Development and Validation of Operant Cocaine Self-Administration in Rodents

Matthew Pomrenze
Department of Psychology and Neuroscience
Dr. Donald Cooper

Dr. Robert Spencer, Department of Psychology and Neuroscience
Dr. Ken Krauter, Department of Molecular, Cellular, and Developmental Biology
Dr. Robert Kuchta, Department of Chemistry and Biochemistry

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Abstract:

Drug addiction is a debilitating psychiatric disorder characterized by compulsive drug seeking despite negative consequences. Due to its prevalence in our society, the development of reliable models to investigate its epidemiology is critical to its conditional prevention. Here, it is shown that the development and validation of a self-administration model in rodents is an accurate depiction of cocaine abuse in a conditioned environment. Using stable cocaine self-administration and dose-response behavior in un-tampered with animals, the contribution of widespread influences can be assessed and identified as associated risk-factors of drug-seeking behavior. The establishment of a reputable model serves as the foundation for all behavioral research, and proves to be a key determinant for the controlled investigations of drug addiction. The development and validation of the cocaine self-administration model in rodents is an invaluable tool for the field of addiction research.
# Contents

ABSTRACT ........................................................................................................... 2
CONTENTS .......................................................................................................... 3
ACKNOWLEDGEMENTS .................................................................................... 4
CHAPTER ONE: INTRODUCTION ........................................................................ 5
  GENERAL OVERVIEW ....................................................................................... 6
  COCAINE ADDICTION, MOTIVATION, AND GLUTAMATE ......................... 8
  POTENTIATIONS OF DRUG-SEEKING BEHAVIOR .................................. 10
CHAPTER TWO: COCAINE SELF-ADMINISTRATION PARADIGM IN RODENTS ............................................................................................................ 12
  INTRODUCTION .............................................................................................. 13
  MATERIALS AND METHODS ........................................................................ 14
  RESULTS ........................................................................................................ 20
  DISCUSSION ................................................................................................... 25
CHAPTER THREE: DISCUSSION / FUTURE DIRECTIONS ............................. 28
REFERENCES ..................................................................................................... 36
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Chapter One:

Introduction
General Overview:

Drug addiction is a debilitating psychiatric disorder that is characterized by compulsive drug seeking and use despite adverse consequences and a loss of control in limiting intake (Thomas et al., 2008, Renthal and Nestler, 2008, Koob et al., 1998). Due to its rewarding and reinforcing properties, as well as its social repercussions, drug addiction is one of the most prevalent disorders in our society. Research in the realm of drug addiction takes many forms, yet one of the most widely used and accepted is the self-administration model in rodents (Johanson et al., 1976), which simulates a conditioned drug environment in which reinforcing drugs are readily obtainable. A major objective of research in the field of addiction is to replicate real conditions and situations in which drug taking is probable. The development and validation of such a model is necessary for learning about what factors may influence trends in drug taking and drug relapse. Here, we attempt to illustrate the validity of a self-administration paradigm in capturing the essence of easily obtainable drugs in a vulnerable, drug-seeking environment. In order to fully understand how some people are more or less susceptible to drug dependence under certain circumstances, the self-administration model must be developed and utilized in a manner that is relevant to a human condition. This model has the advantage of assessing both the dynamic neurochemical properties of addiction, as well as the motivationally influenced behavioral aspects that lead to dependence.

Emerging evidence suggests that addiction to drugs of abuse usurps reward-related learning and memory processes, ultimately producing long-lasting neuroplastic effects that may underlie relapse to drug seeking (Huang et al., 2011). Synaptic plasticity that occurs in response to chronic cocaine exposure makes up the foundation for a drug-dependent state. Responding to conditioned stimuli associated with drug intake is
precipitated as a consequence of this plasticity, and is generated through a dynamic change in reward and motivational circuitry in the brain (Nestler et al., 2001). It has been shown that there is a common neural circuit for the expression and extinction of fear and drug memories (Peters et al., 2009), indicating that fear and stress provoking stimuli can activate mechanisms responsible for drug seeking and relapse behaviors. If these two responses indeed share common mechanistic domains, it can be inferred that environmental factors that induce fear, anxiety, and stress can influence behaviors towards cocaine usage and relapse.

As one of the more potent causes of anxiety and drug relapse, everyday stressors from our daily environments can be viewed as inimical factors in drug addiction. The interactions between motivational reward circuitry in the CNS and the higher order executive functioning make up a locus for drug-induced decision making and behaviors. The neural mechanisms underlying drug addiction are primarily associated with long-lasting changes in dopamine (DA) neurotransmission in the mesolimbic dopamine system (Koob, 2006, Huang et al., 2011), as well as selective inputs and feedback from the medial prefrontal cortex (mPFC) (Huang et al., 2006). It is the interface between these two circuits that produces a behavioral output encoding for drug-dependence. A more profound look at the timeline of addiction events shows strong vulnerability to continuing and up-regulated drug use after drug extinction has occurred (Koob et al., 2004). The time after drug use has been abolished is coupled with a heightened sensitivity to drug associated stimuli that may elicit learning and memory plasticity that occurred through pairings of the drug with conditioned stimuli. This is thought to be a molecular basis for drug pairing and relapse. The behavioral paradigms in this thesis, primarily cocaine self-
administration, were meant to model situations in which drugs and cue-induced drug taking are prevalent, easily obtainable, and relevant to the human experience.

