ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor, Dr. David Roberts. Throughout my five years in his lab he has provided me with guidance, support and a swift ‘kick in the butt’ when needed. I’ve enjoyed out many scientific conversations which have generally started on one topic and ended with an entire new project that needs to be completed posthaste. I am leaving with a true sense of confidence in my skills and a feeling of independence that I credit to him.

I would also like to thank the members of my committee, Dr.’s Michelle Nicolle, Paul Czoty, Allyn Howlett, Brian McCool and Sara Jones who have provided me with much needed feedback and direction. I would especially like to thank Sara Jones for providing additional mentorship on being a mother in science.

Another source of feedback and support has come from Dr. Caroline Bass, who never hesitates on giving advice when needed. Additionally, I would like to thank the other members of the Robert’s lab, especially Leanne Thomas and Benjamin Zimmer, whom I have enjoyed having as my scientific peers over the years.

I would like to thank my family for all the support and love they have offered. My parents, who helped instill a drive to be educated from an early age and to my husband Scott, who is travelling the same journey as me and who has been the best support and guidance source I could ask for.

Finally I would like to thank NIDA for my funding (F31 DA025443).
# Table of Contents

LIST OF ABBREVIATIONS ........................................................................................................ iv

LIST OF TABLES AND FIGURES ............................................................................................... v

ABSTRACT ............................................................................................................................... vii

CHAPTER ONE - Introduction ....................................................................................................1
  Circadian mechanisms in drug reinforcement .......................................................................3
  Behavioral Indicators of a “Changed Rat” ............................................................................14

CHAPTER TWO – Circadian control of binge-like self-administration in rats .......................32

CHAPTER THREE – Transitions in the addiction process as revealed by a novel hold-down procedure ......................................................................................................................57

CHAPTER FOUR – Brain cocaine levels predict the duration of continuous cocaine consumption ..................................................................................................................89

SUMMARY AND CONCLUSIONS .......................................................................................... 111

APPENDIX .............................................................................................................................. 112

CURICULUM VITAE ............................................................................................................... 144
LIST OF ABBREVIATIONS

CPP: conditioned place preference
CPu: caudate putamen
cry1: cryptochrome 1
cry2: cryptochrome 2
DA: dopamine
DAT: dopamine transporter
DT: discrete trials
ED: every day
EOD: every-other day
HD: hold-down
NAc: nucleus accumbens
per1: period 1
per2: period 2
PR: progressive ratio
STR: striatum
TH: tyrosine hydroxylase
SCN: suprachiasmatic nucleus
PFC: prefrontal cortex
VTA: ventral tegmental area.

Note on naming convention:

CLOCK = the clock protein, Clock = the clock gene, clock = generic name for circadian genes
LIST OF TABLES AND FIGURES

Chapter 1

Figure 1. Graphical representation of Circadian Rhythms……………………………………5

Figure 2. Model of the molecular components of the circadian oscillator………………..7

Table I. DSM-IV criteria for substance dependence……………………………………15

Chapter 2

Figure 1. Probability of taking an available infusion of cocaine………………………..39

Figure 2. Percentage of infusions received during light and dark hours…………………42

Figure 3. Interaction of dose and availability with modeled brain cocaine level……..44

Figure 4. Peak brain cocaine level and duration of cocaine taking ……………………...45

Figure 5. Effects of dose and availability on cocaine intake……………………………47

Chapter 3

Figure 1. Average cocaine intake by hour……………………………………………..…64

Figure 2. Consecutive non-zero infusions for an individual rat…………………………65

Figure 3. Average daily cocaine intake……………………………………………………67

Figure 4. The number of available opportunities to self-administer taken……………69

Figure 5. The average cocaine dose taken………………………………………………71

Figure 6. Rate of cocaine intake under FR1 schedule……………………………………73
Figure 7. Representative example of brain modeling……………………………………….74

Chapter 4

Figure 1. A representative animal……………………………………………………….96

Figure 2. Average hours of consecutive cocaine taking for each session type……..97

Figure 3. Peak and trough brain-cocaine levels across the four conditions……….100

Figure 4. Correlation of trough and the duration………………………………………..102

Appendix

Figure 1. Average total cocaine intake………………………………………………..135

Figure 2. Average intake by session……………………………………………………136

Figure 3. Breakpoints for 0.17 mg/kg on a progressive ratio schedule………………137

Figure 4. Breakpoints for 0.30 mg/kg on a progressive ratio schedule………………138

Figure 5. Breakpoints for 0.56 mg/kg on a progressive ratio schedule………………139

Figure 6. Breakpoints for 1.0 mg/kg on a progressive ratio schedule……………….140

Figure 7. Breakpoints for 1.7 mg/kg on a progressive ratio schedule………………..141

Figure 8. Breakpoints for 3.0 mg/kg on a progressive ratio schedule…………….…142

Figure 9. Breakpoints with and without 10 days of deprivation…………………..…..143
ABSTRACT

CARSON V. DOBRIN

CIRCADIAN MECHANISMS IN COCAINE REINFORCEMENT

Dissertation under the direction of David C.S. Roberts, Ph.D.,
Professor of Physiology and Pharmacology

Our central governing theme has been our belief that there is an endogenous circadian control that underlies (at least in part) self-administration behavior. This control can be over-ridden after extended self-administration and with higher dosing and access parameters. The neurobiological correlates of this control could prove to be a rewarding area of research in the search for better understanding, and ultimately better therapeutics, for drug addiction. The proceeding chapters of this dissertation are aimed elucidating some of the mechanism associated with circadian influences in cocaine reinforcement. The central themes that run throughout are 24-hour cocaine self-administration and the impact of time-of-day and brain cocaine levels on cocaine taking.

What are some of the factors that contribute to the maintenance and termination of a cocaine ‘binge’? Chapter 2, Circadian control of binge-like cocaine self-administration, examines the complex interaction of dose, availability and time of day on the duration of cocaine consumption. The data show that access to very little cocaine was necessary to maintain intake during the dark phase. By contrast, cocaine self-administration during the light phase was strongly influenced by dose and availability. Additionally, continued intake into the light phase was highly correlated with modeled brain cocaine levels. Higher concentrations of cocaine appear to be necessary to sustain drug taking during the inactive phase of the light/dark cycle.
Are maintained brain-cocaine levels are necessary for escalation of drug intake over time or are intermittent intoxicating cocaine events are sufficient? Chapter 3, Transitions in the addiction process revealed by a novel hold-down procedure, gave intermittent 24-hour access to cocaine over an extended period of time using a hold-down (HD) procedure in which animals are able to self-administer as much cocaine as they chose over a 5-min timer period. We were able to show two distinct forms of escalation over time with different intermittent access procedures, showing that sustained blood levels do not appear to be necessary to produce this very important symptom the addiction process.

What is the influence of brain-cocaine levels and time of day on continued bouts of cocaine consumption? Chapter 4, Brain cocaine levels predict the duration of continuous cocaine consumption, combines a DT procedure with a HD procedure to produce different peak and low point (or trough) brain cocaine levels in order to determine which (if any) contributed to continued consumption of cocaine. We show that the duration of continued cocaine consumption is tightly correlated to the trough in brain cocaine levels, where the higher the trough is, the longer the duration of cocaine taking is.

Taken together, the data presented in this dissertation speak to the heavy influence of time of day on cocaine consumption and examine the factors that may contribute to it, such as brain cocaine levels. Overall, it opens the door to investigate time of day specific neurobiological differences that might influence cocaine taking and how they may be overcome by dose, availability and experience. It is well known that circadian genes
have daily cycles, and that these genes act as transcription factors thereby influencing some very important systems in drug abuse and are thus a very good place to start.
CHAPTER ONE

CIRCADIAN MECHANISMS IN DRUG REINFORCEMENT AND BEHAVIORAL INDICATORS OF A “CHANGED RAT”

Carson V. Dobrin and David C.S. Roberts
Introduction

This chapter seeks to place into context three experiments that were designed to investigate the changes in reinforcing effects relative to time of day. Drug addiction, abuse and dependence affect more than 22.5 million people in the United States (SAMHSA 2010). Research in this area is constantly evolving as we learn more about factors that seem to influence this disease. The hunt for reliable treatment and therapeutic options is never ending, and the more we can understand about addiction the better we can target treatments for it. One of the newer factors gaining attention as of late is circadian mechanisms underlying drug reinforcement. Research in this area is really just beginning but it seems that the intricate endogenous circadian system may be involved initially in governing the timing and duration of drug taking. After extended drug taking it may be possible that the circadian system that normally underlies drug taking could become deregulated, thus beginning the downward spiral that is addiction. In the first section of this introduction, *Circadian mechanisms of drug reinforcement*, I will discuss some of the evidence behind the idea that addiction could be influenced by the endogenous circadian system.

The other key factor in developing effective treatments options for addiction is having a reliable and robust animal model that closely mimics the human addiction condition. As we learn more about the factors that possibly govern the addiction phenomenon we must be evolving our animal models to help fit this new expanded view. In the second half of this introduction, *Behavioral Indicators of a “Changed Rat”*, I will be reviewing some of the more common animal models of addiction and underscore some of the evidence that helped guide the formation of this dissertation that focuses on 24-hour cocaine self-administration and the impact of time-of-day and brain cocaine levels on cocaine taking
Circadian mechanisms in drug reinforcement

Explanation of ‘circadian’

Almost all species exhibit daily changes in their behavior and physiology. These changes cannot be simply attributed to the 24-hour cycle imposed by the earth turning on its axis but on an internal timekeeping system within the organism. Circadian rhythms guide daily life in an intricately controlled manner. The word circadian is derived from the Latin terms *circa*, meaning “around”, and *diem*, meaning “day”. There are many different rhythms happening in our bodies every day controlled by an internal biological “clock” including the sleep-wake cycle, body temperature, feeding, hormone expression and glucose metabolism.

In order for a biological rhythm to be considered circadian it must meet three general criteria (Vitaterna et al. 2001). First, it must be self-sustained, meaning a diurnal, or daily, rhythm that occurs under natural conditions must continue to cycle under laboratory conditions devoid of any external time-giving cues (i.e. a light-dark cycle). Second, it must persist with a period of close to but not exactly 24 hours, meaning under conditions devoid of external entraining cues (i.e. constant dark) the behavior must exhibit a time pattern that is close to but not exactly 24 hours. This creates a phenomenon that is known as “free-running” where the behavior being measured shifts slightly every day it is not quite in line with our 24 hour world (see example in figure 1). Finally, it must be able to be entrained by external cues such as a light-dark cycle.

There are three main terms that are often used to describe circadian rhythms (see figure 1). First is amplitude, which is the difference in the level between the peak (high point) and trough (low point) levels of the rhythm. Second is phase, which is the timing of a reference point in the cycle (i.e. peak) relative to a fixed event (i.e. the beginning of the
night phase). Finally there is the period, which is the time interval between phase reference points (i.e. two peaks) (Vitaterna et al. 2001).
Figure 1. Graphical representation of Circadian Rhythms. The red line represents a hypothetical observed rhythm. The y-axis represents the level of the rhythm, while the x-axis is an indicator of the light-dark cycle. Circadian rhythms are explained by their amplitude (overall change from low to high point), phase (time difference between low and high point) and period (time difference between two peaks). Adapted from (Vitaterna et al. 2001).
Molecular basis of circadian rhythms

The last decade has seen a remarkable advance in our understanding of the molecular mechanisms underlying circadian rhythms in mammals. Vitaterna et al. (Vitaterna et al. 1994) used a mutagenesis strategy in mice to find a gene that altered circadian rhythms. The mutation was tracked to a gene on chromosome 5 which they called Clock (which stands for “Circadian Locomotor Output Cycles Kaput”). This discovery opened the door to the characterization of the positive and negative gene-expression feedback loops which in hindsight are remarkably similar to those described for drosophila (see figure 2).

In brief, it has been shown that CLOCK dimerizes with BMAL1 in the nucleus to form a transcription factor that binds to an E-box (enhancer element) domain in the promoter of the Period (Per1, Per2) and Cryptochrome (Cry1, Cry2) genes, thereby inducing their transcription. The PER and CRY protein products dimerize and suppress the CLOCK-BMAL1 activity in a negative feedback loop (Darlington et al. 1998; Gekakis et al. 1998; Griffin et al. 1999; Kondratov et al. 2006; Reppert and Weaver 2002).
Figure 2. A simplified model of the molecular components of the circadian oscillator. CLOCK and BMAL1 form a dimer that binds to the E-box in the 5’ regulatory region of various clock-controlled genes (including per and cry), initiating their transcription. The arrows indicate that the E-box can have interactions with CRE and AP-1, and all three can be acted upon by psychoactive drugs. The PER and CRY proteins then negatively feed-back, preventing the formation of the CLOCK/BMAL1 complex. The time delay between activation and repression leads to oscillations in clock controlled gene expression (adapted from Manev and Uz, 2006).
Herzog (Herzog 2007) has suggested that “all cells are born to oscillate” since period
genes are found in all cells examined so far including neurons and glia. While the
majority of the circadian literature for the past thirty years has focused on the SCN as a
master regulator, or oscillator, of circadian function (for reviews see (Aton and Herzog
2005; Weaver 1998)), it now appears that (secondary) clocks exist throughout the brain
(Dorenbos et al. 2007; Guilding and Piggins 2007). Interestingly, tissues grown in culture
have been shown to exhibit a circadian rhythm, even though they have never been
under the control of the SCN (Yamazaki et al. 2000).

Circadian clock genes also act as transcription factors that can regulate the expression
of non-clock genes (termed clock-controlled genes). In fact, it has been shown that any
gene that possesses an E-box enhancer upstream of its promoter region can have
transcription affected by clock genes acting as transcription factors (Leclerc and
Boockfor 2005). Some of these clock-controlled genes are tyrosine hydroxylase, DA
D1R, DAT, CART, delta opioid receptor, noradrenaline receptor, EAAT1 and mGluR5
(Manev and Uz 2006a). The presence of cAMP response element (CRE) and/or
activating protein 1 (AP-1) sites in the vicinity of an E-box site offer the potential for
cocaine and other stimulant drugs to affect the expression of clock genes. This may be
the mechanism whereby daily methamphetamine and nicotine can entrain rat circadian
activity (Gillman et al. 2007; Kosobud et al. 1998), even in rats with SCN lesions (Iijima
et al. 2002). This makes it possible that Clock genes influence the behavioral effects of
cocaine as well as other psychoactive drugs.
Examples of how drug use can be considered circadian

Humans are known to take and crave many different drugs in a cyclical pattern. For example, nicotine craving is reported to be strongest in the morning at waking time (Shiffman et al. 1997). Alcoholics report the time of day that they take their first drink is most often 9:00-11:00 am independent of wake time and with little variation from day-to-day (Danel et al. 2003). Women have been reported to drink more caffeine in the evening hours, while males drink more caffeine in the morning hours (Wilson 1990). In a unique study by Preston et al (Preston et al. 2009), human cocaine addicts were asked to record their craving on hand-held electronic devices at random intervals during the day. They showed that craving has a diurnal cycle that increases throughout the day. Additionally, abstinent cocaine users have been shown to have severe disruptions in sleep processes (Morgan et al. 2006b).

Markers of DA system activity have also been shown to have a circadian pattern. Rat microdialysis studies have demonstrated that the levels of DA and its metabolites, DOPAC and HVA, cycle in both the NAc and the STR with levels being greater during the dark phase (Castaneda et al. 2004; Paulson and Robinson 1994; 1996). A key enzyme in the biosynthetic pathway of DA, TH, is co-localized in dopaminergic VTA neurons, and its expression is increased in Clock mutant mice (McClung et al. 2005). DA receptors also exhibit cyclic variability; in mice D2 receptor expression is greater in the day, while D3 receptor expression is greater in the night. These differences lead to altered responsiveness to the D2/D3 agonist quinpirole, such that administration of the drug during the day induced hyperactivity, while administration during the night resulted in decreased locomotor activity (Akhisaroglu et al. 2005).
Diurnal variation in drug reward has been illustrated using both locomotor sensitization and conditioned place preference. The degree of locomotor sensitization to cocaine is usually much greater when tested during night (or dark/active) hours (Akhisaroglu et al. 2004; Kurtuncu et al. 2004; Sleipness et al. 2005; Uz et al. 2003; Uz et al. 2002). There seems to be a cyclical feedback loop in circadian influences on cocaine reinforcement as circadian genes including Clock and Period 1 and 2 have been shown to have a role in the expression of cocaine, morphine and alcohol-related behaviors (Abarca et al. 2002; Andretic et al. 1999; Liu et al. 2005b; McClung et al. 2005; Roybal et al. 2007; Spanagel et al. 2005a; Spanagel et al. 2005b) and cocaine itself has been shown to influence the expression of circadian genes (Lynch et al. 2008; Uz et al. 2005; Yuferov et al. 2003), for review see (Manev and Uz 2006b).

