

F31 DA041825 03/1/16 – Present

Mentor: Michael Nader, Ph.D.

Ruth L. Kirschstein National Research Service Award (NIDA)

**Behavioral and pharmacological determinants of cannabinoid reinforcement and effects on cognition in rhesus monkeys**

*Role: PI*
COMPLETED RESEARCH SUPPORT:

T32 AA007565 07/01/2014 – 2/29/16
Mentor: Michael A. Nader, Ph.D.
Institutional Training Fellowship (T32), Collaborative Research on Addiction Initiative at NIH (CRAN) supplement (NIAAA)
Ethanol/cocaine interactions in nonhuman primates
Role: Graduate student