**Cocaine Addiction, Motivation, and Glutamate**

Research investigating the neuronal changes responsible for sustained addiction to drugs of abuse, such as cocaine, has focused primarily on its interactions with the mesolimbic dopamine system. This is due to the critical learning, motivational, and rewarding roles of dopaminergic centers such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc) (Cooper 2002, Fuchs et al., 2004), as well as enhanced dopamine cell impulse activity responsive to drug exposure (Marinelli et al., 2003). These cellular circuits govern much of an organism’s primary reinforcing mechanisms, from satiation of thirst and hunger to sexually driven behaviors (Koob 1996). Drugs of abuse are viewed as compounds that supplant these normal functionalities, and induce malleable changes that can be modulated by a plethora of stimulation (Leshner and Koob, 1999). It is clear that motivation for cocaine is rooted in an enhanced dopamine transmission, but past studies have not been entirely consistent with an obligatory role for dopamine (Kalivas, 2004). A popular hypothesis is that compulsive cocaine use and cocaine relapse is due to drug-induced neuroadaptations in reward-relating learning and memory processes in the mesolimbic dopamine system and glutamatergic corticolimbic circuitry in which the dopamine projections are embedded (Thomas et al., 2008). These adaptations are thought to cause hypersensitivity to cocaine-associated cues, impulsive decision making, and abnormal habit-like behaviors that are insensitive to adverse consequences through the regulation therein by selected signaling cascades, growth factors, and physiological processes implicated in neuroplasticity (Thomas et al., 2008). Some of these include brain-derived neurotrophic factor (BDNF), glutamate
transmission, synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD), and epigenetic effects such as histone modification and chromatin remodeling (Renthal and Nestler, 2008, Huang et al., 2011). If motivation is embedded in the mesolimbic dopamine circuitry, powerful rewiring and synaptic adhering between the mesolimbic and corticolimbic systems can evoke strong motivational activation through specific learning and memory traces in the mPFC and other structures, such as a conditioned stimulus does.

Motivation for cocaine is entrenched in an enhanced dopamine transmission, yet more and more emerging studies demonstrate drug-induced changes in the function of proteins that regulate pre- and postsynaptic glutamate transmission (Knackstedt et al., 2010), and support the role for glutamate in the enduring behavioral characteristics of cocaine addiction, particularly in the PFC (Kalivas, 2004). Indeed, Knackstedt and her colleagues have shown that up-regulated Homer1b/c proteins and the associated reduced surface expression of metabotropic glutamate receptor 5 (mGluR5) and a loss of LTD are compensatory adaptations induced by extinction training that reduce cocaine seeking. These results are corroborated by a study that shows that the positive allosteric modulation of mGluR5 receptors through their interaction with NMDA receptors facilitates the extinction of cocaine-associated contextual memories (Gass et al., 2009). These findings indicate that the glutamatergic system has an influential role in cocaine acquisition and attenuation.

It appears that while many actions of glutamate derive their importance from a stimulatory interaction with the dopaminergic system, there are some glutamatergic mechanisms that contribute to addiction independently (Tzschentke and Schmidt, 2003). The idea of an “addiction memory” supports the ways drug cravings and relapse events
can be induced by the administration of the drug itself or by the exposure to cues associated with drug-taking or the drug effect (Tzschentke and Schmidt, 2003). Indeed, the molecular mechanisms that support normal learning and memory functions are responsible for memories associated with drug use, such as the role of Protein Kinase Mζ in memory maintenance (Li et al., 2011). During drug-free periods, DA input to the PFC normalizes while the glutamergic activity remains in a hypersensitive state, leading to an altered dopamine-glutamate interaction that ultimately leads to aberrant control over compulsive drug-taking behavior (Tzschentke and Schmidt, 2003). Exposure to the drug, stress, conditioned cues, or appropriate electrical stimulation can trigger a full-blown relapse. In these ways, the complex interaction between the dopaminergic and glutamergic circuitry underlie some of the cellular and molecular mechanisms for reward contingent behaviors and relapse in cocaine addiction.