Giving unlimited access to cocaine in a self-administration paradigm is one way to override diurnal variations in drug reward. In fact, one of the earliest observations in the cocaine self-administration literature was that rats and non-human primates given unlimited access to cocaine or other stimulants, self-administered in very long ‘binges’ producing extreme toxicity. If binge/abstinence cycles were allowed to continue, a fatal overdose was inevitable (Bozarth and Wise 1985; Deneau et al. 1969; Johanson et al. 1976). However if dose, price or availability is altered, then the pattern of drug taking is changed considerably. For example, if the maximum hourly intake is restricted (by offering cocaine only during 3 or 4 discrete trials per hour), then cocaine self-administration no longer occurs in binges and rats can be studied round-the-clock for many weeks without signs of toxicity. Interestingly, rats show highly regular circadian patterns of cocaine self-administration with high intake during the dark (or active phase), and lower intake during the light phase (Fitch and Roberts 1993; Roberts and Andrews
This discrete trials procedure offers a unique model to study the circadian nature of cocaine self-administration and the transition to binge-type intake.

**Interaction of drugs with clock genes**

Manipulations of clock genes affect the stimulant and reinforcing effects of cocaine. Andretic et al. (Andretic et al. 1999) showed in drosophila that circadian genes Period, Clock, Cycle and Doubletime influence the expression of behavioral sensitization to cocaine. In the years following this discovery, there has been a growing number of studies examining clock gene knockout mice (Abarca et al. 2002; Debruyne et al. 2007a; b; Liu et al. 2005b; Masubuchi et al. 2001; McClung et al. 2005; Spanagel et al. 2005b; Zghoul et al. 2007). By far the most extensively characterized have been Clock mutant mice. Clock knockouts have enhanced novelty-induced locomotor activity and cocaine conditioned place preference (CPP) (McClung et al. 2005). Somewhat surprisingly, Clock knockouts were shown to exhibit relatively normal circadian rhythms in locomotor activity, suggesting that CLOCK protein is not necessary for circadian rhythms governing this behavior (Debruyne et al. 2006). The same group later showed that a different circadian gene protein product, NPAS2, substitutes for the CLOCK protein in Clock knockout mice, resulting in a relatively normal phenotype (Debruyne et al. 2007a). Behavioral sensitization and CPP to cocaine and morphine is absent in Per1 mutant mice, while Per2 mutant mice exhibit robust cocaine-induced sensitization and CPP (Abarca et al. 2002; Liu et al. 2005b). Per2 mutant mice also have increased basal levels of extracellular glutamate (Spanagel et al. 2005a). Alcohol self-administration is increased in Per2 mutants but unaffected by deletion of Per1 (Spanagel et al. 2005a).

In addition to clock genes influencing the effects of psychoactive drugs, it is also possible that psychoactive drugs, such as cocaine, can influence clock genes. As
alluded to above, the presence of cAMP response element (CRE) and/or activating protein 1 (AP-1) sites in the vicinity of an E-box site offer the potential for cocaine and other stimulant drugs to even further affect the expression of clock genes (Leclerc and Boockfor 2005; Manev and Uz 2006b). Either through direct actions of drugs on the CRE, AP1 or E-box, but also through interactions these elements may have with each other. In fact Lynch (Lynch et al. 2008) showed that 7 days of 24 hour self-administration on a discrete trials (DT) schedule causes an up regulation of a number of genes, including some circadian genes including Clock, Bmal1, Per1 and Cyp101a1. Additionally, human heroin addicts have been shown to have blunted diurnal signaling of both Per1 and Per2 mRNA after heroin abstinence (Li et al. 2009).

**Implications of circadian mechanisms on drug reinforcement on addiction**

Recreational drug use, like most human behaviors, follows a cyclical pattern in which the time of day appears to influence drug use and its effects. Similarly, animal models have shown that the motivation to self-administer cocaine can fluctuate dramatically according to time of day. Understanding the physiological processes which can dampen interest in drug use could provide important therapeutic targets. Additionally, the conversion from recreational use to cocaine binges is a diagnostic transition point in the addiction process. This transition might be viewed as a loss of the circadian influence which would normally control the timing and duration of drug taking. We believe there is some endogenous regulator that influences whether or not an animal will self administer drug that is likely related to the circadian system.
More insight into the circadian mechanisms on drug reinforcement and addiction could provide many new potential therapeutics and treatment strategies. Treatments for circadian rhythm disorders could very promising. One current treatment is bright light therapy, in which a person is exposed to a dose of high intensity light for a short period of time (Bjorvatn and Pallesen 2009). This procedure is thought to phase shift (advance or delay depending on the time of treatment) the circadian rhythm back to a more normal set point. If addicts are affected by an “out of control” circadian system, then bright light therapy could potentially help to re-set the rhythm. In fact, some in-patient addiction treatment centers have begun to incorporated bright light therapy as a tool to fight addiction. Another treatment for circadian rhythm disorders is melatonin. Like bright light therapy, melatonin is thought to work by phase shifting an out of whack circadian cycle (Bjorvatn and Pallesen 2009). Interestingly, it has been shown that melatonin can block locomotor sensitization to cocaine in rats (Sircar 2000) and also helps to reduce anxiety-like behaviors produced during cocaine withdrawal (Zhdanova and Giorgetti 2002). To date there hasn’t been any examination on the effects of melatonin on cocaine self-administration, but it is certainly an interesting avenue of study.

The Roberts lab has consistently shown that under certain access parameters cocaine self-administration takes on a diurnal pattern with greater amounts of drug taken in the dark, or active, hours (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003). This adds an interesting dimension to many self-administration studies currently being conducted that run for short durations of time (i.e. 6 hours). It is possible that certain pharmacological manipulations could have different effects depending on the time they were administered. For example, a drug that shows
no effect during times when self-administration is normal low (light hours) could have drastically different effects in the dark hours. This raises the possibility of receiving false negatives if a substance is not tested for not only a full dose response, but also a range of times throughout the day.

**Behavioral Indicators of a “Changed Rat”**

**What does it mean to be a changed rat? Why do we care?**

There are many different drug self-administration tools that researchers use to study drug taking, seeking, use and dependence. Most often these tools are designed to shadow the DSM-IV criteria for substance dependence in some form (see table I). The investigation into the behavioral and neurobiological correlates of addiction has been growing since the early 1960’s when Weeks (Weeks and Collins 1964) described a procedure for intra-venous self-administration of drugs in rodents. Currently, many researchers are focusing on creating a reliable animal model of addiction and question that most commonly occurs to them is how can we tell that drug exposure through self-administration has made the animal “addicted”, or more realistically models the pathway of addiction?
Table I. DSM-IV criteria for substance dependence (adapted from (Roberts et al. 2007)).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerance</td>
<td>Needing an increasing amount of drug to achieve a desired level of intoxication or effect.</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>Experiencing drug-specific withdrawal symptoms after ceasing consumption; related to taking drug to relieve withdrawal effects.</td>
</tr>
<tr>
<td>Binge</td>
<td>The drug is taken in increasing amounts over longer periods of time.</td>
</tr>
<tr>
<td>Frequent relapse</td>
<td>Unable to sustain ceased drug taking time after time.</td>
</tr>
<tr>
<td>Drug seeking</td>
<td>The time spent acquiring drug increases and takes over the time.</td>
</tr>
<tr>
<td>Drug taking</td>
<td>The time spent taking drug increases and takes over the time.</td>
</tr>
<tr>
<td>Social activity disruption</td>
<td>Drug seeking and taking starts to become a main focus and takes over the time normally spent in other non-drug related activities.</td>
</tr>
</tbody>
</table>
In the effort to find effective treatments and cures for addiction it is essential that we have reliable, robust and realistic animal models to give us insight into the disease. The remainder of this introduction will focus on the most common ways researchers use to display a “changed” or more interesting animal after self-administration drug exposure.

Current self-administration tools available to assess changes

Escalation

Escalation of cocaine intake has become a major theme of the preclinical self-administration literature and the phenomenon has become the focus of considerable theoretical debate. The basic observation is that rats given access to short daily (e.g. 1 hr) cocaine self-administration sessions show very stable levels of intake; however, rats given greater access (e.g. 6 hr/day) show robust increases in the rate of cocaine self-administration (Ahmed and Koob 1998). Typically a 40%-50% increase in daily intake is observed over two weeks of testing (Ahmed and Koob 1998; Gipson et al. 2011; Im et al. 2010; Quadros and Miczek 2009). Long access (LgA) has been shown to produce escalation of drug intake of variety of drugs including cocaine (Ahmed and Koob 1998; 1999), methamphetamine (Kitamura et al. 2006), methylphenidate (Marusich et al. 2010), heroin (Ahmed et al. 2000) and even food (Johnson and Kenny 2010). Escalation has been posited to occur as a result of tolerance (Emmett-Oglesby et al. 1993) or sensitization (Deroche et al. 1999; Lorrain et al. 2000; Piazza et al. 1990; Schenk and Partridge 1997; 2000; Schenk et al. 1993; Suto et al. 2002; Vezina et al. 1999) or from changes in incentive salience (Robinson and Berridge 1993), reward allostasis (Koob and Le Moal 1997; 2001), reinforcing strength (Heyman 1996) or habit
formation/compulsion (Everitt and Robbins 2005) (see (Zernig et al. 2007) for review). After a period of LgA, rats have been reported to exhibit greater relapse responding to cocaine (Ahmed and Koob 1998), and self-administer cocaine to higher breakpoints on a progressive ratio schedule (Allen et al. 2007; Liu et al. 2005a; Paterson and Markou 2003; Wee et al. 2008) and continue to self-administration in the face of adverse consequences (Vanderschuren and Everitt 2004).

The LgA protocol appears to demonstrate that maintained blood levels are responsible for escalation of drug intake. Rats are said to ‘load up” when drug becomes available and then maintain a relatively constant drug level throughout the duration of a session (Ahmed and Koob 1998; Gipson et al. 2011; Im et al. 2010; Quadros and Miczek 2009). The critical difference between the ShA and LgA groups is the duration of maintained cocaine levels. The LgA protocol appears to model a condition that might occur in human users during frequent, sustained binges – a pattern characteristic of an advanced stage of the addiction process.

An unresolved issue is whether escalation of drug intake can occur under conditions characteristic of the beginning stages of drug use. Recreational users might experience a powerful intoxicating event however their drug use may be intermittent. The question is whether intermittent drug experiences that do not result in sustained blood levels are sufficient to move the addiction process forward. Here we examined access conditions that allowed animals frequent opportunities to ‘load up’ but prevented cocaine levels from being sustained.
Behavioral sensitization

Behavioral sensitization, as it relates to self-administration, is considered “as the increased motivation to self administer drugs” (Vanderschuren and Pierce 2010). In 1993, Robinson and Berridge (Robinson and Berridge 1993) coined their ‘incentive salience’ hypothesis to explain increased desire for a drug over time leading to compulsive drug pursuit and drug taking as one explanation for drug addiction. Studies which have been cited as support for this theory have shown that repeated drug pretreatment aids in the acquisition of self-administration, either through lower acquisition doses, faster time frame or increased responding (Covington and Miczek 2001; Horger et al. 1992; Horger et al. 1990; Piazza et al. 1989; Piazza et al. 1990; Pierre and Vezina 1997; 1998; Valadez and Schenk 1994).

More clear evidence of behavioral sensitization in self-administration comes from studies where animals were given long term exposure to a progressive ratio (PR) schedule with limited pre-training. In a PR schedule, the response requirement increases with each successive infusion of drug, so that an animal is required to work harder to obtain drug over the course of the session (Richardson and Roberts 1996). By definition, increases in responding over time on a PR schedule are examples of behavioral sensitization and has been shown to occur repeatedly in the Roberts lab (Liu et al. 2007; Liu et al. 2005a; Morgan et al. 2005; Morgan et al. 2006b; Ward et al. 2006) with both cocaine and heroin.

As hinted above, escalation in drug use over time can also be thought of as an example of incentive salience. The most pervasive example of this is the Long-Access Escalation model (Lg-A, (Ahmed and Koob 1998)). In some cases, rats that have escalated intake with either cocaine or heroin have been shown to display increased motivation to self
administer (as measured through a PR schedule) (Lenoir and Ahmed 2008; Orio et al. 2009; Paterson and Markou 2003; Wee et al. 2008), however, that is not always the case (Liu et al. 2005a; Morgan et al. 2006a). Whether or not escalated animals are behaviorally sensitized still remains unclear.

**Drug seeking, relapse and reinstatement**

Drug seeking refers to “behavioral patterns aimed to search, acquire and forage for the drug when it is not readily available” (O'Brien and Gardner 2005). This phenomenon is central to addiction, and is featured as one of the DSM-IV criteria for drug dependence (see table I). In animals, drug seeking can be measured using a reinstatement model, where when animals who have reached a stable level of responding, in some cases followed by extinction (a series of days where the lever is presented but not reinforced by a drug infusion), and a period of time off (forced deprivation), are then presented with a trigger which ‘reinstates’ responding on the lever despite no drug infusion being received when the lever is pressed. The most common triggers used are stress, cue and a drug prime. The degree of reinstatement responding is often compared across groups or treatment conditions.

In the strictest terms, drug reinstatement is not really self-administration, as the animals are not actually self-administering anything. Additionally, it can be difficult to compare to humans (where extinction does not really exist and forced deprivations are rare).

**Binge behavior – circadian break down**

Binge-like cocaine intake has been well characterized in self-administration studies in animals and this work has highlighted the importance of dose and availability of drug in sustaining a binge. Rats and non-human primates given unlimited access to cocaine
exhibit prolonged binges (Deneau et al. 1969; Johanson et al. 1976) that last 11-22 hours (Fowler et al. 2007). However binge-like intake can produce considerable toxicity and, with repeated binge cycles, can result in high levels of lethality (Bozarth and Wise 1985). Studies designed to examine binge-like patterns of cocaine self-administration usually place constraints on intake in order to avoid toxicity and overdose. For example, session lengths have been restricted to 16-72 hrs and the number of sessions have been limited (Covington and Miczek 2005; Fowler et al. 2007; Mutschler et al. 2001; Tornatzky and Miczek 2000).

A Discrete Trials (DT) procedure is another method of constraining drug intake and has revealed a strong circadian influence on the pattern of cocaine (Fitch and Roberts 1993). Typically, on an FR1 schedule, rats will self-administer cocaine in the range of 8-10 mg/kg/hr (Ahmed and Koob 1998; Brebner et al. 2000). A DT schedule can restrict hourly intake through limitations on the number of discrete trials per hour. For example, access to a dose of 1.5 mg/kg/inj during 5 discrete trials per hour only slightly limits maximum hourly intake to 7.5 mg/kg/hr. Rats exposed to this schedule show binge-like intake, self-administering cocaine during virtually every trial for as long as 4 days (Fitch and Roberts 1993). If greater restrictions are placed on cocaine availability (i.e fewer trials/hr or smaller doses) then cocaine self-administration becomes largely confined to the dark (active) phase of the light/dark cycle (Fitch and Roberts 1993; Roberts et al. 2002). The observation that this diurnal cycle of cocaine intake persists in the absence of external light-dark entraining cues (Bass et al. 2010), suggests that there is a strong endogenous or internal regulator of cocaine self-administration. A circadian regulator appears to be one mechanism involved in the termination a drug taking binge.
Whether time-of-day influences the initiation and maintenance of cocaine intake appears to depend on dose and access conditions. It has been well established that unlimited access to cocaine results in high intake binges lasting 11-22 hours (Bozarth and Wise 1985; Deneau et al. 1969; Fowler et al. 2007; Johanson et al. 1976; Tomatzky and Miczek 2000). However, by using a DT procedure to limit hourly intake, diurnal patterns of cocaine self-administration emerge. It has been repeatedly shown that a predictable rhythm of drug intake is associated with the day/night cycle when hourly intake is constrained (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003).

Summary

Due to logistical and equipment limitations, not many laboratories study 7 days a week, 24-hour access self-administration; however it is an area of research that should not be over-looked. It is our belief that under some conditions, there is an endogenous circadian control that underlies (at least in part) self-administration behavior. That control can be over-ridden after extended self-administration and with higher dosing and access parameters. The neurobiological correlates of that control could prove to be a rewarding area of research in the search for better understanding, and ultimately better therapeutics, for drug addiction. The proceeding chapters of this dissertation are aimed at answering some of the central questions alluded to above. The central themes that run throughout are 24-hour cocaine self-administration and the impact of time-of-day and brain cocaine levels on cocaine taking.

Firstly, what are some of the factors that contribute to the maintenance and termination of a binge? Chapter 2, Circadian control of binge-like cocaine self-administration,
examines the complex interaction of dose, availability and time of day on the duration of cocaine consumption. The data show that access to very little cocaine was necessary to maintain intake during the dark phase. By contrast, cocaine self-administration during the light phase was strongly influenced by dose and availability. Additionally, continued intake into the light phase was highly correlated with modeled brain cocaine levels. Higher concentrations of cocaine appear to be necessary to sustain drug taking during the inactive phase of the light/dark cycle.

Secondly, we investigated whether maintained blood levels are necessary for escalation of drug intake over time or whether intermittent intoxicating cocaine events are sufficient. Chapter 3, *Transitions in the addiction process revealed by a novel hold-down procedure*, gave intermittent 24-hour access to cocaine over an extended period of time using a hold-down (HD) procedure in which animals are able to self-administer as much cocaine as they chose over a 5-min timer period. We were able to show two distinct forms of escalation over time with different intermittent access procedures, showing that sustained blood levels do not appear to be necessary to produce this very important symptom the addiction process.

Finally, we examined more clearly the influence of brain-cocaine levels and time of day on continued bouts of cocaine taking. Chapter 4, *Brain cocaine levels predict the duration of continuous cocaine consumption*, combines a DT procedure with a HD procedure to produce different peak and low point (or trough) brain cocaine levels in order to determine which (if any) contributed to continued consumption of cocaine. We show that the duration of continued cocaine consumption is tightly correlated to the
trough in brain cocaine levels, where the higher the trough is, the longer the duration of cocaine taking is.

Taken together, the data presented in this dissertation speak to the heavy influence of time of day on cocaine consumption and examine the factors that may contribute to it, such as brain cocaine levels. Overall, it opens the door to investigate time of day specific neurobiological differences that might influence cocaine taking and how they may be overcome by dose, availability and experience. It is well known that circadian genes have daily cycles, and that these genes act as transcription factors thereby influencing some very important systems in drug abuse and are thus a very good place to start.
REFERENCES


Suto N, Austin JD, Tanabe LM, Kramer MK, Wright DA, Vezina P (2002) Previous exposure to VTA amphetamine enhances cocaine self-administration under a


CHAPTER TWO

CIRCADIAN CONTROL OF BINGE-LIKE SELF-ADMINISTRATION IN RATS

Carson V. Dobrin, Leanne N. Thomas, Benjamin A. Zimmer,
David C. S. Roberts

Submitted for publication to *Psychopharmacology* April, 2011
(revisions pending)
Introduction

Individuals diagnosed as cocaine dependent characteristically consume large amounts of drug during extended binges (First and Tasman 2010; Gawin 1989; 1991; Gawin and Kleber 1985). Such binges can occur one to three times per week, with each binge lasting 8 to 24 hours (Gawin 1989). Drug availability, environmental context and current withdrawal state have all been reported to factor into the initiation of a binge (Gawin 1989). However, for a variety of ethical and practical reasons, the influences contributing to the maintenance and termination of a binge in human addicts have not been systematically studied. A better understanding of the biological mechanisms that underlie cocaine bingeing behavior, specifically the factors that contribute to the timing and termination of intake, would be useful when designing treatment strategies for cocaine addiction.

Binge-like cocaine intake has been well characterized in self-administration studies in animals and this work has highlighted the importance of dose and availability of drug in sustaining a binge. Rats and non-human primates given unlimited access to cocaine exhibit prolonged binges (Deneau et al. 1969; Johanson et al. 1976) that last 11-22 hours (Fowler et al. 2007). However binge-like intake can produce considerable toxicity and, with repeated binge cycles, can result in high levels of lethality (Bozarth and Wise 1985). Studies designed to examine binge-like patterns of cocaine self-administration usually place constraints on intake in order to avoid toxicity and overdose. For example, session lengths have been restricted to 16-72 hrs and the number of sessions have been limited (Covington and Miczek 2005; Fowler et al. 2007; Mutschler et al. 2001; Tornatzky and Miczek 2000).
A Discrete Trials (DT) procedure is another method of constraining drug intake and has revealed a strong circadian influence on the pattern of cocaine use (Fitch and Roberts 1993). Typically, on an FR1 schedule, rats will self-administer cocaine in the range of 8-10 mg/kg/hr (Ahmed and Koob 1998; Brebner et al. 2000). A DT schedule can restrict hourly intake through limitations on the number of discrete trials per hour. For example, access to dose of 1.5 mg/kg/inj during 5 discrete trials per hour only slightly limits maximum hourly intake to 7.5 mg/kg/hr. Rats exposed to this schedule show binge-like intake, self-administering cocaine during virtually every trial for as long as 4 days (Fitch and Roberts 1993). If greater restrictions are placed on cocaine availability (i.e fewer trials/hr or smaller doses) then cocaine self-administration becomes largely confined to the dark (active) phase of the light/dark cycle (Fitch and Roberts 1993; Roberts et al. 2002). The observation that this diurnal cycle of cocaine intake persists in the absence of external light-dark entraining cues (Bass et al. 2010), suggests that there is a strong endogenous or internal regulator of cocaine self-administration. A circadian regulator appears to be one mechanism involved in the termination a drug taking binge.

The purpose of the present studies was to determine the conditions under which binge-like intake of cocaine can be regulated by the day/night cycle. A discrete trials procedure was used to examine how constraining hourly intake would affect binge length. Twenty-four hour sessions began in the middle of the dark phase (when a high probability of self-administration would be expected) and the diurnal pattern was assessed. We predicted that increased cocaine availability would increase the probability of a binge continuing into the light phase. The behavioral data were used to model cocaine levels in the brain. The data show that continued cocaine intake into the light phase is tightly coupled to predicted brain cocaine levels.
Methods

Animals. Male, Sprague-Dawley rats (Harlan, Indiana USA) weighing 300-350 g at the time of surgery were used in all experiments. After arrival animals acclimated in the vivarium for a minimum of 3 days before surgery. Throughout acclimation and over the entire course of the study rats were maintained on a reverse 12 hour light-dark cycle (lights on at 3:00 pm) and had ad libitum access to food and water. Rats were housed in custom made steel operant chambers (30 x 30 x 30 cm). All procedures were conducted under approval of the Animal Care and Use Committee at Wake Forest University Health Sciences.

Surgery. All surgeries were preformed under aseptic conditions. Animals were anesthetized (100 mg/kg ketamine & 8 mg/kg xylazine, i.p.) and implanted with chronically indwelling Silastic® jugular catheters (CamCaths, Cambridgeshire, UK). An externalized access port was anchored dorsally on the back and the tubing was passed over the shoulder and into the jugular vein (Roberts and Goeders 1989). A stainless steel tether was used to protect the Tygon® tubing that attached the access port to the swivel (Instech Laboratories, Inc., Plymouth Meeting, PA., USA) which was located above the operant conditioning chamber. An infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) located outside the chamber was connected to the swivel. Catheters were flushed daily with heparinized saline in order to maintain patency. In total, 35 surgeries were preformed. Twelve rats were excluded either because they did not acquire self-administration behavior or their catheter lost patency during the experiment.
Drugs. Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC, USA), was dissolved in a solution of sterilized saline (0.9%) and passed through a microfilter (0.45-μm pore size).

Self-administration. Approximately 3 days after surgery rats began cocaine self-administration training. Subjects were given access to cocaine (0.75 mg/kg, delivered in 0.1 ml sterile saline over 4 sec) on an FR1 schedule during daily 6 hour sessions. Intake was restricted to a maximum of 20 infusions per session. Acquisition was defined as two consecutive days with 20 injections and consistently spaced inter-injections intervals. After meeting acquisition criteria rats were given access to cocaine using a discrete trials (DT) procedure during 24h sessions which began at 10 am. During each trial, a lever was extended into the test cage and a single lever response (FR1) resulted in the delivery of a cocaine infusion and termination of the trial. If no response occurred then the trial was terminated after 10 min. Separate groups of rats (N = 5-6) were tested using 3, 4, 5 or 6 discrete trials per hour. Each group was tested with an ascending series of cocaine doses (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg) during 24h sessions. Each 24h session was separated by a 24h drug-free period.

Data analysis. All statistics were performed using SigmaPlot (version 11). A two-way repeated measures analysis of the variance (ANOVA) was used to test the effect of number of discrete trials per hour (Trials) and unit dose of cocaine (Dose). Trials was a between groups comparison and Dose was a within groups comparison. Separate analyses were performed on the percentage of total infusions self-administered during the dark and light phases. Peak brain cocaine levels and hours of consecutive cocaine taking were compared using a linear regression model. The dose-response function for total cocaine intake per 24 hr session was analyzed using an area under the curve.
(AUC) analysis and a two way ANOVA. A Holms-Sidak test was used for all post hoc analyses. All data are represented as mean ± SEM unless otherwise noted.

**Modeling brain cocaine levels.** A two-compartment model developed by Pan (1991) was used to mathematically estimate whole brain levels of cocaine. The model has been calibrated for rats receiving a chronic IV cocaine regimen. Briefly, we used the equation

\[
c = \frac{dk}{v(\alpha - \beta)} (e^{-\beta t} - e^{-\alpha t})
\]

where c is the concentration in the brain, d is the dose, k is the rate of flow from the blood to the brain [0.233], v is volume of the brain [0.044], \( \alpha \) and \( \beta \) [0.642 and 0.097 respectively] are constants representing the flow of cocaine between the blood and brain compartments and the elimination of cocaine from the body, and t is the time in minutes since the last infusion. The total concentration of cocaine was determined by calculating each infusion of cocaine independently then summing them in 5 second intervals (Stuber et al. 2005b).
Results

Increased dose or availability resulted in an increased probability of self-administering cocaine during the light phase. Figure 1 illustrates the mean probability of a cocaine infusion being self-administered during each hour of the 24h session for the groups tested with 3, 4, 5 or 6 trials per hour (Panels A-D respectively) with cocaine doses ranging from 0.1 to 1.7 mg/kg. Note that for all unit doses of cocaine the probability of a trial resulting in a cocaine infusion is relatively high (>80%) during the initial five hours of the session which corresponds to the end of the dark phase. With the lower doses (0.1, 0.17 and 0.3 mg/kg/inj) the probability of an injection being self-administered drops to low levels (<10%) at light onset and remains low until the beginning of the dark phase 12 hours later. Higher unit doses (1.0 – 1.7 mg/kg) and/or increased availability per hour (5 or 6 trials/hr) resulted in greater cocaine intake especially during the light hours. Higher doses appeared to maintain responding for proportionally longer periods. When given 4 or 5 trials per hour at the highest dose tested (1.7 mg/kg) rats self-administered during 95-100% of all trials (i.e. throughout the entire session). Animals given six opportunities per hour (Figure 1D) consistently maintained their intake around 70% of available infusions throughout the light hours (10:00 am – 10:00 am, dark hours indicated by gray bars).
Figure 1. Probability of taking an available infusion of cocaine for different dose and access parameters across a 24-hr time period. Points represent the average the probability (percentage) of taking an available infusion of cocaine across a range of cocaine doses (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg) and number of trials per hour [3 trials/hour (A), 4 trials/hour (B), 5 trials/hour (C) and 6 trials/hour (D)] across a 24-hr time period. Dark hours are indicated by the grey background. As dose or availability was increased the probability of administering an available infusion of cocaine in the light hours also increases.
Manipulations of dose and availability have a greater impact on total cocaine intake during the light phase compared to the dark phase. Figure 2 shows the percentage of total infusions self-administered during either the light phase (white bars) or dark phase (black bars) across a range of doses (0.1-1.7 mg/kg) and access conditions (3-6 discrete trials per hour). For the majority of access parameters intake in the dark was consistently around 60-80% of the total available infusions (per 12 hour dark time period). A two-way repeated measures ANOVA revealed a significant effect of Trials $F_{(3,92)} = 3.46, p = 0.04$, and Dose $F_{(5,92)} = 4.26, p = 0.002$ but no significant Trials x Dose interaction $F_{(15, 92)} = 0.76, p = 0.72$. Intake in the light was more sensitive to access parameters, as lower doses (0.1-0.3 mg/kg) of cocaine intake ranged from 2-17% regardless of the number of trials per hour. The number of trials per hour had a larger influence for the higher doses of cocaine (0.56-1.7 mg/kg) resulting in an inverted-U function with the greatest probability of intake occurring in the 5 trials/hr condition. A two-way repeated measures ANOVA failed to reveal a significant Trials effect $F_{(3,92)} = 3.46, p = 0.14$, but did reveal a significant effect of Dose $F_{(3,92)} = 106.30, p < 0.001$. A significant Trials x Dose interaction was observed $F_{(15,92)} = 3.09, p < 0.001$. Note that at 5 trials per hour and 1.7 mg/kg the rats self-administered close to 100% of the available infusions in a 24 hour time period (about 200 mg/kg/session).
**Figure 2.** Breakdown of percentage of infusions received during both the light and dark hours. Bars represent the percentage of total infusions received over 24 hours in DT 3, 4, 5 and 6 at a range of cocaine doses (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg) during light (white bars) and dark (black bars) with a maximum of 100% per 12-hours. A Holm-Sidak post hoc analysis reveals a significant interaction of trials per hour and cocaine dose at 0.56 mg/kg between 5 (48.33 ± 7.12) and 3 (13.89 ± 3.86) and 4 (17.08 ± 7.13) trials per hour and also between 6 (41.90 ± 11.84) and 3 trials per hour. † indicates significance compared to 5 trials per hour, # indicates significance compared to 6 trials per hour. The regulation of cocaine intake by dose and availability is revealed exclusively during the light phase.
Figure 2.

Percentage of Total Infusions Received (per 12 hours in light and dark)

- Dark
- Light

Number of Discrete Trials Per Hour

Cocaine Dose (mg/kg)
Higher brain levels of cocaine are associated with longer cocaine binges. Figure 3 illustrates the modeled brain cocaine concentrations (µM) for four representative animals given access to cocaine during 3, 4, 5 or 6 trials/hr access. The pattern of self-administration was evaluated using six unit doses of cocaine (0.1 -1.7 mg/kg/inj) tested in an ascending order during 24 hr sessions (every other day). The modeling shows that the doses and access conditions tested here produce a range of patterns of cocaine levels in the brain. With low doses and infrequent access conditions drug levels fail to accumulate. Peak drug levels remain below 2 µM. With higher doses and access conditions, however, appreciable oscillating levels of cocaine were sustained. As expected, longer binges (defined as consecutive trials in which a cocaine injection was self-administered) were associated with the dose and trial conditions that resulted in higher sustained levels of cocaine. Figure 4 summarizes the relationship between the mean duration of a binge and the peak brain cocaine concentration (i.e. the peak point in modeled brain level per trial) for each of the 24 conditions tested (6 doses x 4 access conditions). The peak brain concentration was found to significantly correlate ($r = 0.91$, $p < .01$) with the number of hours of consecutive drug taking. At lower trials per hour (3/hour) and lower doses of cocaine (0.1 and 0.17 mg/kg), when brain levels are unable to rise above 5 µM, cocaine taking was restricted to the dark hours (indicated by the gray bars). However, during the light phase, higher brain levels were associated with sustained cocaine taking. A brain level of 15-20 µM was associated with binges that were sustained throughout the entire 24 hour session.
Figure 3. Interaction of dose and availability with modeled brain cocaine level. Individual brain cocaine level (µM) models for rats responding on at 3 trials/hour (A), 4 trials/hour (B), 5 trials/hour (C) and 6 trials/hour (D) at a range of cocaine doses (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg), indicated on the right hand side). Dark hours are indicated by the gray bars. A scale indicating 10 µM is shown on the left. Brain cocaine levels play a role in the duration of continuous cocaine taking in the light phase.
Figure 4. Peak brain cocaine level and duration of cocaine taking are correlated. Points represent peak brain cocaine level and the hours of consecutive cocaine taking broken down by trial number (3, 4, 5 and 6) and cocaine dose (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg) over the 24-hr time period. Dark hours are indicated by the gray bars. The duration of cocaine taking increases as brain cocaine levels rise.
Five and six trials/hr at the highest dose (1.7 mg/kg/inj) produced similar levels of drug intake. Figure 5 shows the cumulative cocaine intake (mg/kg/24 hours) for 3, 4, 5 and 6 trials per hour and a range of doses (0.1-1.7 mg/kg). A two-way repeated measures ANOVA revealed a significant Trials effect $[F_{(3,95)} = 12.56, p < 0.001]$, a significant Dose effect $[F_{(5,95)} = 168.72, p < 0.001]$ and a significant Trial x Dose interaction $[F_{(15,95)} = 4.28, p < 0.001]$. An area under the curve analysis confirmed no significant difference in intake between 5 and 6 trials per hour (146.9±3.9 and 135.9±20.1 mg/kg/24 hour respectively), despite the fact that the 6 trials/hr group had the potential to self-administer a great deal more.
Figure 5. Effects of dose and availability on cocaine intake. Points represent the average (±SEM) intake for the 24-hr time period. Cocaine intake (mg/kg/24 hours) for DT 3-6 for the range of cocaine doses (0.1, 0.17, 0.3, 0.56, 1.0 and 1.7 mg/kg). There is no significant difference between intake at 5 and 6 trials per hour at any point. A Holm-Sidak post hoc analysis reveals a significant interaction of trials per hour and cocaine dose at 1.0 mg/kg between 5 (100.33 ± 5.66) and 3 (43.83 ± 1.99) trials per hour and at the 1.7 mg/kg dose between 5 (200.88 ± 1.48) with 4 (145.52 ± 15.17) and 3 (92.93 ± 10.68) trials per hour. † indicates significance compared to 5 trials per hour, @ indicates significance compared to 3 trials per hour. There appears to be an upper limit in preferred cocaine intake over a 24-hour time period.
Discussion

Stimulant users characteristically binge (First and Tasman 2010); however, very little is known about the factors contributing to the maintenance and termination of these binges. Previous preclinical studies have suggested that circadian mechanisms can influence cocaine self-administration if hourly intake is restricted; however, time-of-day may have little effect with unlimited access. Here we performed a systematic manipulation of cocaine dose and access parameters in order to characterize the conditions under which binge-like intake comes under circadian control. The present data show that access to very little cocaine was necessary to maintain intake during the dark phase. By contrast, cocaine self-administration during the light phase was strongly influenced by dose and availability. Continued intake into the light phase was highly correlated with modeled brain cocaine levels. Higher concentrations of cocaine appear to be necessary to sustain drug taking during the inactive phase of the light/dark cycle.