**Potentiation of Drug-Seeking Behavior**

Conditioned fear responses offer an analog to drug addiction in that they are both subject to molecular “memories”, and activation in the mPFC through glutamatergic transmission. Recent evidence indicates that the mPFC is critical for the extinction of both fear and drug-seeking behaviors (Peters et al., 2009). In the mPFC-NAc path, glutamate released from the Prelimbic - mPFC region within the NAc Core triggers relapse for both cocaine and heroin (McFarland et al., 2003, 2004; LaLumiere and Kalivas 2008). The analogous pathways between fear and addiction conditioning fosters powerful implications for how two psychiatric conditions, anxiety and addiction, may be precipitated. The same stressful, environmental cues that bring on anxiety attacks could be responsible for triggering relapse behaviors, manifest through neuroplastic adaptations that occur within the PFC.
The notion that repeated cocaine exposure and stress can have a potent impact on the circuitry and excitability of the PFC has been shown by many (McFarland et al., 2003, 2004; Dong et al., 2004; Dong et al., 2005; Huang et al., 2006; LaLumiere and Kalivas 2008; Knackstedt et al., 2010). Furthermore, it has been demonstrated that glucocorticoid hormone responses are enhanced in rats previously exposed to uncontrollable stress, and that this response is necessary for a stress-enhanced potentiation of the rewarding properties of some drugs of abuse (Der-Avakian et al., 2005). These findings are an important step in understanding how the PFC and other stress regulating centers can incite drug-seeking behaviors. It has been shown that the conditional loss of the Transient Receptor Potential Channel 5 (TRPC5), a receptor implicated in sensory perception and some neurodegenerative diseases (Nestler, Hyman, and Malenka, 2009), in the PFC of mice results in an increased sensitivity to cocaine and increased drug seeking. Thus, the physiology of the PFC may be playing a central role in the mediation of drug seeking behavior subsequent to chronic cocaine exposure. This being the case, it is plain to see that PFC can be viewed as a physiological substrate for drug addiction and relapse. Potentiation of drug seeking behavior, both endogenous and intrinsic, seem to converge in the complex circuitry of the PFC. Susceptibilities to drug taking and seeking behavior is potentiated by changes in cortical circuitry and changes in the environment. By manipulating the inputs to the PFC in the face of cocaine accessibility, such as genetic knock-downs in the PFC or the control over stressful events within a cocaine self-administration model, we can begin to get a clearer depiction of how drug seeking behavior is established, maintained, and reinstated.
Chapter 2:

Cocaine Self-Administration Paradigm
Introduction:

Cocaine addiction is a psychiatric disorder characterized by compulsive drug seeking and use despite negative consequences, with a high prevalence in developed countries. In order to investigate certain risk factors involved in its development, a proper model is necessary, and thus its replication in a controlled setting is critical to its experimental analysis. The design of an appropriate self-administration model in mice and rats is required in order to use genetically modified animals with the aim of clarifying some of the mechanisms involved in cocaine relapse, spanning from environmental cues to biological states. In humans, relapse to cocaine and heroin abuse or the intense desire for these drugs can be generated by re-exposure to the consumed drug, by stimuli usually associated with drug intake, and by stress (Bossert et al. 2005). In the rodent, operant intravenous drug self-administration is the most complete and reliable approach to investigate the addictive potential of cocaine (Soria et al. 2008). In the past, many studies conducted in mice to evaluate the rewarding properties of drugs of abuse have been performed in the conditioned place preference (CPP) paradigm. At the present time, thousands of studies have confirmed the successful modeling of cocaine-use maintenance, extinction, and relapse using self-administration paradigms, and enable researchers to further investigate the roles of operant influences on cocaine-seeking behavior. The literature in the field yields a considerable amount of investigations concerning drug self-administration in rats as opposed to mice. In fact, there are almost 5 times as many peer-reviewed studies using rats over mice, despite recent genetic tools and molecular advances designed to be applied to mice. This method, in mice and rats, helps elucidate the contributions conditioned stimuli and other salient influences have on the epidemiology of drug abuse. Using protocols to model cocaine addiction has revealed
enduring neuroplasticity in glutamatergic synapses in the NAc (Knackstedt et al. 2010), as well as countless other homeostatic changes associated with cocaine use.

Animals that learn to deliver reinforcing drugs to themselves serve as powerful tools for replicating reward-contingent behavior. Cocaine self-administration in the rodent is able to model cocaine addiction in the human to a reliable extent (Pickens and Thompson, 1968). The paradigm extends into an analog of withdrawal and relapse as extinction and reinstatement are conducted. Animals that are self-administering to a stable extent typically extinguish their drug-seeking behaviors after the rewarding drug is absent. A subsequent drug challenge, usually with a drug prime or an associated cue presentation, facilitates the reinstatement of drug-seeking behavior, analogous to relapse. Within these parameters, researchers can investigate the contributions specific drug-instigating factors lend towards the probability and robustness of a relapse event. The self-administration paradigm makes it possible to translate the familiar influences humans face when dealing with drug addiction into an easily manipulated model in rodents.

Materials and Methods

Methods

The self-administration project began with the construction of multiple self-administration apparatuses. The SA boxes were built from custom pieces of acrylic, steel mesh for grated floors, and a host of photo-sensors and LED lights (Figure 2.1). Using solvent cement and a drill press, the appropriate acrylic pieces were glued together and holes were drilled for active and inactive portals. There is a reinforcing portal and a non-reinforcing portal, one of which is located on the back wall and another on the front retracting door at random sequences. In order to completely randomize portal locations,
there is the option for a second portal on the front. The portals consist of a hole with an infrared photo-sensor located just inside with a white LED. The penetration of the active beam results in the activation of the LED and an infusion of about 40 uL cocaine solution over 3 seconds and a time out time of 5 seconds for the reinforcing portal and no consequences for the non-reinforcing portal. Electronics were put together by hand and soldered for custom connections using National Instruments breadboards and data acquisition boards, which relay recordings to a custom designed software program generated through LabView. Med Associates syringe pumps were programmed into LabView as well, and used for the activity-dependent infusions of drugs or vehicles. The first two self-administration boxes were completed in the month of May, and from there two other boxes were subsequently constructed. Each box contains 6 operant chambers, allowing 6 animals to be trained at a time per box (Figure 2.4). Separate boxes were allocated to mice and to rats and set up on different floors of the animal facility to prevent any abnormal behaviors due to the detection of each other’s presence. Once all electronics and mechanics were set up and confirmed as functional, preliminary self-administration experiments began shortly thereafter.