Whether time-of-day influences the initiation and maintenance of cocaine intake appears to depend on dose and access conditions. It has been well established that unlimited access to cocaine results in high intake binges lasting 11-22 hours (Bozarth and Wise 1985; Deneau et al. 1969; Fowler et al. 2007; Johanson et al. 1976; Tornatzky and Miczek 2000). However, by using a DT procedure to limit hourly intake, diurnal patterns of cocaine self-administration emerge. It has been repeatedly shown that a predictable rhythm of drug intake is associated with the day/night cycle when hourly intake is constrained (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003). The highest probability of cocaine intake is during the last 5
hrs of the dark phase and declines at the time of transition to the light phase. The present experiment was designed to characterize the dose-response relationship associated with binge termination during the light phase. In agreement with previous reports, animals in the present study consistently self-administered 60-80% of available cocaine infusions during the first 5 hours of the session (dark hours) regardless of dose or the number of trials per hour (figure 2). It appears that access to very little cocaine in necessary to maintain self-administration behavior. Whether cocaine self-administration continued into the light phase was shown to be markedly dependent on dose and access conditions. At low doses (0.1-0.3 mg/kg) or with fewer trials (3 and 4 DT at 0.56 mg/kg), cocaine intake during the light phase was less than 20% of available infusions. At higher doses (1.0 and 1.7 mg/kg) and more frequent trials (5 or 6 trials/hr at 0.56 mg/kg) intake during the light phase ranged from 50-100% of available infusions, showing that the diurnal rhythm of circadian intake can be over-ridden with high cocaine dose and availability.

Modeling brain cocaine levels provides some insight into the factors that contribute to the maintenance and termination of cocaine taking (figure 3). The range of access conditions used here produced a wide spectrum of brain levels. Cocaine concentrations never exceeded 5 µM when access was restricted to lower doses (0.1 – 0.3 mg/kg). Predictably, higher doses and access conditions allowed drug concentrations to oscillate within a much higher range. Note that animals given access to the highest dose of cocaine (1.7 mg/kg/inj) self-administered approximately the same amount of drug regardless of whether they were given 5 or 6 trials/hr (Figure 5). Rats in the 5 trials/hr group self-administered at virtually every opportunity through the day/night cycle while animals in the 6 trials/hr group self-administered during only 70% of the trials. However, since the drug intake and blood levels maintained by both groups are virtually
equivalent, we interpret the constant but reduced level of responding (70%) for the 6 trials/hr group (Figure 1D) to reflect a preferred rate of drug intake. These data suggest that 6 trials per hour access to a high dose (1.7 mg/kg/inj) does not constrain intake and is equivalent to what might be expected with unlimited hourly access.

Figure 3 illustrates the relationship between modeled brain cocaine concentration and the probability of a cocaine binge extending into the light phase and beyond. Animals were unlikely to self-administer cocaine after the light change if access conditions capped brain levels to 5 µM and below, suggesting there is a threshold for drug intake to continue into the light. If access conditions permit higher brain levels of cocaine, either with more trials per hour or higher doses of cocaine, the duration of cocaine taking increases in a predictable manner into the light hours. This finding is made clearer when the average duration of cocaine taking and the corresponding peak brain cocaine level is graphed for each access and dose combination (figure 4).

The accuracy of the equations used to model brain-cocaine concentrations in the current study has been well established. Self-administration (Ahmed and Koob 2005; Samaha et al. 2002; Zernig et al. 2007), electrophysiology (Nicola and Deadwyler 2000; Peoples and Cavanaugh 2003; Peoples et al. 2007; Peoples et al. 2004), microdialysis (Wise et al. 1995b) and voltammetric (Hermans et al. 2008; Stuber et al. 2005a; Stuber et al. 2005b) studies have all used these equations to illustrate the temporal relationship between modeled brain-cocaine concentration, NAc dopamine levels (Hermans et al. 2008; Shou et al. 2006; Wise et al. 1995b) and cocaine-induced locomotor behavior (Shou et al. 2006). We believe these equations closely model actual brain-cocaine levels however it is important to note that the conclusions drawn were based on relative changes and not the absolute magnitude.
Two important points must be made regarding the DT protocol used in the current experiment. First, it differs in from the DT procedures used previously in this lab (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003), where rats were given round-the-clock access for many consecutive days (tested every day). In the present studies rats were tested every-other day. We previously reported that animals tend to binge on the first day of access and their drug taking becomes more diurnal on the days following (Fitch and Roberts 1993). Pilot studies have shown that giving every-other day access to cocaine recapitulates the first day binge; a drug free day appears to engender binge-like behavior. Second, rats were given access in an increasing dose paradigm. Results were not different in a similar paradigm where the doses were run in a descending manner (Roberts unpublished observation) leading us to conclude that the order of dose testing did not affect the results.

The concept of diurnal variation in drug reward has received increasing experimental support. We and others have shown that limiting intake to a specific number of discrete trials per hour reveals the cyclical nature of cocaine intake (Fitch and Roberts 1993; Negus et al. 1995; Roberts et al. 2002), with the majority of cocaine being consumed during dark (active) hours. The cyclical nature to cocaine self-administration that persists despite external entraining cues (i.e. becomes free running in a constant dim environment) (Bass et al. 2010) suggests an endogenous control over the motivation to take drug. The degree of locomotor sensitization and conditioned place preference to cocaine is usually much greater when tested during the dark (active) hours (Akhisaroglu et al. 2004; Kurtuncu et al. 2004; Sleipness et al. 2005; Uz et al. 2003; Uz et al. 2002). It has been shown that the behavioral effects of some drugs of abuse including cocaine,
morphine and alcohol, are influenced by circadian genes such as Clock and Period 1 and 2 (Abarca et al. 2002; Andretic et al. 1999; Liu et al. 2005b; McClung et al. 2005; Roybal et al. 2007; Spanagel et al. 2005a; Spanagel et al. 2005b). In addition the expression of circadian genes are influenced by drugs of abuse (Lynch et al. 2008; Uz et al. 2005; Yuferov et al. 2003), for review see (Manev and Uz 2006b), suggesting a positive feedback loop that becomes increasingly out of control with continued drug intake. It should be noted that even small doses of cocaine can affect biological rhythms as low dose/availability cocaine self-administration entrains physiological rhythms such as core body temperature (Tornatzky and Miczek 1999).

A better understanding of the neurobiological mechanisms underlying cocaine bingeing has implications for developing treatment strategies for addicts. In this study it was shown that self-administration was much more sensitive to changes in dose and availability in the light hours as compared to the dark hours. Since testing in the dark phase is relatively unaffected by changes in dose or trials/hr whereas the reverse is true in the light phase, we suggest that the most sensitive timeframe to test putative therapeutic agents for their ability to dampen binge intake would be during the transition from dark to light.


CHAPTER THREE

TRANSITIONS IN THE ADDICTION PROCESS AS REVEALED BY A NOVEL HOLD-DOWN PROCEDURE

Carson V. Dobrin, Benjamin A. Zimmer, David C. S. Roberts

In preparation for submission to Psychopharmacology September, 2011
Introduction

Escalation of drug intake is an important criterion in the diagnosis of drug dependence (APA 2000; Cami and Farre 2003; Gawin 1991; WHO 1992). Escalation is exhibited with many different kinds of drugs of abuse and involves increasing drug use over time while decreasing time spent on other non-drug related activities. Escalation of drug use is one of the clear signs that recreational drug use is becoming more compulsive.

Escalation of cocaine intake has become a major theme of the preclinical self-administration literature and the phenomenon has become the focus of considerable theoretical debate. The basic observation is that rats given access to short daily (e.g. 1 hr) cocaine self-administration sessions show very stable levels of intake; however, rats given greater access (e.g. 6 hr/day) show robust increases in the rate of cocaine self-administration (Ahmed and Koob 1998). Typically a 40%-50% increase in daily intake is observed over two weeks of testing (Ahmed and Koob 1998; Gipson et al. 2011; Im et al. 2010; Quadros and Miczek 2009). Long access (LgA) has been shown to produce escalation of drug intake of variety of drugs including cocaine (Ahmed and Koob 1998; 1999), methamphetamine (Kitamura et al. 2006), methylphenidate (Marusich et al. 2010), heroin (Ahmed et al. 2000) and even food (Johnson and Kenny 2010). Escalation has been posited to occur as a result of tolerance (Emmett-Oglesby et al. 1993) or sensitization (Deroche et al. 1999; Lorrain et al. 2000; Piazza et al. 1990; Schenk and Partridge 1997; 2000; Schenk et al. 1993; Suto et al. 2002; Vezina et al. 1999) or from changes in incentive salience (Robinson and Berridge 1993), reward allostasis (Koob and Le Moal 1997; 2001), reinforcing strength (Heyman 1996) or habit formation/compulsion (Everitt and Robbins 2005) (see (Zernig et al. 2007) for review). After a period of LgA, rats have been reported to exhibit greater relapse responding to
cocaine (Ahmed and Koob 1998), and self-administer cocaine to higher breakpoints on a progressive ratio schedule (Allen et al. 2007; Liu et al. 2005a; Paterson and Markou 2003; Wee et al. 2008) and continue to self-administration in the face of adverse consequences (Vanderschuren and Everitt 2004).

The LgA protocol appears to demonstrate that maintained blood levels are responsible for escalation of drug intake. Rats are said to ‘load up’ when drug becomes available and then maintain a relatively constant drug level throughout the duration of a session (Ahmed and Koob 1998; Gipson et al. 2011; Im et al. 2010; Quadros and Miczek 2009). The critical difference between the ShA and LgA groups is the duration of maintained cocaine levels. The LgA protocol appears to model a condition that might occur in human users during frequent, sustained binges – a pattern characteristic of an advanced stage of the addiction process.

An unresolved issue is whether escalation of drug intake can occur under conditions characteristic of the beginning stages of drug use. Recreational users might experience a powerful intoxicating event however their drug use may be intermittent. The question is whether intermittent drug experiences that do not result in sustained blood levels are sufficient to move the addiction process forward. Here we examined access conditions that allowed animals frequent opportunities to ‘load up’ but prevented cocaine levels from being sustained.

The present design incorporated two novel features. Firstly, access to cocaine was provided during 5 min discrete trials (DT) which were presented intermittently throughout
the 24 h day/night cycle. Separate groups were tested with various inter-trial intervals for two weeks. Secondly, a newly described hold-down procedure (Morgan et al. 2009) was used to control delivery of cocaine. In this procedure the amount of cocaine the animal receives is dependent upon how long the lever is held down. That is, the syringe pump delivered drug for the duration that the lever was depressed. We have recently shown that the dosage of cocaine that rats self-administer during 5 min trials is dependent upon the current brain level of cocaine (Zimmer 2011). Specifically, the amount of drug self-administered was equal to the dose necessary to reach a preferred blood level. In effect, the HD trial provides an opportunity for subject to ‘load up’ within a 5 min timeframe. Here we show that animals that experienced repeated high levels of cocaine, but were unable to maintain those levels, show escalation of drug intake.

**Methods**

**Animals.** Male, Sprague-Dawley rats (Harlan, Indiana USA) weighing 300-350 grams at the time of surgery were used in all experiments. After arrival animals acclimated in the vivarium for a minimum of 7 days before surgery. Throughout acclimation and over the entire course of the study rats were maintained on a reverse 12 hour light dark cycle (lights on at 3:00 pm) and had *ad libitum* access to food and water. Rats were housed in custom made steel operant chambers (30 x 30 x 30 cm). All procedures were conducted under approval of the Animal Care and Use Committee at Wake Forest University Health Sciences.
Surgery. All surgeries were performed under aseptic conditions. Animals were anesthetized (100 mg/kg ketamine & 8 mg/kg xylazine, i.p.) and implanted with chronically indwelling Silastic® jugular catheters (house made). An externalized access port was anchored dorsally on the back and the tubing was passed over the shoulder and into the jugular vein (Roberts and Goeders 1989). A stainless steel tether was used to protect the Tygon® tubing that attached the access port to the swivel (Instech Laboratories, Inc., Plymouth Meeting, PA., USA) which was located above the self-administration chamber. An infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) located outside the chamber was connected to the swivel. In total, 32 surgeries were performed. 13 rats were excluded either because they did not acquire self-administration behavior or their catheter lost patency during the experiment.

Drugs. Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC, USA), was dissolved in a solution of sterilized saline (0.9%) and passed through a microfilter (0.45-μm pore size). A 2.5 mg/ml concentration was used for acquisition and 5 mg/ml was used for the remainder of the study (pump speed 1.6 ml/min).

Self-administration. Approximately 3 days after surgery rats began cocaine self-administration training. Subjects were given access to cocaine (0.75 mg/kg, delivered in 0.1 ml sterile saline over 4 sec) on an FR1 schedule during daily 6 hour sessions. Intake was restricted to a maximum of 20 infusions per session. Acquisition was defined as two consecutive days with 20 injections and consistently spaced inter-injections intervals. After meeting acquisition criteria rats were given access to cocaine using a hold down (HD) procedure for three hours. After rats were able to consistently titrate their brain
cocaine levels (usually 2-4 days) they were switched to a DT-HD procedure (hereon referred to as 5-55, 5-25 and 5-10) where the animals were given 5 min to hold down the lever and receive drug followed by a time out period (55, 25 or 10 min) for 24 hours and 21 days. After completing the 21 days rats were tested using a 3 HR FR1 test to check catheter patency.

**Data analysis.** All statistics were performed using SigmaPlot (version 11). All data are represented as mean ± SEM unless otherwise noted.

**Modeling brain cocaine levels.** A two-compartment model developed by Pan (Pan et al. 1991) was used to mathematically estimate whole brain levels of cocaine. The model has been calibrated for rats receiving a chronic IV cocaine regimen. Briefly, we used the equation

\[
c = \frac{dk}{v(\alpha - \beta)} (e^{-\beta t} - e^{-\alpha t})
\]

where c is the concentration in the brain, d is the dose, k is the rate of flow from the blood to the brain [0.233], v is volume of the brain [0.044], \( \alpha \) and \( \beta \) [0.642 and 0.097 respectively] are constants representing the flow of cocaine between the blood and brain compartments and the elimination of cocaine from the body, and t is the time in minutes since the last infusion. The total concentration of cocaine was determined by calculating each infusion of cocaine independently then summing them in 5 second intervals (Stuber et al. 2005b).
Results

Rats escalate intake over time. Figure 1 A, B and C represent the average hourly cocaine intake in week 1 (black line) vs week 3 (gray line) for 5-55 (A), 5-25 (B) and 5-10 (C) HD self-administration respectively over a 24 hour time period. The dark hours are indicated by the dark grey background. Intake has a cyclical pattern for 5-55 and 5-25, with greater intake occurring in the dark vs. light hours, however intake under 5-10 is relatively constant throughout the day. Note also that intake increases more in the dark than the light hours from weeks one to three. The most dramatic increase in intake was observed with the 5-25 paradigm. A two-way repeated measures ANOVA revealed a significant effect of WEEK for 5-55 [5-55: $F_{(1,132)} = 15.33$, $p = 0.008$], a significant effect of TIME [5-55: $F_{(22,132)} = 13.79$, $p <0.001$, 5-25: $F_{(22,110)} = 16.84$, $p <0.001$ and 5-10: $F_{(22,88)} = 3.35$, $p <0.001$] and a significant interaction of WEEK and TIME for 5-55 and 5-25 [5-55: $F_{(22,132)} = 1.66$, $p = 0.043$, and 5-25: $F_{(22,110)} = 3.12$, $p <0.001$]. As an example of how rats increase intake over time, figure 2 illustrates consecutive non-zero cocaine infusions for an individual rat on 5-55 HD across the 21 day time period. Note the increase in infusion size from around 1.6 early on to 4.0 mg/kg at the end.
Figure 1. Average cocaine intake by hour for week 1 (black circles and lines) and week 3 (gray circles and line) for rats under a 5-55 HD schedule (A), a 5-25 HD schedule (B) and a 5-10 HD schedule (C).
Figure 2. Consecutive non-zero infusions for an individual rat over the course of the 21-day HD exposure on a 5-55 schedule. A linear regression analysis reveals a significant correlation ($p < 0.001$, $r^2 = 0.24$).
The degree of intake escalation varies with access parameters. Figure 3 represents the average daily cocaine intake in week 1 (black bar) and week 3 (gray bar) for 5-55, 5-25 and 5-10 HD self-administration. Note that intake increases differentially across all paradigms. A two-way repeated measures ANOVA revealed a significant effect of GROUP \( F_{(2,15)} = 11.90, p < 0.001 \), a significant effect of WEEK \( F_{(1,15)} = 9.05, p = 0.009 \) and no significant interaction between GROUP and WEEK.
Figure 3. Average daily cocaine intake for week 1 (black bars) and week 3 (gray bars) for the different access parameters 5-55, 5-25 and 5-10. Data is presented as average cocaine intake (mg/kg/day) for each group. A Holms-Sidak post hoc test revealed that within 5-25 there was a significant increase in the average cocaine dose self-administered from week 1 to week 3 [t=2.27, p=0.038].
In some cases escalation is a result of increasing the number of intoxication events per 24 hours. Figure 4 represents the average number of injection opportunities taken per 24 hours in week 1 (black bar) and week 3 (gray bar) for 5-55, 5-25 and 5-10 HD self-administration. Notice that there is no difference for the 5-55 or 5-25 groups and that the highest increase was observed with the 5-10 group. A two-way repeated measures ANOVA revealed a significant effect of GROUP \( F(2,15) = 67.39, p < 0.001 \), a significant effect of WEEK \( F(1,15) = 7.05, p = 0.018 \) and no significant interaction of GROUP and WEEK.
Figure 4. The number of available opportunities to self-administer taken per 24-hr time period for weeks 1 (black bars) and week 3 (gray bars). The data are presented as the number of opportunities taken in 24 hours for each group. A Holms-Sidak post hoc test revealed that within 5-10 there was a significant increase in the number of intoxicating events from week 1 to week 3 \[t=2.77, p=0.014\].
In other cases escalation is a result of increasing the average cocaine dose per intoxication event. Figure 5 represents the average cocaine dose self-administered per 5 min in week 1 (black bar) and week 3 (gray bar) for 5-55, 5-25 and 5-10 HD self-administration. Note that the increases for 5-55 and 5-25 were much greater than that observed with 5-10. A two-way repeated measures ANOVA revealed a significant effect of WEEK \[F_{(1,15)} = 17.66, \ p < 0.001\] and no significant effect of GROUP or significant interaction of GROUP and WEEK.
Figure 5. The average cocaine dose taken for the three groups tested (5-55, 5-25 and 5-10) for week 1 (black bar) and week 3 (gray bar). Data are presented as the average dose taken (mg/kg) for each of the three groups. A Holms-Sidak post hoc test revealed that within 5-55 and 5-25 there was a significant increase in the number the average dose taken from week 1 to week 3 [5-55: t=3.13, p=0.007, 5-25: t=3.21, p=0.006].
An escalation in rate was also observed under some circumstances. Figure 6 represents the average rate (mg/kg/hour) of cocaine intake during FR1 sessions that were run either before the 21 day HD experience (pre, black bar) or after the 21 day HD experience (post, gray bar) for each group tested. Note the large increase in post rate for 5-55 and 5-25 and the modest increase for the 5-10 group. A two-way repeated measures ANOVA revealed a significant effect of GROUP \( [F_{(2,12)} = 4.59, \ p =0.033] \), a significant effect of DAY \( [F_{(1,12)} = 14.33, \ p =0.003] \), and no significant interaction of GROUP and DAY.
Figure 6. Rate of cocaine intake under FR1 schedule both before exposure to 21 days of HD (pre, black bar) and after exposure to 21 days of HD (post, gray bar). Data is represented as rate (mg/hr) for each of the three groups tested (5-55, 5-25 and 5-10). A Holms-Sidak post hoc test revealed that within 5-55 and 5-25 there was a significant increase in rate from pre to post [5-55: t=2.50, p=0.028, 5-25: t=3.61, p=0.004].
**Figure 7.** A representative example of modeled brain-cocaine concentrations for each of the different access parameters; 5-55, 5-25 and 5-10. The dark period is indicated by the black bars. Note that the for 5-55, brain levels return to close to zero after each trial, whereas brain levels don’t get lower than 5 µM for the 5-10 group.
Discussion

Escalation of drug intake is an important criterion in the diagnosis of drug dependence (APA 2000; Cami and Farre 2003; Gawin 1991; WHO 1992) and the escalation phenomenon has become a major focus of the preclinical self-administration literature. It has been repeatedly demonstrated that daily long access (6 hour) cocaine self-administration sessions result in an increased rate of drug intake. Modeling the brain cocaine levels shows a rapid spike at the beginning of a LgA session followed by maintained blood levels for the duration of the 6-hr period. The present experiments addressed whether maintained blood levels are necessary for escalation of drug intake or whether intermittent intoxicating events are sufficient.

In the present experiments, subjects were given access to brief (5 min) self-administration trials that allowed for sharp rises in brain cocaine concentrations. Three groups, with different inter-trial intervals (ITI), were tested round-the-clock for three weeks. In the group tested with a 55 min ITI, calculated brain cocaine concentrations where shown to decline to near zero levels between each trial; there was therefore no opportunity for sustained blood levels in this group. Subjects could, however, 'load up' to a (presumably) preferred blood lever during each hourly 5 min trial and are thus were frequently exposed to intoxicating events. Two additional groups (5-25, 5-10) were tested with ITIs of 25 and 10 min, which resulted in 5 min discrete trails being offered twice or four times per hour respectively. The different access frequencies produced predictably different oscillatory patterns of brain cocaine concentrations (Figure 1). In the 5-25 Group, brain concentrations of cocaine decline to low levels but were not entirely cleared when animals self-administer cocaine during consecutive trials. Animals in the 5-10 Group were able to sustain higher brain concentrations.
The three groups illustrate how different access conditions can produce very different patterns of self-administration. The 5-55 group self-administered very high quantities (big spikes) in a circadian fashion. They usually self-administered during about half of all trials offered, and these were generally restricted to the dark (active) phase. By contrast, the 5-10 group was able to maintain drug levels and their patterns were less circadian (or diurnal) – more binge-like. They generally self-administered all throughout the day, regardless of it being their active cycle or not. The 5-25 group was in between both of these as the started out very circadian, and then over time increased the number of opportunities to self-administer well into the light hours.

The different patterns of self-administration revealed two distinct forms of escalation. In the current study we illustrate that escalation can occur in at least two ways. First, rats can escalate by increasing the amount of cocaine self-administered during a trial. The other way is by increasing the number of trials during which cocaine is self-administered. (i.e. positive trials). Access to the 5-55 condition resulted in a substantial (75%) increase in cocaine intake during the 5 min trial but the number of ‘positive trials’ did not increase significantly (from about 50% of available). By contrast, the 5-10 group did not significant increase drug intake during a trail, instead the number of positive trials increased substantially. The 5-25 Group showed a combination of both types of escalation.

We found that total intake was a predictor of one kind of escalation (increasing the number of trials over time) but not the other. Animals self-administering under the 5-55, 5-25 and 5-10 paradigms averaged 531.7 ± 103.0, 754.3 ± 159.3 and 1243.6 ± 152.2 mg/kg of cocaine respectively). By contrast, LgA animals consume close to 1500 mg/kg. As expected, the groups given more frequent access self-administered greater quantities of cocaine over the 21 days. The 5-10 Group, which self-administered the greatest
amount of cocaine showed the greatest increase in binge-like behavior (more positive trials). This reinforces what has been shown before, that more access to cocaine (which leads to more consistent levels of drug in the system across the session) leads to a greater duration of cocaine taking (or binge-like behavior). Interestingly, the 5-10 group showed no increase in within trial intake during the 3 hr FR1 test.

We compared changes in rate of intake before and after the 21 days of HD. Escalation in rate does not seem to correlate with either of the other two kinds of escalation described above. Interestingly, the 5-55 Group showed about a 75% increase in both FR rate and the average dose self-administered during each 5 min trial from the first to third week of testing. Similarly, the 5-25 group showed about a 100% increase in FR rate that corresponded to about a 100% in average dose taken. The 5-10 group, however, showed a 26% increased in the number of opportunities taken and no significant increase in either rate or average dose taken. It is possible that the 5-10 group had more trouble switching between the HD operant and a FR operant (as they were very experienced on the HD operant by the end of the 21 day trial) so we are unsure whether this non-significant effect might be the result of a schedule confound.

Round-the-clock access to cocaine has been studied previously using a DT procedure however, the critical difference between the previous reports and the present studies involves the dosing procedure. All previous reports using a DT procedure used a fixed unit dose (i.e. an experimenter-selected dose). Instead of allowing free access on an FR1 schedule, which would allow rats typically to self-administer about 8-10 mg/kg/hr, a DT schedule could limit intake proportionately to unit dose and trails per hour. For example, a DT schedule that made available a dose of 1.5 mg/kg three times per hour would effectively restrict intake to a maximum of 4.5 mg/kg/hr. Such restrictions allow
for continual 24h/day access to be studied over many weeks without risk of toxicity (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003). From this work we have learned that higher doses or access parameters lead to binge-like intake, self-administering cocaine during virtually every trial for as long as 4 days (Fitch and Roberts 1993). If greater restrictions are placed on cocaine availability (i.e. fewer trials/hr or smaller doses) then cocaine self-administration becomes largely confined to the dark (active) phase of the light/dark cycle (Fitch and Roberts 1993; Roberts et al. 2002). Additionally we observed that the diurnal cycle of cocaine intake persists in the absence of external light-dark entraining cues (Bass et al. 2010), suggesting that there is a strong endogenous or internal regulator of cocaine self-administration. One issue with a DT procedure that uses a unit dose is that a single injection with a fixed ITI does not allow animals to ‘load up’ and achieve a preferred drug level. This suppression of the maximum amount of intake, on the one hand may help reveal control processes, such as a diurnal rhythm, that influence cocaine intake; but on the other hand, the observed results might have little applicability to a real world setting. We felt it was important to provide the opportunity for animals to attain a preferred blood level and then assess the influence of the day/night cycle.

We predicted that if we allow rats to “load up” or take as much as they want during the 5 minute trial that the diurnal rhythm might disappear as the rats were no longer restricted to a specific unit dose. We found however, that frequency of access played a large role initially, with the 5-55 and 5-25 groups both displaying circadian patterns of intake, while the 5-10 group maintained a consistent level of intake regardless of the time of day. Across the 21-day experiment, the 5-55 group remained relatively circadian throughout while the 5-25 group started to increase their intake into the light hours.
We have recently developed a novel hold-down (HD) self-administration procedure was (Morgan et al. 2009; Zimmer 2011) that allows animals to choose the amount of drug self-administered rather than receiving an experimenter chosen unit dose. In this procedure the amount of cocaine the animal receives is dependent upon how long the lever is held down. That is the syringe pump is delivering drug as long as the lever is depressed. It has been shown that the dose an animal self-administers at any given time is dependent upon the current brain level of cocaine (Zimmer 2011). The coupling of the DT and HD procedures allows is to examine changes in cocaine dose/brain level preference while allowing round-the-clock access to cocaine. Long term access to cocaine under this type of schedule could allow for the investigation of increasing dose over time.

The amount of drug an animal chooses to self-administer at any given time is a moving target. Rats self-administered different doses depending on the time of day, with higher amounts taken in the dark hours. Additionally, the amount of cocaine self-administered changed over the course of the study, with greater increases observed during the dark hours. The idea that animals may titrate their brain levels within a ‘preferred’ range is not a new one (Tsibulsky and Norman 1999; Wise et al. 1995a). The ability to get to this ‘preferred’ brain level of cocaine, which we have shown varies depending on the time of day, is important for 24-hour studies. Offering one unit dose makes it difficult to get to the ‘preferred’ level, in some cases it might not be enough and in others it may overshoot the desired level, which could be aversive to the animal. Additionally we show that the maintenance of a steady blood level of cocaine is not necessary in order to see escalation of rate and intake over time.
We have previously shown that animals will readily self-administer cocaine in 5 minute periods (Zimmer 2011). Additionally, it allows us to maximize the number of large doses self-administered (taken when brain cocaine levels are low) as forcing a range of time-out periods (using the DT procedure) causes brain cocaine levels to decline. We hypothesized that these repeating loading periods are important in the addiction process, and thereby wanted to examine the effect repeated loading would have on escalation. In the current study we examined the effects of 21 day cocaine self-administration, spanning a range of reasonable access parameters using a combination of DT and HD procedures. We found that escalation can occur in a number of dimensions that aren’t necessarily related nor is one better than the other. The three groups used in the present experiment span a range of reasonable access parameters. The 5-55 group gets one 5 min trial per hour, giving them a relatively low-level of access. In contrast, the 5-10 group gets four 5 min trials per hour, giving them a relatively high level of access. The 5-25 group, receiving two 5 min trials per hour, serves as a good middle ground between the two.

We show that escalation can occur in a number of different dimensions. The LgA procedure examines the rate of drug intake during a fixed-length (6 hr) cocaine self-administration session. Here we illustrate that escalation can occur in a number of dimensions, namely increasing dose per intoxication event and increasing the overall duration and frequency of intoxication events. The various different DT schedules used engendered different patterns of intake. Rats under a 5-55 schedule escalated their intake solely through increasing the dose per intoxication event. Rats under a 5-25 schedule escalated their intake both by increasing the dose per intoxication event and increasing the number of intoxication events. Rats under a 5-10 schedule escalated their intake solely by increasing the number of intoxication events. Escalations in rate of
intake were observed for both the 5-55 and 5-25 rats, but not the 5-10 rats. This suggests that escalation is a complex phenomenon brought on in a number of different ways.

The combination of the HD and DT procedures allows for the examination of aspects of escalation that were otherwise difficult to understand using a standard 6 hour escalation procedure, namely circadian influences and different types of escalation. Using these procedures animals were able to self-administer a preferred dose of cocaine in a spiking manner repeatedly in a 24 hour time period over the duration of the study. This spiking type of cocaine administration is in contrast to maintaining cocaine blood levels at a constant level for shorter durations of time as is observed with 6 hour FR1 schedule (LgA).

The time of day influences the duration of responding in regards to blood levels. The observation that rats under both a 5-55 and 5-25 HD schedule display a circadian pattern of drug intake can be understood by examining modeled brain cocaine levels. As we previously reported continued intake into the light phase was highly correlated with modeled brain cocaine levels (Dobrin 2011). Figure 7 illustrates the modeled brain cocaine levels for representative rats under the various HD schedules. Notice that, under a 5-55 schedule, the brain levels return to zero after each intoxication event and so it is not surprising that their intake does not extend much beyond the dark hours. Rats responding under a 5-25 schedule can keep their brain-cocaine levels above zero, but they are still relatively low. Under a 5-10 schedule, however, rats can maintain brain-cocaine levels above 5 μM, and thus their intake extends into the light hours. Rats responding under a 5-10 schedule take about as much cocaine (10 mg/kg) per day as
do rats on FR1 schedules 8-10 mg/kg/hr (Ahmed and Koob 1998; Brebner et al. 2000) indicating that we are at the upper level of cocaine availability per day.