Once the SA boxes were up and running, numerous animals were catheterized using a standard catheterization protocol for mice and rats. Catheters are fabricated in two parts; using Silastic tubing (0.012 cm gauge for mice, 0.020 cm gauge for rats) as an intravenous guide cannula and syringe needles (22.5 gauge for rats, 23 gauge for mice) with the bevels snapped off and the metal plumbing forced back up through the plastic syringe dispenser and bent at a right angle to allow a connection with the tubing (Figure 2.2). The plumbing and plastic dispenser were adhered to nylon mesh using multiple liquid latex layers. Silastic tubing with bound heat shrink tubing was inserted into the
right jugular vein and anchored to the surrounding tissue with fine suture thread using the
heat shrink as a landmark (Figure 2.3a/b). The opposite end of the tubing was channeled
subcutaneously through the back of the animal and super-glued to the back-mount. After
the stability of the Silastic tubing – needle interface was ensured, the incisions on the
back and chest were securely stapled, reinforced with VetBond, and covered with
antibacterial ointment. Patency of catheters is confirmed each day by injecting Heparin-
saline mixture into the vein.

The self-administration protocol took about four months or so to refine, and is
producing stable self-administering rats and mice. Our training dose of cocaine for mice
is 0.75 mg/kg/infusion and 0.50 mg/kg/infusion for rats. C57BL/6J mice (20-30 grams)
are subjected to a 12-hour binge acquisition session, where they are either allowed to
finish out the 12 hours or the contents of the syringe (10 mL 0.75 mg/kg/infusion cocaine
hydrochloride or 0.9% NaCl saline). Mice start on a Fixed-Ratio 1 (FR-1), where one
nose poke earns a reward, for 3-hour sessions and advance up to an FR-3 if poking
activity and infusion levels are high (minimum 20 infusions). Once activity is maintained
at an FR-3 for five days with discrimination to the active port, dose-response curves are
taken at 0.1 mg/kg/infusion, 0.3 mg/kg/infusion, 1.5 mg/kg/infusion, and 3.0
mg/kg/infusion. Dosages are administered for a minimum of 2 days, and are completely
randomized to avoid any unwanted biasing, extinction, or titration of the drug.

Sprague Dawley Rats weighing ~250 grams when arrived are allowed a one week
acclimation to the facility, residing in a reversed dark-light cycle, and given food and
water ad libitum. Rats are then subjected to a periodic-infusion acclimation session
consisting of a 50 uL infusion of cocaine or saline every 120 seconds coupled to the LED
for one hour instead of a food-reward training acclimation session. They are then put into
a self-administration paradigm at an FR-3. Once rats self-infuse 20 infusions, they are advanced to an FR-5 for the remainder of the sessions. Rats that do not exhibit discrimination for five days are excluded from the studies. After dose response curves are collected for mice, we will perform progressive ratio paradigms and determine the animals’ motivational break points. In rats, stable self-administration and discrimination for a minimum of 5 days is followed by an extinction period. The number of days it takes to extinct as well as the progression of decreasing infusion rates will serve as home cage controls (HC) for future stress experiments. These data will form the baselines for our future manipulations.

Figure 2.4. Self-administration boxes are able to house up to 6 animals at a time, each with a reinforcing and a non-reinforcing portal located on opposite sides of the chamber, one on the back wall and one on the retracting door. Each portal is fitted with a photo-sensor that relays a signal to a LabView fixed-ratio program that activates each respective syringe pump to yield an appropriate infusion. The syringes are connected to Silastic tubing that is in turn connected to a commutator that serves as a swivel for the animal’s freedom of motion. The swivel is further connected to another line of Silastic tubing covered with a steel-coiled sheath that is connected to the animal’s catheter. It is through this tightly regulated system that drug delivery is regulated and ensured.
**Figure 2.1.** The chamber is set up so the animal can nose poke into either the front portal or the back portal with equal ease (front door open). Each hole contains an infrared photo-sensor that relays a signal back to the LabView interface once the beam is broken. Rats and mice learn to associate the drug or saline and a white LED that illuminates the entire cage with the nose poke, and acquire reward-contingent behaviors.

**Figure 2.2.** Back-mounts were constructed using syringe needles bent into conformation and inserted back into the plastic dispenser. They were then latexed to a mesh disc with a larger mesh on top in a “sandwich” method. After the Silastic tubing was inserted into the vein, they were channeled subcutaneously through the back and superglued to the metal plumbing sticking out the front. Backmounts were then capped.