Using a DT schedule, our lab has shown a strong circadian influence on the pattern of cocaine self-administration. The greater the restrictions on availability (i.e. fewer trials/hr or smaller doses) the more likely that cocaine intake will be confined to the dark (active) phase of the light/dark cycle (Fitch and Roberts 1993; Roberts et al. 2002). Additionally we have shown that this cycle persists even in the absence of external entraining cues (light-dark cycle) and therefore is likely regulated by some endogenous mechanism (Bass et al. 2010). These data are consistent with a growing literature documenting the involvement of circadian influences in drug reinforcement (Abarca et al. 2002; Akhisaroglu et al. 2004; Andretic et al. 1999; Fitch and Roberts 1993; Kurtuncu et al. 2004; Liu et al. 2005b; Lynch et al. 2008; Manev and Uz 2006b; McClung et al. 2005; Negus et al. 1995; Roberts et al. 2002; Roybal et al. 2007; Sleipness et al. 2005; Spanagel et al. 2005a; Spanagel et al. 2005b; Uz et al. 2005; Uz et al. 2003; Uz et al. 2004; Uz et al. 2002; Yuferov et al. 2003). The hypothesis addressed here is that endogenous circadian mechanisms serve to control drug intake and that a dysregulation of these systems is involved in cocaine binges. Animals self-administering under the 5-55 and 5-25 paradigms both display circadian patterns of intake, with greater amounts being self-administered in the dark hours vs the light. The circadian pattern is overcome as access increases, as the 5-10 rats displayed a relatively consistent level of intake over the 24 hour time period.

While the literature shows that LgA conditions produce robust increases in drug intake, the present results show that sustained blood levels do not appear to be necessary to produce this very important symptom in the addiction process. It is unclear which
represents the way in which human addicts consume cocaine, whether they maintain a consistent blood level over the course of a binge, or whether they consume cocaine in a repeated spiking manner over time. Addicts report seeking the rush of fast rising blood levels (through high dose binges) and data on drug taking frequency (every 10-30 min) indicate that inter-use intervals (either smoked or by injection) would probably cause dramatic oscillations of drug levels (Gawin 1991). We are interested in the fast rising edge of brain cocaine levels and whether they would be sufficient in themselves to result in escalation.
References


reinforcing properties: role of NMDA receptors. Psychopharmacology (Berl) 111: 332-8.


WHO (1992) The ICD-10 Classification of Mental and Behavioural Disorders


CHAPTER FOUR

BRAIN COCAINE LEVEL PREDICTS DURATION OF COCAINE CONSUMPTION

Carson V. Dobrin and David C. S. Roberts

In preparation for submission to *Psychopharmacology* September, 2011
### Introduction

Extended cocaine binges are characteristic of cocaine dependent individuals (First and Tasman 2010; Gawin 1989; 1991; Gawin and Kleber 1985). Often these binges occur multiple times a week lasting as long as 24 hours (Gawin 1989). Although we know some things about the factors that contribute to the initiation of a binge (drug availability, environmental context and current withdrawal state, (Gawin 1989)) we know little about the contributing factors into the maintenance and termination of a binge. When it comes to designing treatment strategies, a better understanding of the biological mechanism that contribute to cocaine bingeing behavior (specifically those that underlie the timing and termination of intake), could prove to be quite useful.

It is well known that animals, both non-human primates and rats, will self administer cocaine in binge-like behavior when given the right dose and availability parameters. When given unlimited access to cocaine animals will exhibit prolonged durations of consecutive cocaine taking, often lasting 11-22 hours (Deneau et al. 1969; Fowler et al. 2007; Johanson et al. 1976). One drawback to unlimited access is that after extended periods there are often considerable amounts of toxicity and lethality (Bozarth and Wise 1985). It is possible to avoid some of these deleterious effects, and still study binge-like behavior, by placing some constraints on availability or intake. One common technique is to simply restrict the hours of access or the number of overall sessions an animal is exposed to (Covington and Miczek 2005; Fowler et al. 2007; Mutschler et al. 2001; Tornatzky and Miczek 2000).

A Discrete Trials (DT) procedure, which is another method that constrains cocaine intake, has been useful for studying binge intake and for documenting interactions with the day-night cycle (Bass et al. 2010; Brebner et al. 2000; Espana et al. 2010; Fitch and
Discrete trails can be scheduled for a fixed number of times per hour round-the-clock for many days or weeks. The maximum hourly intake of drug is determined by the unit dose and the number of discrete trials per hour. If access is restricted to less than 4.5 mg/kg/hr (e.g., 3 trials/hr x 1.5 mg/kg/inj) then cocaine self-administration becomes largely confined to the dark (active) phase of the light/dark cycle ([Fitch and Roberts 1993; Roberts et al. 2002]). The observation that this diurnal cycle of cocaine intake persists in the absence of external light-dark entraining cues (Bass et al. 2010) suggests that there is a strong endogenous or internal regulator of cocaine self-administration.

This diurnal pattern of cocaine intake can be overcome with increased access to cocaine. At one extreme, Fitch and Roberts (1993) showed that rats given access to 7.5 mg/kg/hr on a DT schedule (1.5 mg/kg/inj x 5 discrete trials per hour) showed binge-like intake, self-administering cocaine during virtually every trial for as long as 4 days. More recently, a detailed parametric analysis of unit dose and trial frequency revealed that the probability of cocaine self-administration is determined by an interaction of the diurnal cycle and the drug levels in the brain at the beginning of a trial (Dobrin et al., Chapter 1). It appears that little, if any, cocaine is required to be on board for animals to self-administration during the dark phase; animals continue to self administer cocaine regardless of dose and current brain cocaine levels during the active phase of the light/dark cycle. However, during the light phase, self-administration becomes dependent on brain cocaine levels; higher drug levels were necessary for cocaine intake to be sustained.

It is important to note that a DT procedure using fixed unit doses can constrain cocaine blood levels in an important way that should be considered when interpreting the results.
described above. The DT procedure forces a time-out period between each injection thus ensuring a decline in blood levels between trials. This has been used to advantage to investigate the minimum blood level that would sustain responding. However, the procedure also constrains the upper blood level that can be reached. During a typical free access (FR1) session, rats tend initially to self-administer several injections of cocaine in rapid succession producing a fast rise in blood levels (Ettenberg et al. 1982; Wilson et al. 1971). This has been termed the ‘loading phase’ (Tornatzky and Miczek 2000). A relatively constant injection rate is seen thereafter resulting in the maintenance of what has been speculated to be a ‘preferred’ drug level (Oleson et al. 2009). The DT procedure does not allow injections to be self-administered at short intervals and modeling of brain concentrations (see below) have shown that the presumed ‘preferred’ drug concentration is never achieved. It remains unclear how preventing animals from achieving high blood/brain levels might affect binge-like responding.

In the present study a combination of procedures were used that not only allowed a loading phase to occur but also ensured a decline in blood levels between trials. Instead of the discrete trial resulting in the delivery of a fixed unit dose, animals were given access to a drug lever that controlled the delivery of cocaine on a novel hold-down procedure (Morgan et al. 2009). Under this schedule the pump delivered cocaine for the duration that the lever was being held down. A five minute trial was used which we have found to be sufficient for animals to ‘load up’ to high blood levels (Zimmer 2011). A range of inter-trial intervals (10, 15, 25 and 55 min) were investigated which produced proportionate degrees of decline in cocaine blood levels. Here we confirm previous results showing that the continuation of a binge into the light phase depends on how low blood levels have dropped when the opportunity of self-administer is presented; the peak levels of cocaine do not appear to be an important factor.
Methods

Animals. Male, Sprague-Dawley rats (Harlan, Indiana USA) weighing 300-350 grams at the time of surgery were used in all experiments. After arrival animals acclimated in the vivarium for a minimum of 7 days before surgery. Throughout acclimation and over the entire course of the study rats were maintained on a reverse 12 hour light dark cycle (lights on at 3:00 pm) and had ad libitum access to food and water. Rats were housed in custom made steel operant chambers (30 x 30 x 30 cm). All procedures were conducted under approval of the Animal Care and Use Committee at Wake Forest University Health Sciences.

Surgery. All surgeries were performed under aseptic conditions. Animals were anesthetized (100 mg/kg ketamine & 8 mg/kg xylazine, i.p.) and implanted with chronically indwelling Silastic® jugular catheters (house made). An externalized access port was anchored dorsally on the back and the tubing was passed over the shoulder and into the jugular vein (Roberts and Goeders 1989). A stainless steel tether was used to protect the Tygon® tubing that attached the access port to the swivel (Instech Laboratories, Inc., Plymouth Meeting, PA., USA) which was located above the self-administration chamber. An infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) located outside the chamber was connected to the swivel. In total, 12 surgeries were preformed. 4 rats were excluded either because they did not acquire self-administration behavior or their catheter lost patency during the experiment.

Drugs. Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC, USA), was dissolved in a solution of sterilized saline (0.9%) and passed through a microfilter (0.45-μm pore size). A 2.5 mg/ml concentration was used
for acquisition and 5 mg/ml was used for the remainder of the study (pump speed 1.6 ml/min).

**Self-administration.** Approximately 3 days after surgery rats began cocaine self-administration training. Subjects were given access to cocaine (0.75 mg/kg, delivered in 0.1 ml sterile saline over 4 sec) on an FR1 schedule during daily 6 hour sessions. Intake was restricted to a maximum of 20 infusions per session. Acquisition was defined as two consecutive days with 20 injections and consistently spaced inter-injections intervals. After meeting acquisition criteria rats were given access to cocaine using a hold down (HD) procedure for three hours. After rats were able to consistently titrate their brain cocaine levels (usually 2-4 days) they were switched to a DT-HD procedure (hereon referred to as 5-55, 5-25, 5-15 and 5-10) where the animals were given 5 min to hold down the lever and receive drug followed by a time out period (55, 25, 15 or 10 min) for 24 hours every-other day.

**Data analysis.** All statistics were performed using SigmaPlot (version 11). All data are represented as mean ± SEM unless otherwise noted. One-way analysis of variance (ANOVA) in conjunction with a Fisher LSD post hoc test or a linear regression was used to determine significance.

**Modeling brain cocaine levels.** A two-compartment model developed by Pan (Pan et al. 1991) was used to mathematically estimate whole brain levels of cocaine. The model has been calibrated for rats receiving a chronic IV cocaine regimen. Briefly, we used the equation

\[ c = \frac{dk}{\nu(\alpha - \beta)} (e^{-\beta} - e^{-\alpha}) \]
where $c$ is the concentration in the brain, $d$ is the dose, $k$ is the rate of flow from the blood to the brain [0.233], $v$ is volume of the brain [0.044], $\alpha$ and $\beta$ [0.642 and 0.097 respectively] are constants representing the flow of cocaine between the blood and brain compartments and the elimination of cocaine from the body, and $t$ is the time in minutes since the last infusion. The total concentration of cocaine was determined by calculating each infusion of cocaine independently then summing them in 5 second intervals (Stuber et al. 2005b).

**Results**

The hours of consecutive cocaine taking increases with increased access (see figure 1 for a representative animal). Increased access leads to longer durations of cocaine taking such that 5-55 < 5-25 < 5-15 ≤ 5-10 (see figure 2). Data is displayed as average hours of consecutive cocaine taking for each of the different session types. A one-way repeated measures ANOVA revealed a significant effect of session type [$F(3,21) = 14.04, p < 0.001$]. Note that animals when self administering on the 5-55 paradigm average 6 hours of intake, which is the length of the initial dark period.
Figure 1. A representative animal self-administering on a 5-55, 5-25, 5-15 and 5-10 HD schedule. Data is displayed as modeled brain-cocaine level for a 24-hr time period. The time the lights were out are indicated by the black bars. Notice that the various conditions lead to different peak and trough values. When self-administering on a 5-55 schedule the modeled brain-cocaine level returns to zero after every trial, in contrast when self-administering on a 5-10 schedule, the modeled brain-cocaine level stays between 5-15 µM the whole time.
**Figure 2.** Average hours of consecutive cocaine taking for each session type; 5-55, 5-25, 5-15 and 5-10. Note that the duration of cocaine taking increases as access increases. A Fisher LSD post hoc test indicated 5-25, 5-15 and 5-10 were all significantly different than 5-55 (indicated by *, p<0.01) and that 5-15 and 5-10 were significantly different from 5-25 (indicated by †, p<0.05).
There is relatively no difference in the peak brain-cocaine concentration across the different access parameters. Animals were allowed to self-administer as much as they wanted for each 5 min trial, and that did not change much across conditions (see figure 3a). The data are displayed as the average modeled peak brain cocaine level (per 24 hour session) for each of the different session types. A one-way repeated measures ANOVA revealed a significant effect of session type \( F(3,21) = 10.34, p = 0.02 \). Further analysis with a Fisher LSD test indicated the only significant difference was with the 5-55 session type. Note that the average preferred brain cocaine level ranged from 10-12 µg regardless of the session type.

Modeled trough or low point in brain cocaine concentrations was different across conditions. With increased access animals were able to maintain higher brain cocaine levels (despite the time out period) and therefore brain cocaine levels increased with increasing access parameters; 5-55 < 5-25 < 5-15 < 5-10 (see figure 3b). The data are displayed as the average modeled trough brain cocaine level (per 24 hour session) for each of the different session types. A one-way repeated measures ANOVA revealed a significant effect for session type \( F(3,21) = 124.03, p < 0.001 \).
Figure 3. Illustrations of the peak and trough brain-cocaine levels across the four conditions; 5-55, 5-25, 5-15 and 5-10. A) Average modeled peak brain-cocaine concentration across the four conditions. Note that the level doesn’t change much between 5-25, 5-15 and 5-10. A Fisher LSD post hoc test indicated 5-25, 5-15 and 5-10 were all significantly different than 5-55 (indicated by *, p<0.05). B) Modeled average trough brain-cocaine concentration across the four conditions. Note the step-wise increase as access increases. A Fisher LSD post hoc test indicated 5-25, 5-15 and 5-10 were all significantly different than 5-55 (indicated by *, p<0.001), 5-15 and 5-10 were significantly different from 5-25 (indicated by †, p<0.001) and that 5-10 was significantly different from 5-15 (indicated by @, p<0.001).
Figure 3.

A.

B.
**Modeled trough brain cocaine levels correlate with hours of consecutive cocaine taking.** There is no significant correlation with the peak ($p=0.37$, data not shown) brain level values and the duration of cocaine taking. There is however a significant correlation with the trough ($r^2 = 0.44$, $p < 0.001$, see figure 4) brain cocaine levels and the duration of cocaine taking where the higher the level is, the longer the bout of cocaine taking persists.
Figure 4. Correlation of trough brain-cocaine levels and the duration (in hours) of consecutive cocaine taking for each of the four different conditions; 5-55, 5-25, 5-15 and 5-10. Note that the trough level tends to be greater for higher access conditions.
Discussion

Binge cocaine administration is typical of cocaine addicts. Rodents also binge, however, previous literature is based on the maintaining of a particular blood level over time rather than a number of discrete intoxicating events that occur in close proximity to one another which could be more indicative of the nature of a human cocaine binge. We had previously shown that brain cocaine levels play a crucial role in the continuation of a bout of responding into the light hours (see Chapter 1); however we were unsure what aspect of the brain cocaine level (peak or trough) that influenced the continuation of consumption. Using a combination of a DT and HD procedure we were able to allow animals to self administer to their preferred brain cocaine level and various time out periods engendered different trough values. Here we show that the trough, or low point, in brain cocaine levels correlates with the hours of consecutive cocaine taking, where higher trough levels are indicative of longer durations of consumption.

Previous DT studies have taken advantage of constraining hourly intake in order to allow long term 24 hour self-administration without the deleterious toxic effects seen in other unlimited access studies (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003). This is done by allowing the administration of one unit dose a set number of times per hour. We have observed that animals will exhibit circadian-like patterns of intake, with the majority of cocaine being self administered in the dark hours, at lower doses and trials per hour (Roberts et al. 2002). When the dose of cocaine or the number of opportunities per hour increases, the circadian control is often overcome, and animals self administer large amounts regardless of the time of day. Allowing animals to only self administer cocaine on the DT schedule every other day we find a recapitulation of the first day binge often observed in DT studies (Roberts
unpublished observation). Using this every other day pattern we are able to examine binge-like behavior, and are not confounded by animals taking a day off after heavy intake (which is often observed with high dose and trials per hour combinations).

The current study uses the DT schedule in a similar manner; hourly intake is constrained in order to achieve 24 hour access without toxicity. One difference is that animals are able to self administer an unlimited amount during each trial as opposed to self administering an experimenter-selected unit dose. Here we used an every-other day paradigm because we were interested solely in the duration of cocaine intake for the various different access parameters (and therefore the blood levels resulting from them) and wanted to use a procedure that is conducive to observing a recapitulation of the binge over days.