**Figure 2.3.** Catheterization surgery takes an average of about 25 minutes to complete. An incision about 1 cm in length is made and stretched open using retractors for an optimal window. Silastic tubing is inserted into the right jugular vein and secured to the surrounding tissue. A) shows preparation for inserting the tube (arrow pointing to jugular) and B) shows just after the tube was inserted and securely tied down.
Problems and Solutions
Throughout this project, there were a variety of obstacles that were encountered. In terms of mechanics, problems with the catheters, boxes, and syringe pumps emerged after self-administration behavior was very poor. After adopting a new surgery protocol and catheter fabrication method that uses different materials such as latex instead of dental cement and is more reliable, drug delivery into the jugular vein was secured (Figure 2.3). To ensure that cocaine was being pumped into the jugular vein itself, the infusion volume was increased to 50 uL and plastic syringes replaced original glass ones. Mice initially showed a lack of exploration within the chambers, so to address this problem mice were switched to a reversed 12 hour light-dark cycle and a 12 hour binge acquisition session. This put the animals in a very active state and their nose-poking increased substantially. The final behavioral problem that was experienced was poor discrimination between the active (cocaine delivering) and inactive ports. To troubleshoot this problem, white LED lights were installed in the ports, shining a light that illuminated the entire chamber in the active port when their nose broke the nose-poke beam. One last manipulation in the process of being worked out is replacing one of the portals to a hole across the length of the chamber to the back wall. At this point, these two interventions have substantially increased our rate of discrimination at FR-3 for mice (Figure 2.5). Since the rat box was completed after most of these problems were dealt with, the rats have had much smoother training. With all of these solutions put forth, except for the portal on the back wall, we have been able to get several rats to discriminate for cocaine at an FR-5, and numerous mice at FR-3.
Figure 2.5: After switching the animals to a reverse dark-light cycle and switching the LED’s, poking responses and infusion rates increased substantially (a), as well as discrimination between the active and inactive portals (b).

Results

After months of troubleshooting the construction of the boxes, proper functioning of the electronics and software, and optimal activity of the animals, preliminary self-administration sessions began daily. The initial goal was to get as many mice and rats installed with catheters as possible, and establish stable self-administration behavior. We were able to get rats and mice to respond for cocaine or saline for a minimum of five days with robust discrimination between the reinforcing and non-reinforcing portals, as well as a display of stable infusion rates (Figure 2.6a/b). Our criteria for advancement in the self-administration design is a minimum of 20 active infusions per each 2 hour session for rats and each 3 hour session for mice for a minimum of 5 days, with a minimum of a 70% bias to the reinforcing portal. Animals that did not meet these criteria were discontinued and omitted from the study. Shown is the graph for a group of mice that met our criteria and advanced to the dose-response phase of the study. Shown are the graphs for the 3 sessions of self-administration prior to dose-response training (Figure 2.7a/b/c), indicating robust
discrimination, stable infusion rates, and a satisfaction of the >70% bias criterion. Since the earliest 2 sessions out of the minimum 5 contained high levels of variance between the subjects, only the last 3 are shown. As portrayed below, we were able to obtain successful self-administration for mice and rats with intact catheters and functional hardware and software.

After stable self-administration behavior was observed, mice that met criteria graduated to a dose-response regimen, and rats that met criteria were subjected to an extinction regimen. Mouse and rat cocaine infusion rates were compared to saline controls using one-way ANOVA (p < .005 and p < .05 for third session prior to dose-response and two sessions prior to dose response, respectively for mice, and p < .005 and p < .05 for second trial prior to extinction and third and first session leading up to extinction, respectively for rats). The mouse dose-response was taken at two doses below and two doses above the training dose, and illustrates a clear titration in drug intake (Figure 2.7d). Statistical analysis was performed using a repeated measures ANOVA, followed by a post hoc analysis using a Fisher’s PLSD test. We were able to show an overall effect of cocaine dose (p < 0.05) and significant differences (p < 0.01) in effect for doses differing in relation to the training dose (Table 2.1). These data illustrate a clear effect of cocaine on the mice, as well as a motivational and sensitivity measurement across doses. This preliminary data serves as baseline measurement in un-tampered with animals for our future manipulations (genetic knock-outs, acute nicotine exposure, optogenetics, etc.). Once graduated from the dose-response regimen, mice are brought back to the training dose of cocaine (0.75 mg/kg/inf) and allowed to return to stable administration for several days. Once established, a progressive-ratio session follows consisting of a set increase of the fixed-ratio as the animals infuse more and more. This experiment is used to determine the “break
point” for self-administration in the cocaine-addicted mouse. It serves as a useful model for the working drive or motivation of the mice for cocaine.