We have shown that the dose an animal self administers on a HD schedule is dependent upon current brain cocaine levels (with lower brain levels leading to higher doses) (Zimmer 2011). In the current study we show that continued intake is dependent upon the trough, or low point, in brain cocaine levels. Allowing animals to self administer as much as they want for each trial we were able to show that the preferred brain cocaine level remains relatively unchanged with access parameters, and overall is in the range of 10-13 µM. It did not matter where the brain cocaine level started, as it varied with the different access parameters, animals brought their levels up to a consistent level every time. This supports the idea that there may be a preferred brain cocaine level.

This protocol was designed so that animals were unable to maintain a consistent brain cocaine level over time. Forcing time out periods caused brain levels to fall from the preferred level. Unlimited access to cocaine results in long durations of cocaine consumption often lasting 11-22 hours (Fowler et al. 2007). These animals are taking
cocaine in a manner that maintains their brain cocaine levels and a constant level. We were interested in the mechanisms that come in to play when animals are in a drug seeking mode. Meaning we wanted to better understand some of the mechanisms that may come in to play when an animal is looking to continue a bout of cocaine taking, rather than maintaining their current brain level. We found that the low point or trough in brain cocaine levels is tightly correlated with continued cocaine consumption, where at some point in the light hours, brain levels can fall below a certain level, resulting in ceased responding. The fact that this type of control exists in the light hours but not the dark hours suggests there may be some type of endogenous circadian underpinnings underlying the behavior.

Here we modeled brain cocaine levels with equations that have been previously well established using a variety of procedures to illustrate the temporal relationship between modeled brain-cocaine concentration, NAc dopamine levels (Hermans et al. 2008; Shou et al. 2006; Wise et al. 1995b) and cocaine-induced locomotor behavior (Shou et al. 2006). These procedures include self-administration (Ahmed and Koob 2005; Samaha et al. 2002; Zernig et al. 2007), electrophysiology (Nicola and Deadwyler 2000; Peoples and Cavanaugh 2003; Peoples et al. 2007; Peoples et al. 2004), microdialysis (Wise et al. 1995b) and voltammetric (Hermans et al. 2008; Stuber et al. 2005a; Stuber et al. 2005b).

Circadian variability in drug reward is becoming an increasingly popular area of study, as an understanding of the neurobiological correlates of decreased interest in drug consumption could lead to new treatment avenues. A cyclical nature in cocaine intake, where the majority is consumed in the dark hours, is revealed when self-administration is limited to a specific number of discrete trials per hour (Fitch and Roberts 1993, Roberts, 2002; Negus et al. 1995). We have shown that this cyclical pattern of intake persists
despite the absence of external entraining cues such as a light dark cycle (Bass et al. 2010), suggesting the possibility of endogenous regulator of cocaine intake. Others have shown that there appears to be a cyclical nature to other drug-related behaviors such as locomotor sensitization and conditioned place preference to cocaine (Akhisaroglu et al. 2004; Kurtuncu et al. 2004; Sleipness et al. 2005; Uz et al. 2003; Uz et al. 2002). Additionally, circadian genes such as Clock and Period have been shown to influence the behavioral effects of drugs of abuse (Abarca et al. 2002; Andretic et al. 1999; Liu et al. 2005b; McClung et al. 2005; Roybal et al. 2007; Spanagel et al. 2005a; Spanagel et al. 2005b) and drugs of abuse influence circadian genes (Lynch et al. 2008; Uz et al. 2005; Yuferov et al. 2003), for review see (Manev and Uz 2006b). This suggests a positive feedback loop where drugs influence circadian genes and circadian genes influence the behavioral effects of drugs which could become increasingly out of control over continued exposure.

In the current study we show that the duration of continued cocaine consumption is tightly correlated to the trough in brain cocaine levels, where the higher the trough is, the longer the duration of cocaine taking is. Better understandings of the biological mechanisms that come in to play during bouts of cocaine taking are important for the formation of new treatment strategies. These data suggest that there could be a circadian underpinning controlling continued cocaine taking and would be a good avenue of continued investigation.
REFERENCES


SUMMARY AND CONCLUSIONS
This dissertation consists of three experiments that were designed to investigate the changes in the reinforcing effects of cocaine relative to time of day. Chapter 2, *Circadian control of binge-like cocaine self-administration*, examined the complex interaction of dose, availability and time of day on the duration of cocaine consumption. The data show that access to very little cocaine was necessary to maintain intake during the dark phase. By contrast, cocaine self-administration during the light phase was strongly influenced by dose and availability. Additionally, continued intake into the light phase was positively correlated with modeled brain cocaine levels. Higher concentrations of cocaine appear to be necessary to sustain drug taking during the inactive phase of the light/dark cycle.

In chapter 3, *Transitions in the addiction process revealed by a novel hold-down procedure*, we investigated whether maintained blood levels are necessary for escalation of drug intake over time or whether intermittent intoxicating cocaine events are sufficient. Here we gave intermittent 24-hour access to cocaine over an extended period of time using a hold-down (HD) procedure in which animals are able to self-administer as much cocaine as they chose over a 5-min time period. We illustrated two distinct forms of escalation over time with different intermittent access procedures, showing that sustained blood levels do not appear to be necessary to produce this very important symptom the addiction process.

In the final chapter, *Brain cocaine levels predict the duration of continuous cocaine consumption*, we examined the influence of brain-cocaine levels and time of day on continued bouts of cocaine taking by combining a DT procedure with a HD procedure to produce different peak and low point (or trough) brain cocaine levels in order to determine which (if any) contributed to continued consumption of cocaine. We showed that the duration of continued cocaine consumption is tightly correlated to the trough in
brain cocaine levels, such that the higher the trough is, the longer the duration of cocaine taking is.

In the appendix we examined whether giving animals long-term exposure on a discrete trials DT schedule increase responding on a PR schedule assessed after the DT exposure and found two key things. First, giving animals every-other day access to cocaine increases the overall amount of cocaine self-administered, as compared to animals experiencing the same number of sessions every day. Second we found that at most of the doses tested, every-other day access lead to increased breakpoints (as compared to every-day access to the same dose) across the dose response curve except for the highest dose tested.

Taken together, these data highlight some critical themes in cocaine addiction research as it relates to circadian influences in cocaine reinforcement: escalation of intake over time, binge-like cocaine intake and the influence of brain cocaine levels on cocaine consumption.

**Circadian influences**

An understanding of the neurobiological correlates of decreased interest in drug consumption is important as it could lead to new treatment avenues, making circadian variability in drug reward a valuable area of study. A cyclical nature of cocaine intake, in which the majority is consumed in the dark hours, is revealed when self administration is limited to a specific number of discrete trials per hour (Fitch and Roberts 1993, Roberts, 2002 ; Negus et al. 1995). We have shown that this cyclical pattern of intake persists despite the absence of external entraining cues such as a light/dark cycle (Bass et al. 2010), suggesting the possibility of endogenous regulator of cocaine intake. Others have shown that there appears to be a cyclical nature to other drug-related behaviors such as
locomotor sensitization and conditioned place preference to cocaine (Akhisaroglu et al. 2004; Kurtuncu et al. 2004; Sleipness et al. 2005; Uz et al. 2003; Uz et al. 2002). Additionally, circadian genes such as Clock and Period have been shown to influence the behavioral effects of drugs of abuse (Abarca et al. 2002; Andretic et al. 1999; Liu et al. 2005; McClung et al. 2005; Roybal et al. 2007; Spanagel et al. 2005a; Spanagel et al. 2005b) and drugs of abuse influence circadian genes (Lynch et al. 2008; Uz et al. 2005; Yuferov et al. 2003), for review see (Manev and Uz 2006). This suggests a positive feedback loop in which drugs influence circadian genes and circadian genes influence the behavioral effects of drugs which could become increasingly out of control over continued exposure.

**Binge cocaine intake**

One behavioral phenomenon that parses apart cocaine users and cocaine addicts is a transition from recreational drug use to binge-abstinence cycles (First and Tasman 2010). Very little is known about the factors contributing to the maintenance and termination of these binges. Previous preclinical studies have suggested that circadian mechanisms can influence cocaine self-administration if hourly intake is restricted; however, time-of-day may have little effect with unlimited access. We showed that access to very little cocaine was necessary to maintain intake during the dark phase. By contrast, cocaine self-administration during the light phase was strongly influenced by dose and availability. Continued intake into the light phase was highly correlated with modeled brain cocaine levels. Higher concentrations of cocaine appear to be necessary to sustain drug taking during the inactive phase of the light/dark cycle. Additionally, we show that the trough, or low point, in brain cocaine levels correlates with the hours of consecutive cocaine taking, where higher trough levels are indicative of longer durations of consumption. Taken together these results provide clear evidence of circadian
variability in cocaine self-administration in rodents. One implication of these results is the possibility that recreational human drug use may initially be controlled (at least partially) by underlying circadian mechanisms, and the transition from recreational to binge-abstinence cycles could be a result of a breakdown of these circadian influences. There are established treatment options for circadian disorders (for example bright light therapy and melatonin) that could potentially help a disrupted circadian cycle get back on track.

**Escalation of drug intake**

Escalation of drug intake is an important criterion in the diagnosis of drug dependence (APA 2000; Cami and Farre 2003; Gawin 1991; WHO 1992) and the escalation phenomenon has become a major focus of the preclinical self-administration literature. It has been repeatedly demonstrated that daily long-access (LgA, 6 hour) cocaine self-administration sessions result in an increased rate of drug intake. Modeling the brain cocaine levels shows a rapid spike at the beginning of a LgA session followed by maintained blood levels for the duration of the 6-hr period. We showed that maintained blood levels are not necessary for escalation of drug intake, and that intermittent intoxicating events are sufficient. Additionally, we showed that escalation can occur in a number of dimensions, namely increasing dose per intoxication event and increasing the overall duration and frequency of intoxication events. The various different DT schedules used engendered different patterns of intake. Rats under a 5-55 schedule escalated their intake solely through increasing the dose per intoxication event. Rats under a 5-25 schedule escalated their intake both by increasing the dose per intoxication event and increasing the number of intoxication events. Rats under a 5-10 schedule escalated their intake solely by increasing the number of intoxication events. Escalations in rate of intake were observed for both the 5-55 and 5-25 rats, but not the 5-10 rats. This suggests that escalation is a complex phenomenon brought on in a number of different
ways. Until the factors underlying these different forms of escalation are better elucidated, we may never fully understand the phenomenon of escalation in humans. However, the idea that escalation could come in different forms in general could be important in the clinical setting, as different treatment strategies may better target these different forms. For example, an individual who escalates their intake by increasing the number of intoxication events that occur within a 24-hr time period, may be better suited for treatment strategies designed at targeting circadian influences. By contrast, an individual who escalates their intake by increasing the amount of drug they take per intoxication event, may be better suited for treatment strategies designed to target compulsive behavior.

**Discrete trial procedures**

Whether time-of-day influences the initiation and maintenance of cocaine intake appears to depend on dose and access conditions. It has been well established that unlimited access to cocaine results in high intake binges lasting 11-22 hours (Bozarth and Wise 1985; Deneau et al. 1969; Fowler et al. 2007; Johanson et al. 1976; Tornatzky and Miczek 2000). However, by using a DT procedure to limit hourly intake, diurnal patterns of cocaine self-administration emerge. It has been repeatedly shown that a predictable rhythm of drug intake is associated with the day/night cycle when hourly intake is constrained (Bass et al. 2010; Brebner et al. 1999; Espana et al. 2010; Fitch and Roberts 1993; Lynch and Roberts 2004; Roberts and Andrews 1997; Roberts et al. 2002; Roberts et al. 2003). The highest probability of cocaine intake is during the last 5 hrs of the dark phase and declines at the time of transition to the light phase. In the appendix we show that every-other day access leads to greater cocaine intake and higher cocaine breakpoints on a PR schedule (at all except the highest dose tested). We also showed that access to very little cocaine in necessary to maintain self-
administration behavior in the dark hours, however, whether cocaine self-administration continued into the light phase was shown to be markedly dependent on dose and access conditions. We found that the diurnal rhythm of circadian intake can be over-ridden with high cocaine dose and availability.

One issue with the DT procedure that uses a unit dose is that a single injection with a fixed inter-trial-interval (ITI, time between trials) does not allow animals to ‘load up’ and achieve a preferred drug level. This suppression of the maximum amount of intake, on the one hand may help reveal control processes, such as a diurnal rhythm, that influence cocaine intake; but on the other hand, the observed results might have little applicability to a real world setting. We felt it was important to provide the opportunity for animals to attain a preferred blood level and then assess the influence of the day/night cycle.

**Hold-down procedures**

Until recently giving rats control over the self-administered dose was impossible. However, a novel hold-down (HD) self-administration procedure was established (Morgan et al. 2009; Zimmer 2011) that allows animals to choose a preferred amount of drug rather than receiving an experimenter-chosen unit dose. In this procedure the amount of cocaine the animal receives is dependent upon how long the lever is held down. That is the syringe pump is delivering drug as long as the lever is depressed. It has been shown that the dose an animal self-administers at any given time is dependent upon the current brain level of cocaine (Zimmer 2011). The coupling of the DT and HD procedures allows is to examine changes in cocaine dose/brain level preference while allowing round-the-clock access to cocaine. Long-term access to cocaine under this type of schedule could allow for the investigation of increasing dose over time.
We found that the preferred dose of cocaine at any given time is a moving target. Rats self-administered different doses depending on the time of day, with higher amounts taken in the dark hours. Additionally, the preferred amount of cocaine changes over the course of a study, with greater increases observed during the dark hours. The ability to get to a preferred brain level of cocaine depending on the time of day is important, especially for 24-hour studies. Offering one unit dose makes it difficult to get to a preferred level, in some cases it might not be enough and in others it may over-shoot the desired level, which could be aversive to the animal. Additionally we show that the maintenance of a steady blood level of cocaine is not necessary in order to see escalation of rate and intake over time.

The data collected using different ITIs in chapters 2 and 3 provided information on how a brief abstinence period influences drug taking during the 5 min trial. We have previously shown (Zimmer 2011), using daily 6hr sessions, that the dosage self-administered is proportional to the calculated or modeled blood levels.

**A possible disconnect between bingeing and escalation**

This dissertation focuses on modeling two distinct (but often hard to separate) aspects of cocaine addiction, binge use and escalation over time. Binge cocaine taking refers to repeated cocaine use lasting anywhere from hours to days and occurring multiple times per week (Gawin 1989). In this dissertation we modeled bingeing by reporting consecutive injections of cocaine, where brain levels stayed above zero. In chapters 2 and 4 of this dissertation we report that brain levels in the light hours are a predictor of continued cocaine intake, such that higher levels are necessary for longer durations of cocaine taking. Escalation of intake refers to a more long-term increase in overall drug taking. We reported escalation both as an increase in overall dose per opportunity and
as an increase in the overall opportunities taken, both which increase the amount of drug consumed per 24 hours. In chapter 3 of this dissertation we report that intermittent spikes, which force brain-cocaine levels to decrease, are sufficient for producing escalation of intake, and thus that maintained brain-cocaine levels are not necessary for escalation. Initially it may seem that these two points are in contrast with one another, where we state that maintained brain levels are not necessary for escalation (chapter 3) and that brain levels are important for bingeing (chapters 2 and 4), so an additional clarification needs to be made. The argument is that there is a point which brain cocaine levels need to stay above, particularly in the light hours, in order to see binge-like cocaine self-administration. This is not related to the observation that maintained blood levels are sufficient to produce escalation of intake over time. We have not tested whether the occurrence of binge-like self-administration is necessary for long-term escalation of intake. In order to test this we would need to prevent the occurrence of consecutive cocaine taking (most likely by long time-out periods) and see if these animals still escalate over time.

**Modeling brain-cocaine levels**

Throughout this dissertation, a two-compartment model developed by Pan (Pan et al. 1991) was used to mathematically estimate whole brain levels of cocaine. The model has been calibrated for rats receiving a chronic IV cocaine regimen. The total concentration of cocaine was determined by calculating the effects of each infusion of cocaine independently then summing them in 5 second intervals (Stuber et al. 2005b).

Modeling brain cocaine levels provides some insight into the factors that contribute to the maintenance and termination of cocaine taking. Allowing a range of access conditions produces a wide spectrum of brain levels. Predictably, higher doses and
access conditions allowed drug concentrations to oscillate within a much higher range. The observation that rats self-administering under lower-access conditions (i.e. 5-55 and 5-25) display a circadian pattern of drug intake can be understood by examining modeled brain cocaine levels. Continued intake into the light phase is highly correlated with modeled brain cocaine levels (Chapter 1). Under a 5-55 schedule, brain levels return to zero after each intoxication event and so it is not surprising that rat’s intake does not extend much beyond the dark hours. Rats responding under a 5-25 schedule can keep their brain-cocaine levels above zero, but they are still relatively low. Under access conditions that allow more intake, for example a 5-10 schedule, rats can maintain higher brain cocaine levels and thus their intake extends into the light hours. Rats responding under a 5-10 schedule take about as much cocaine (10 mg/kg) per day as do rats on FR1 schedules 8-10 mg/kg/hr (Ahmed and Koob 1998; Brebner et al. 2000) indicating that we are at the upper level of cocaine intake per day.

The accuracy of the equations used to model brain cocaine concentrations in the current study has been well established. Self-administration (Ahmed and Koob 2005; Samaha et al. 2002; Zernig et al. 2007), electrophysiology (Nicola and Deadwyler 2000; Peoples and Cavanaugh 2003; Peoples et al. 2007; Peoples et al. 2004), microdialysis (Wise et al. 1995b) and voltammetric (Hermans et al. 2008; Stuber et al. 2005a; Stuber et al. 2005b) studies have all used these equations to illustrate the temporal relationship between modeled brain-cocaine concentration, NAc dopamine levels (Hermans et al. 2008; Shou et al. 2006; Wise et al. 1995b) and cocaine-induced locomotor behavior (Shou et al. 2006). We believe these equations closely model actual brain cocaine levels however it is important to note that the conclusions drawn were based on relative changes and not the absolute magnitude.
It is possible, however, that some of the assumptions underlying the brain-cocaine model equation (i.e. the constants) used here could change over time, especially after long-term, high intake cocaine self-administration. The constants used are c (the concentration in the brain), d (the dose), k (the rate of flow from the blood to the brain [0.233]), v (volume of the brain [0.044]), α (the flow of cocaine between the blood and brain compartments [0.642]), β (the elimination of cocaine from the body), and t (the time in minutes since the last infusion). It is not unreasonable to wonder if some of these might change after cocaine exposure, specifically those representing the rate of flow from the blood to the brain, the volume of the brain, and the elimination of cocaine from the body. For example, it has been shown that long term cocaine users have decreased gray matter in the orbitofrontal cortex (Alia-Klein et al. 2011) as compared to non-drug users and over time. This raises the possibility that within our animal model there could be some changes in overall brain volume as a result of long-term cocaine exposure over time. It is important to note that the changes observed in humans happen over the period of many years while the period of drug exposure in our rodents is no more than several months.

Perhaps the biggest potential discrepancy is with the constant β, or the elimination of cocaine from the body. The pharmacokinetic profile of cocaine in the periphery and how that may change over time needs to be accounted for. Cocaine is rapidly eliminated from the brain, returning to half-peak levels within 17-30 min (Gorelick 2009). In humans, 95% of cocaine is metabolized in the liver, where cocaine is broken down by carboxylesterases (primarily) and butrylcholineserases (also present in plasma, brain and lungs) into benzoylecgonine (primary metabolite present in urine) and ecgonine methylester. The remaining cocaine is metabolized into norococaine in the liver. In rodents the latter pathway is predominant (Gorelick 2009). It has been shown that the
half-life of cocaine in chronic cocaine users (as compared to occasional users) is longer, despite the fact that benzoylecgonine urine levels remain the same (Moolchan et al. 2000). Additionally, it has been suggested that there is a difference in the process of accumulation of cocaine in the body which results in a prolonged elimination period in heavy users (Jufer et al. 2000). These data raise the possibility that, at least in humans, the rate constant for elimination of cocaine can change over time in association with heavy cocaine use. If this holds true in rodents then a changing rate of elimination could affect our modeling results, however, if anything we would be underestimating the level of cocaine in the brain as the rate constant would become longer, not shorter.

One other discrepancy that needs to be addressed in regards to modeling the brain cocaine level is the differences observed in overall level across laboratories using similar equations. In this dissertation we report brain cocaine levels that range from 0 to around 30 µM. Others have reported a wide range of brain cocaine levels such as 0-0.4 µM (Ahmed and Koob 2005), 0-4 µM (Samaha et al. 2002), 0-25 µM (Nicola and Deadwyler 2000), 0-12 µM (Peoples et al. 2007), 0-15 nM (Wise et al. 1995a) and 0-20 µM (Stuber et al. 2005b). The difference in modeled brain cocaine level across studies is a factor that needs to be figured out. It is important to reiterate that even if our levels are off in the calculations, the conclusions drawn were done so using relative change, not overall magnitude.

All together, these potential discrepancies highlight the importance of experimentally verifying the brain cocaine levels modeled here with actual brain cocaine levels measured by microdialysis. There is a possibility that the constants used in the equation to model brain cocaine levels could change over time simply due to the prolonged cocaine exposure. Verifying the modeled levels could answer this question.
A ventral to dorsal shift

In this dissertation we argue that intermittent intoxicating events (repeated loading of brain cocaine levels) are sufficient for producing a behavioral phenotype associated with addiction; the escalation of intake over time. This is in contrast to the more commonly used long access model (Ahmed and Koob 1998) where escalation is observed after maintaining a constant brain cocaine level every day for 6 hours. One important question to address is why, when given unlimited access to cocaine with no time-out periods to force brain cocaine levels down, animals maintain constant blood levels rather than repeatedly loading within a session?

One phenomenon that has been widely discussed in the literature is the so-called ‘ventral to dorsal shift’ or ‘spiraling’ whereby cocaine initially exerts the majority of its effects on the ventral striatum (vSTR) and after repeated exposure there is a shift where the functional activity is now centered more in the dorsal striatum (dSTR) (Everitt and Robbins 2005; Haber 2003; Haber et al. 2000; Porrino et al. 2007; Vanderschuren et al. 2005). It has been further reported that the vSTR, along with the PFC, is more responsible for impulsive actions, and more specifically the vSTR core is associated with drug seeking (Everitt and Robbins 2005). In contrast, the dSTR has been shown to be more important in compulsive behaviors, or drug taking (Belin and Everitt 2008). This shift has been shown to occur after long-term cocaine use (across sessions) that is representative as a transition from a behavior that occurs across one neural pathway gradually towards another. Transitions in neural pathways often occur as the usage of one becomes more preferred and is thus made stronger over another, for example, the improvement in performance after practice. It is possible that there could be some sort of ‘within-session shift’ whereby the beginning of a self-administration session is typified by impulsive, vSTR-regulated behavior that transitions (after within-session cocaine
exposure) to a compulsive, dSTR regulated behavior. Brain-cocaine levels would likely play a role in this shift, such that high levels lead to a shift to dSTR regulated behavior and low or zero levels lead back to vSTR regulated behaviors. These within-session shifts could be representative of future, long-term consequences of cocaine and become stronger over time until a point where the compulsive behavior is more common. One way to test this idea is to use an intrastriatal disconnection procedure (Belin and Everitt 2008) which in theory should prevent the shift from occurring (at least to some extent). If by doing this the animals do not show a proclivity towards maintained brain cocaine levels over time, yet still self-administer intermittent spikes it could mean more attention should be given to this line of thought.

Future Directions

Taken together these data suggests that there is a rhythm of drug taking that occurs in cocaine self-administration. The question of what may underlie that rhythm needs to be specifically addressed. The most likely suspect, and one that is assumed throughout this dissertation, is a neurobiological circadian influence. One way to test this (using behavioral measures) is by examining the influence of melatonin rhythms on cocaine self-administration. Melatonin is a hormone synthesized and released by the pineal gland with the highest amount being released at night (regardless of the species being diurnal or nocturnal). This cycle of melatonin production relies on input from the suprachiasmatic nucleus (SCN) and is one of the most well characterized physiological circadian rhythms. Interestingly, it has been shown that exogenous melatonin can block locomotor sensitization to cocaine in rats (Sircar 2000) and also helps to reduce anxiety-like behaviors produced during cocaine withdrawal (Zhdanova and Giorgetti 2002). Additionally, commonly observed daily differences in the expression of locomotor sensitization and CPP to cocaine (both greater during the light hours) is abolished in
animals that either do not have rhythmic melatonin expression (Uz et al. 2002) or in pinealectomized mice (Uz et al. 2003). The influence of melatonin rhythms on cocaine self-administration has not been examined. In a rodent, the pineal gland sits outside the blood-brain barrier, dorsal to the brain, just underneath the skull. This is an area easy to access, and thus could be lesioned or removed with little damage to other brain structures. One might expect that the typical pattern of cocaine self-administration observed (i.e. greater in the dark hours) might be disrupted in the absence of melatonin rhythms. If this is the case then it suggests that an intact circadian melatonin rhythm plays a role in cocaine self-administration.

Summary and final conclusions

A better understanding of the neurobiological mechanisms underlying cocaine bingeing has implications for developing treatment strategies for addicts. In chapter 2 we showed that self-administration was much more sensitive to changes in dose and availability in the light hours as compared to the dark hours. Since testing in the dark phase is relatively unaffected by changes in dose or trials/hr whereas the reverse is true in the light phase, we suggest that the most sensitive timeframe to test putative therapeutic agents for their ability to dampen binge intake would be during the transition for dark to light.

While the literature shows that LgA conditions produce robust increases in drug intake, in chapter 3 we showed that sustained blood levels do not appear to be necessary to produce this very important symptom the addiction process. It is unclear which represents the way in which human addicts consume cocaine, whether they maintain a consistent blood level over the course of a binge, or whether they consume cocaine in a repeated spiking manner over time. Addicts report seeking the rush of fast rising blood
levels (through high dose binges) and data on drug taking frequency (every 10-30 min) indicate that inter-use intervals (either smoked or by injection) would probably cause dramatic oscillations of drug levels (Gawin 1991). We are interested in the fast rising edge of brain cocaine levels and whether they would be sufficient in themselves to result in escalation.

In Chapter 4 we showed that the duration of continued cocaine consumption is tightly correlated to the trough in brain cocaine levels, where the higher the trough is, the longer the duration of cocaine taking is. Better understandings of the biological mechanisms that come in to play during bouts of cocaine taking are important for the formation of new treatment strategies. This data suggests there could be a circadian underpinning controlling continued cocaine taking and would be a good avenue of continued investigation.

Taken together, the data presented in this dissertation speak to the heavy influence of time of day on cocaine consumption and examine the factors that may contribute to it, such as brain cocaine levels. Overall, it opens the door to investigate time of day specific neurobiological differences that might influence cocaine taking and how they may be overcome by dose, availability and experience. It is well known that circadian genes have daily cycles, and that these genes act as transcription factors thereby influencing some very important systems in drug abuse and are thus a very good place to start.
REFERENCES


WHO (1992) The ICD-10 Classification of Mental and Behavioural Disorders


APPENDIX

BINGE-ABSTINENCE SELF-ADMINISTRATION INCREASES MOTIVATION FOR COCAINE

Carson V. Dobrin, Leanne N. Thomas and David C.S. Roberts
**Rationale:** In humans, the shift from recreational cocaine use to binge-abstinence cycles is diagnostic of a serious transition in the addiction process. Developing an animal model of this transition would help address the underlying neurobiology. It has been well established that unlimited access models produce extreme toxicity and lethality (Bozarth and Wise, 1985; Deneau et al., 1969; Johanson et al., 1976). Here we were interested in elucidating exposure conditions that do not produce such extreme toxicity or tolerance, but provide us with a reliable rodent model of increased motivation to self-administer cocaine.

**Objectives:** Will giving animals long-term exposure on a discrete trials (DT) schedule increase responding on a progressive ratio (PR) dose response curve assessed after the DT exposure. In a DT schedule, the number of infusions per hour is restricted to a specific amount of discrete trials. This allows for different patterns of self-administration by manipulating dose, price or availability. Often under these schedules, the first day is characterized by a binge, with intake extending well into light cycle. Here we use discrete trials 3 or DT3, with 3 opportunities available per hour.

**Materials and Methods:** Male Sprague-Dawley rats (300-350g) were housed under a reverse 12-h light/dark cycle with *ad libitum* access to food and water. Rats were implanted with indwelling venous catheters (CamCaths, UK) using previously established procedures (Roberts and Goeders, 1989). They were allowed to recover from surgery for at least 3 days before beginning cocaine self-administration. After meeting training criterion (one stable day, 20 infusions of 0.75 mg/kg), the schedule was changed to a discrete trials three (DT3) where the lever was available to be pressed three times per hour (every 20 min). Half of the rats self-administered cocaine (0.3, 0.56, 1.0 or 1.7 mg/kg/inf) every day, and the other half every other day. After 10 sessions at PR dose response curve (0.56-3.0 mg/kg for all groups, 0.17 and 0.30 was added for 1.0
and 1.7 DT3 groups) was assessed. In a PR schedule, the number of responses made in order to receive the last reinforcer is defined as the final ratio, and the breakpoint is defined as the total number of infusions received. Additionally, for the 1.0 mg/kg DT3 animals another group was given every-day access to the DT schedule, followed by 10 days of deprivation before the PR dose response curve was assessed.

**Results:** As dose was increased, so was the average intake across sessions (Figure 1). Additionally, animals that self-administered every-other day consumed more cocaine than animals self-administering every day. We also observed a recapitulation of the first day "binge" (high level of intake) in animals that were self-administering every-other day (Figure 2). Figures 3-8 illustrate the range of PR dose response curves ran. Overall, we observed increased breakpoints in animals that self administered lower doses on the DT schedule (0.3 – 1.0 mg/kg) and ran every-other day as opposed to every day (see Figure 6 for the best example). Interestingly, animals that ran every-other day at the highest dose tested (1.7 mg/kg) had decreased breakpoints as compared to those running every day. Finally we observed that deprivation after exposure to the DT3 schedule can increase breakpoints across the board (Figure 9).

**Conclusions:** We found that we can recapitulate binge typically observed on day 1 in cocaine self-administration under a DT schedule by giving rats one day off between sessions. Additionally we found that deprivation from self-administration is an essential component in increasing progressive ratio responding. Finally we observed that escalation of cocaine intake is not necessary to increase responding on a progressive ratio schedule.
Figure 1. Average cocaine intake across the 10 DT sessions for the range of doses (0.3-1.7 mg/kg) and access conditions (every day, ED and every-other day, EOD). * denotes significance on independent sample t-test, p<0.05. Note that intake increases with dose.
Figure 2. Average intake by session for the various doses tested (0.3-1.7 mg/kg) both every-other day (filled symbols) and every day (open symbols). Note the higher level of intake in animals running every-other day.
Figure 3. Breakpoints for 0.17 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (1.0 and 1.7 mg/kg). An independent samples t-test indicated significance (*, p<0.05).
Figure 4. Breakpoints for 0.30 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (1.0 and 1.7 mg/kg). An independent samples t-test indicated significance (*, p<0.05).
Figure 5. Breakpoints for 0.56 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (0.3-1.7 mg/kg). An independent samples t-test indicated significance (*, p<0.05).
Figure 6. Breakpoints for 1.0 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (0.3-1.7 mg/kg). An independent samples t-test indicated significance (*, p<0.05).
Figure 7. Breakpoints for 1.7 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (0.3-1.7 mg/kg).
Figure 8. Breakpoints for 3.0 mg/kg on a progressive ratio schedule for naïve and rats exposed to DT3 schedule for 10 days at the various doses tested (0.3-1.7 mg/kg). An independent samples t-test indicated significance (*, p<0.05).
Figure 9. PR dose response curves for animals exposed to 10 days of DT3 at 1.0 mg/kg every day. Before assessing the PR dose response curve, one group (DEP) was given 10 days of forced deprivation. Note that after deprivation breakpoints are increased.
CURRICULUM VITAE

Education

2006-Present  Wake Forest University Medical School, Neuroscience program

   PhD candidacy advancement May 26, 2009

   Expected completion December 2011

2004  Bachelor of Science, Psychology, Chemistry (minor), University of Colorado at Denver *Summa Cum Laude*

Academic and Professional Honors

2010  Hot Topics Graduate Student Seminar chosen speaker

2009  Society for Neuroscience Graduate Student Travel Award

2008  Neuroscience graduate student research seminar – 2\textsuperscript{nd} place

2007-2010  National Institute on Drug Abuse: Circadian influences in cocaine reinforcement (pre-doctoral NRSA)

2006-present  Member, Western North Carolina Society for Neuroscience

2005-present  Member, Society for Neuroscience

2004  Research funding award: Undergraduate Research Opportunities Program, University of Colorado

2003  Research funding award: Psychology Faculty Undergraduate Research Fund, University of Colorado

Research Experience

2006-present  Dissertation work in laboratory of Dr. David Roberts: Circadian influences in cocaine reinforcement

2004-2006  Professional Research Assistant for Dr. Nancy R. Zahniser, University of Colorado Health Science Center

2002-2004  Undergraduate research under the direction of Dr. Richard Allen, University of Colorado at Denver

   - Honors Thesis: A Comparison of the Abuse Potential of Morphine Butorphanol
Publications


Presentations, National Meetings