Rats are brought through the same protocol as mice, with the exception of the acquisition phase, which consists of the non-contingent delivery of cocaine paired with a light cue for one hour. After the first hour, the program is switched to contingent delivery at an FR-1 for one more hour. After the acquisition, rats are allowed to self-administer either cocaine or saline at an FR-5 for a minimum of 5 days with robust discrimination to the active portal along with stable infusions rates. Just as it is for mice, a minimum of 20 infusions per session is required for advancement in the experiment. As shown, despite varying differences in active and inactive responding (Figure 2.8a), cocaine has a significant effect over that of saline (Figure 2.8b). Once self-administering to a stable extent for 5 days, rats are brought through a drug extinction regimen. With the hope of exposing rats to different forms of stress (Escapable Stress/Inescapable Stress/Home Cage Control), we expose stress the day after they meet criterion, and wait 7 days in between the stress event and extinction initiation. In this way, we can assess the influence of differing types of stress on extinction and a spontaneous recovery of activity 2 weeks later. By increasing our sample size in the future, we expect this data to become even more stable in the future. Using these and future data as a basal measurement for motivation and reward contingency for rats, we plan on investigating the influence stress has on self-administration and drug-seeking behavior in the near future.
Figure 2.6. Cocaine self-administration in C57Bl/6J mice. (a) Values represent mean number of active vs. inactive responding (+/− s.e.m.) per 3 hours for 5 consecutive days at 0.75 mg/kg/infusion training dose on FR-3 (n=9). (b) Values represent mean number of infusions (+/− s.e.m.) on 0.75 mg/kg/infusion per 3 hours for the respective 5 sessions (n=9).

Table 2.1: Dose response: repeated measures ANOVA indicate a significant main effect of dose (p = 0.0111) and subsequent Fisher’s PLSD post hoc comparison reveals significant differences in the number of infusions between high (1.5 and 3.0 mg/kg/inf) and low (0.1 and 0.3 mg/kg/inf) doses (p < 0.01).
Figure 2.7. Cocaine self-administration and dose-response in C57Bl/6J mice. (a) Values represent mean number of active vs. inactive responding (+/- s.e.m.) for last 3 sessions of self-administration at training dose (n=5). (b) Values represent mean percent responding to active portal (+/- s.e.m.) versus inactive portal at training dose (n=5). (c) Values represent mean number of infusions cocaine (n=5) versus saline (n=5) for last 3 sessions at training dose leading up to dose-response (+/- s.e.m.). **(p < .005) *(p < .05). (d) Values represent mean number of infusions (+/- s.e.m.) for 2 sessions at each given dose (n=5). Repeated measures ANOVA indicate a significant effect of dose (p=0.0111). Red data point represents the training dose.
Figure 2.8. Cocaine self-administration for Sprague Dawley rats. (a) Values represent mean number of active vs. inactive responding (+/- s.e.m.) per 2 hours for last 3 sessions leading up to extinction regimen (n=6). (b) Values represent mean number of infusions (+/- s.e.m.) per 2 hour sessions for same 3 sessions for cocaine (n=6) vs. saline (n=9) treated rats at a training dose of 0.5 mg/kg/infusion cocaine. One-way ANOVA indicates significant difference between cocaine and saline treated animals. **(p < .005) *(p < .05).

Discussion

The present study shows a propensity of the rodent towards cocaine self-administration and further drug-seeking behaviors. It was demonstrated that mice and rats are capable of being trained to self-administer to a high standard and exhibit large discrimination to the location of the drug-delivery site. These experiments were essentially a demonstration that drug-seeking behavior, specifically cocaine self-administration, can be instilled within rodents. This has been demonstrated many times in the past, but it is the first time it has been successfully carried out and established in the Cooper Laboratory here at CU. We started this project from the ground up, constructing and fabricating every piece of equipment from the self-administration apparatuses and catheters to the recording
software and electronics. I personally have spent about one full year in the Cooper Laboratory, and essentially built a paradigm that will be maintained for as long as cocaine addiction studies continue to be done. The success of the self-administration setup is reflected in the significance in dose-responses and active discrimination between reinforcing drug delivery sites. This discrimination and dose-response titration shows us that cocaine has an overall effect (p < 0.05) on rodents and their subsequent behaviors. This is exactly what our rodent models were meant to portray, thus giving validation to the model itself. If we can predict how these subjects will respond and behave in the face of cocaine challenges, we can make even more valid predictions about how those behaviors may change in the presence of experimental (biological, environmental, genetic, etc.) manipulations.

The collective results give us confidence in our future experimental manipulations. Much of this phase of the project has been spent identifying fundamental problems with the protocol and troubleshooting them. In order to establish and maintain optimal behavior in the animals, every condition of the self-administration paradigm needed to be mastered and refined. This objective was accomplished after numerous changes to the conditions, including a reverse dark-light cycle, a more salient conditioned stimulus (LED light), and ensured delivery of the drug to the target (animals’ brain). As expected, we observed numerous characteristics of drug-induced states in our animals, including conditioned place-preference to the reinforcing portals, high locomotor activity, and stereotypy behaviors. As self-administration continues to be carried out in mice and rats, variable behavior is always expected, but our criteria for advancement remains rigid, thus ensuring that animals included in our experimental manipulations are exhibiting behaviors that meet our standard for addiction and are consistent. Using our collective data for portal
discrimination, high cocaine infusion rates, and a descending dose-response curve, we can further examine the ways in which these behavioral parameters change in the presence of control over an exogenous, stressful event and the absence of the TRPC5 gene in selective neuronal populations in the forebrain. This baseline phase of the cocaine self-administration project is fundamental to any behavioral experiments that analyze the influence of changing conditions (explicit and implicit) on drug-seeking behaviors.
Chapter 3:

Discussion / Future Directions
The use of self-administration in addiction research is an extremely powerful approach to elucidating critical changes that occur within the pathogenesis of drug dependence. It is possible to map out a series of events that happen in the timeline of an addictive state, from a behavioral and physiological point of view (Koob et al., 2004). The self-administration paradigm makes it very straightforward to test the individual properties of specific neural circuits in the face of cocaine addiction at almost any point in an addiction cycle. We have the advantage over acute doses of the drug because chronic exposure translates into a pathological addictive state where motivation and sustained access are present, characteristic of real drug addicts. By demonstrating that mice and rats do acquire cocaine intake to a significantly larger degree than control animals, and display changing effects from different doses, we have developed a protocol that the entire laboratory can use and do a variety of research on. Although initially a challenge to develop, the self-administration paradigm serves as a reliable behavioral tool that enables subsequent manipulation and assessment of user-defined variables. This is an exciting junction in our research because we are now able to ask some very important and relevant questions that may uncover some key events that occur in the process of addiction. It is from this baseline of behavioral data that this thesis was established that we can begin to explore new realms of influence on cocaine intake, and make reasonable hypotheses about what may affect drug-seeking and dose-response trends such as sensitivity, impulsivity, and motivation.

This thesis was initially concerned with the development of a reliable paradigm that would accurately model psychostimulant addiction in humans. Using experimental procedures that proved successful in eliciting cocaine-reinforced behavior in rodents in the past (Pickens and Thompson, 1968), we were able to replicate the protocol using our own
custom setup and resources. With the inauguration of stable cocaine self-administration and a fitting dose-response curve serving as basal data sets, we can begin to take the research further in multiple directions. Using our self-administration data as a baseline measurement in un-tampered with animals, we have quite a few experiments lined up that will assess the changes that occur in a variety of conditions. Future experiments include a relevant model for the impacts of different types of stress, the impact of the knock-down of the TRPC5 receptor on cocaine unit dose-response, and the co-administration of other drugs of abuse like nicotine and MDMA on subsequent cocaine self-administration. These manipulations are meant to uncover genetic contributions and accurately model real life events that humans go through that may affect their susceptibilities and vulnerabilities to drug taking, drug-seeking behaviors, and relapse.

Using selected groups of Sprague Dawley rats, we plan to continue on with an experimental design used by a Postdoctoral Fellow in the Cooper Lab, Michael Baratta, that imposes varying degrees of stress on the different groups. Using a paradigm known as stressor controllability (Baratta et al., 2007), we will expose three different groups of rats to either escapable (controllable) tail shock (ES), yoked inescapable (uncontrollable) tail shock (IS), or home cage control treatment (HC). Our current study has rats set up for the three differing conditions prior to an extinction phase of self-administration. Once rats self-administer to our criteria, they experience either IS, ES, or HC, given a 7 day recovery period to avoid any generalized stress effects, and are put back into an extinction paradigm where all cues and conditions are the same, except for an exchange of saline for cocaine. In this paradigm, we hypothesize that control over stress (ES) will facilitate extinction to a significant extent over uncontrolled stress (IS) and home cage control (HC). It has already been demonstrated that ES helps relinquish conditioned fear responses over IS (Baratta et
al., 2007), and since fear and addiction circuitry is believed to overlap and originate in regions of the PFC (Peters et al., 2009), we believe that drug-seeking behavior may be affected in these same ways. After these experiments are completed, we plan to impose the same ES/IS/HC design but at different points in the timeline of the self-administration. Acquisition to cocaine self-administration will be challenged by the ES/IS/HC occurring in rats naïve to the self-administration, and thus translating into a human condition that is interpreted as stressful life events prior to any drug use or history. Reinstatement of self-administration will be analyzed through the occurrence of ES/IS/HC treatment after extinction to a set criterion (responding to the extent of saline controls) has established. By inducing stress at this time point, we can imitate a human condition in which withdrawal and abstinence from a drug of abuse has occurred, and stressful life events influence vulnerability to drug relapse. This design has importance because it may indicate how novel stimuli, such as control over stress, may foster the impedement of drug cravings and drug-seeking behaviors.

In a more advanced design of the same experiment, we plan to integrate a novel technique in the field of neuroscience into our stressor controllability design. Optogenetics is an invaluable tool that enables the precise stimulation or silencing of a specific population of neurons at a millisecond time resolution (Nagel et al., 2003). The procedure entails infecting promoter-specific neurons in the CNS through an adenovirus mechanism with a DNA strand that codes for a light-sensitive protein. These proteins form functional ion channels on the cell membrane and become permeable to cations or anions when light of the correct wavelength is absorbed, either eliciting or inhibiting neuronal action potentials. In this manner, we can turn on or off selective neural circuitry that may be involved with an innate perception of stress that translates into an increased probability for
future drug taking. For each of the three time points in which we induce stress on our animals, we plan to optogenetically activate and silence specific regions of the PFC and subiculum just prior to the stressing events. To do this, we will transduce a Channelrhodopsin-2 gene and a Halorhodopsin gene into different rats using an adeno-associated virus (AAV-CaMKII-hChR2-eYFP, AAV-CamKII-eNpHR 3.0-eYFP) that infects Ca\(^{2+}\)/Calmodulin-dependent protein kinase (CaMKII) promoter-specific neurons, those being glutamatergic neurons. Once sufficient expression is recognized in the appropriate regions through immunohistochemistry and fluorescence imaging, we will begin our behavioral experiments using optogenetics in vivo. This is a very exciting research venture. If cocaine self-administration is significantly inhibited or changed at any of the three points in the drug exposure timeline, we can say with confidence that those selected circuits are involved with cocaine acquisition, extinction, or reinstatement, which is an extremely compelling argument for the underlying mechanisms supporting sustained cocaine addiction.

One other manipulation that is currently underway is the cocaine self-administration of TPRC5 receptor knock-down mice, though due to the lack of time there is no preliminary data finalized or shown. This line of mice was created through the crossing of C3H/HeJ trpc5flx mice that express loxP sites flanking exon 5 of the TRPC5 gene with another line of C3H/HeJ mice carrying a transgene directing the expression of Cre recombinase. Through cre-directed homologous recombination events, the flanked exon 5 is excised from the genome rendering the TRPC5 protein dysfunctional in PFC neurons. Using wild-type mice that do not express cre as controls, we can investigate whether loss of the TRPC5 receptor function in the PFC affects cocaine self-administration and responding to differential doses of cocaine. Layer 5 pyramidal neurons in the PFC exhibit a delayed
after-depolarization (dADP), which is a Gq-coupled receptor mediated period of heightened excitability following brief bursts of action potentials (Fowler et al. 2007 and Sidiropoulou et al. 2009), which may serve as a way for neurons to temporarily hold salient reward related information. Pharmacological and expression data point towards the TRPC5 channel as a candidate for mediating the dADP in the PFC (Fowler et al. 2007). Some of Dr. Cooper’s recent collaborative work has demonstrated that robust decreases in dADP amplitudes in response to bursts of action potentials occur in regional and temporal specific TRPC5 knock-down brain slices following repeated cocaine exposure. This TRPC5-mediating effect of lasting plasticity of the dADP may be to promote drug-seeking behavior and alter short-term working memory processing. This same study also showed that TRPC5 knock-down mice exhibit a significant increased sensitivity to the locomotor activating effects of cocaine, as well as an increase in the rewarding properties of cocaine in the cocaine conditioned place preference paradigm. These results may suggest that TRPC5 may modulate the rewarding properties of cocaine, and that loss of these channels in the PFC causes an increase in vulnerability to drug-seeking behavior. Our objective is to see whether or not these same genetic conditions affect cocaine self-administration and dose responding. Any differences between genotypes may indicate the TRPC5 receptor’s role in mediating motivation and sensitivity to the reward-contingent properties of cocaine. These studies are currently being conducted, and very well may corroborate Dr. Cooper’s work.

As it stands, cocaine self-administration is one of the most useful tools researchers have to study the drug-seeking propensities that drugs of abuse can cause. Furthermore, its use in a variety of animal models has the capacity to offer fruitful clues as to how many different factors, environmental to intrinsically biological, can affect the vulnerabilities an organism has to this condition. Within the field of the neuroscience of addiction, the
literature offers about 5 times as many if not more peer reviewed articles investigating the self-administration of cocaine in rat models than it does in mouse models. Historically, self-administration in the rat is the far more popular juncture in drug addiction research. In this age of progressively advancing scientific methods, including an abundance of genetic tools and techniques in molecular biology, it seems logical and appropriate to use these to our advantage in addiction studies. Since most of these advances are designed to for the mouse as opposed to the rat, there should be a focus on using these tools in mice in addiction research. Thus, the analysis of self-administration in the mouse is at the forefront of an emerging era of science. A major point of this thesis is to show that the self-administration of drugs of abuse coupled with the advanced genetic and molecular tools accessible today is an incredibly important integration capable of stimulating insight on some of the mechanisms and behavioral enigmas that have confounded much research in the past. It is up to modern research to take neuroscience and biology multiple steps further and try to exploit the fundamental mechanisms that support the pathological states we observe. In light of the epidemiology of cocaine use, abuse, and relapse, we can use the methods that have been retained over decades of research in concert with the modern tools and techniques that have gained such appraisal to meaningfully add to a knowledge base and propel the field of neuroscience in an enlightening direction.

This independent experience in the laboratory has given me valuable training in how to properly prepare and perform behavioral experiments. This is something that no class on behavioral pharmacology could have given me. It is especially exciting to be able to take this experience and skill-set and apply them in the future for experiments that are of my own design. A major goal of my undergraduate research experience is to gain a diverse array of skill-sets and techniques that I can use in my own research in graduate school and
beyond. The work done throughout this thesis is a portrayal of what I have been able to accomplish as an undergraduate researcher at the University of Colorado. I am excited to apply this method in multiple directions, which we have already begun to do, and potentially find some very interesting results. Using this research as a baseline, the Cooper Laboratory can continue to carry out self-administration experiments and continue asking important questions for many years to come. As long as long-lasting addiction to psychostimulants and other drugs remains prevalent in our society, there will be the need for state-of-the-art research designs that attempt to expose some of the key factors leading to drug-dependence and addiction vulnerability.
References:


