FACTORS UNDERLYING NATURAL REWARD DEVALUATION BY COCAINE: EFFECTS OF DOSE AND EXERCISE

Jennifer Green Stevenson

A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Psychology (Behavioral Neuroscience).

Chapel Hill
2015

Approved by:

Regina M. Carelli

Linda A. Dykstra

Stacey B. Daughters

Donald T. Lysle

Mitchell J. Picker

ABSTRACT

Jennifer Green Stevenson**:** Factors underlying natural reward devaluation by cocaine: effects of dose and exercise
(Under the direction of Regina M. Carelli)

In a preclinical model developed in the Carelli lab, when a palatable saccharin solution predicts impending, but delayed, cocaine availability the saccharin solution becomes devalued, as evidenced by the emergence of aversive taste reactivity during intraoral tastant infusion (Wheeler et al. 2008, 2011). Importantly, this negative affective state predicts the motivation to self-administer cocaine. The primary goal of this work is to extend the current knowledge of this preclinical model of natural reward devaluation and examine several other variables that may influence this process. In the original set of studies (Wheeler et al., 2008, 2011) only a single dose of cocaine was used. The first aim examined if rats receiving varying doses (0.167, 0.33, 0.66 mg/inf) of cocaine paired to the same concentration of saccharin (0.15%), would alter taste reactivity to the predictive cue in a dose-dependent manner. This study revealed that the emergence of negative affect and the associated increase in motivation for the drug was not dependent on cocaine dose. Exercise has been shown to reduce cocaine-seeking in animal models. Additional studies examined if exercise (i.e., access to a running wheel in their home cage), either *following* (Aim 2) or *prior to* (Aim 3) training on the preclinical model would reverse or attenuate the development of the negative affective state, and the motivation to consume cocaine. The results of Aim 2 indicate a trend toward a protective effect against the established negative affective state, however these results were not strong. Further, exercise had some protective effect against the motivation to consume cocaine when rats were reintroduced to

the paradigm, although it did not completely reverse the negative affective state. The results of Aim 3 showed exercise does have a protective effect against the development of the negative affective state in this model, however, the motivation to consume cocaine continued after it had developed. Taken together, the results of these studies indicate that although the development of the negative affective state observed in our preclinical model is not cocaine dose-dependent, it can be altered to some degree by physical activity.

To my family

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Regina M. Carelli, for her many years of guidance and support during my graduate career. She has been a great role model and advisor as I have worked to become an independent scientist and scientific writer. In addition I would like to thank my co-advisor, Dr. Linda Dykstra, for her support. She has assisted in my academic training such as developing my writing skills which helped me to become a better scientific writer. I would also like to thank my dissertation committee for their direction during the planning and completion of this dissertation. I thank all of my current and former lab mates in the Carelli lab for their support in and out of the lab. In particular, our postdocs Dr. Robert Wheeler, Dr. Michael Saddoris, Dr. Elizabeth West, and Dr. Travis Moschak provided assistance with statistical analysis and academic training that helped me to become a better scientist. I could not have completed this work without the help of our technicians, Jessica Briley, Laura Ciompi, Fei Fei Wang, and Elina Thomas. I also thank the National Institute on Drug Abuse, for many years of funding. Finally, I thank my family, especially my husband, LeVaughn, and parents for many years of support and encouraging me to pursue my personal and academic goals. None of this would have been possible without your support.

PREFACE

This dissertation was prepared in accordance with guidelines set forth by the University of North Carolina Graduate School. This dissertation consists of a general introduction, three chapters of original data, and a general discussion chapter. Each original data chapter includes an introduction, methods, results, and discussion section. A complete list of the literature cited throughout the dissertation is included at the end. References are listed in alphabetical order and follow the format of the journal, Behavioural Pharmacology.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BLA	Basolateral Amygdala
DSM-V	Diagnostic Statistical Manual, Fifth Edition
EE	Environmental Enrichment/Environmental Condition
EMG	Electromyography
EX	Exercise
Fig	Figure
FR-1	Fixed ratio 1
IC	Isolated Condition
INT	Inter-press Interval
LCD	Liquid Crystal Display
m/week	Meters Per Week
NAc	Nucleus Accumbens
rev/week	Revolutions Per Week
SC	Social Condition
SED	Sedentary
VTA	Ventral Tegmental Area
USV	Ultrasonic Vocalization

CHAPTER 1

GENERAL INTRODUCTION

Cocaine is one of the oldest known psychoactive addictive drugs (Volkow 2010). According to the Foundation for a Drug Free World, cocaine is the second most traded drug in the world (Foundation For a Drug Free World 2015). Current statistics show that international confiscations of cocaine have increased slightly within the past year (United Nations Office on Drug and Crime 2014), with the largest quantities intercepted in South America, followed by North America. Interestingly the National Survey on Drug Use and Health (2013) reported that cocaine use has declined significantly since cocaine's height in popularity during the 1980s. However, since 2008 there are over 600,000 new cocaine users per year that are 12 years and older, which indicates that cocaine continues to be used in the current population.

Cocaine addiction is characterized by cycles of compulsive drug use, abstinence, loss of control over drug intake, and relapse (Koob 2008, Koob and Le Moal 2008, Koob and Volkow 2010) despite adverse consequences (Diagnostic and Statisitcal Manual of Mental Disorders 2013). Research has helped in the understanding of how cocaine produces high hedonic impact (Koob and Le Moal 2002). Hedonic impact or "liking" for sensory pleasures is a critical aspect of reward, and excessive 'liking' (i.e. high hedonic impact) of particular rewards such as drugs of abuse might contribute to excessive consumption and to addiction (Pecina et. al. 2006). For instance, cocaine is widely acknowledged as a drug reward with high hedonic impact and may be consumed more than natural rewards (i.e. food) when given a choice (Ahmed 2010). Thus in this

way, hedonics contribute to cocaine abuse. Currently there are no effective medications to change the hedonic impact or craving that is placed on cocaine. Although some medications have been considered, either they have shown high abuse potential (Sofuoglu 2010, Amato et al. 2011) or they have not been effective in reducing craving for cocaine (Fox and Sinha 2014). Effective treatments are needed, but developing pharmacological treatments for addiction is difficult because of the underlying neurobiology varies over time as the disease progresses (Lynch et al. 2013). Thus investigators continue to search for the optimal treatment for cocaine addiction by examining the neurobiological mechanisms that may be involved in the disease.

Cocaine Addiction: Neurobiological Mechanisms

Scientists have found that brain regions that are activated by different reinforcing stimuli such as food and water are also activated by cocaine (Carelli, Ijames et al. 2000, Kelley and Berridge 2002, Robbins and Everitt 1996). Brain regions that process reward related information include the ventral tegmental area (VTA) and its dopaminergic projections to the nucleus accumbens (NAc) (National Institute on Drug Abuse 2010, Koob and Volkow 2010). The neurobiological mechanisms associated with drug addiction are the mesocorticolimbic dopamine system and its connections in the basal forebrain (Koob and Le Moal 1997). For all psychostimulants including cocaine, the facilitation of dopamine neurotransmission in the mesocortiolimbic dopamine system appears to play a critical role for the acute and chronic reinforcing effects of drugs of abuse. Interestingly, these are the same neural regions that are activated during goal-directed behaviors for natural rewards such as food and water (Carelli and Deadwyler 1994, Koob and Volkow 2010). As such, numerous studies have focused on this neural system in their examination of the neurobiological mechanisms underlying drug (i.e. cocaine) and natural rewards.

The NAc is anatomically situated to process reward-related information and influence goal-directed behaviors (Green 2012). The NAc receives limbic information from a variety of cortical and subcortical structures including the basolateral amygdala, prefrontal cortex, and hippocampus (Ghitza et al. 2004, Koob and Volkow 2010). In turn, the NAc sends efferent projections through the ventral pallidum to thalamic, brainstem somatomotor, and autonomic effector sites (Ghitza et al. 2004). Given this anatomy, the NAc has been described as a 'limbic motor interface' that integrates information about memory, drive and emotion, and influences goal-directed behavior (Mogenson et al. 1980). The NAc is comprised of two primary subregions that include the core and shell that have slightly different afferent and efferent projections indicating that they contribute differently to reward related behaviors. For example, the NAc shell receives limbic inputs from the basolateral amygdala (BLA) and ventral subiculum (major output region of the hippocampus) while the NAc core receives similar inputs from the BLA and parahippocampal regions of the hippocampus (Ito and Hayen 2011). Animal research has shown that reinforcing stimuli increase levels of dopamine in the nucleus accumbens, thus increasing the neural activity in that region (Wise 2006, Schultz 1998, Phillips et. al. 2003).

Cocaine blocks dopamine transporters, thus preventing this transmitter from being "recycled" (i.e., taken back up into neurons that release it), and causing excessive amounts of dopamine in the synapse. It is believed that the excessive amount of dopamine is responsible for cocaine's euphoric effects (Nestler 2005). Given the increased amount of dopamine in the synapse, it has been suggested that cocaine's euphoric effects last longer than the euphoric effects elicited by other reinforcers (i.e. food) (Di Chiara and Bassareo 2007).

Cocaine Addiction: Craving and Negative Affective State

The dual affect model (Baker et al. 1987) and the elaborated model of desire (Kavanagh et al. 2005) predict that craving for the drug can produce negative and positive affective states. In the dual affect model of craving developed by Baker and colleagues (1987) both negative and positive affective states are associated with drug craving/seeking behavior. Negative affect in addicts can be triggered by cues associated with the drug, by drug withdrawal, and by information that the drug is not available. Positive affect in addicts can be triggered by small doses of the drug, by cues paired with the drug, and by information that the drug is available. Over a history of drug use, addicts typically go through multiple cycles of withdrawal that is alleviated by drug self-administration. These experiences promote learning to take drugs to avoid the aversive aspects associated with withdrawal (Tiffany 1990, Tiffany 2010).

In addition, addicts learn to take drugs to relieve negative mood states associated with everyday life. In fact a study conducted by Robbins and colleagues (2000) illustrated the impact of negative mood state associated with withdrawal on cocaine seeking. These investigators examined negative and positive mood states of cocaine dependent outpatients who served as subjects in their study. Subjects were exposed to non-drug cues (i.e. instructional audiotape) and cocaine cues (i.e. cocaine paraphernalia) in a laboratory setting. Next, the subjects rated their moods before and after the cues were presented. The subjects self-reported mood states did not change with the presentation of the non-drug cues. However, following the presentation of drug cues, their negative mood states increased.

The emergence of negative affective states associated with drug consumption plays an important role in craving and relapse (Harris et al. 2005, Fox et al. 2008, Fox et al. 2007a, Newton et al., 2003, Sofuoglu et al., 2003). For example, Fox and colleagues (2008) examined whether there were changes in a stress response and craving in abstinent cocaine

dependent subjects versus a demographically matched non-addicted control group. These investigators presented 5 minute images of stressful situations, drug/alcohol related situations, and neutral-relaxing situations and then requested the subjects to rate their craving, anxiety, and emotion. Abstinent cocaine dependent subjects reported significantly higher craving, anxiety, anger, fear, and sadness ratings as compared to controls in response to stressful and drug/alcohol related situations. This finding suggest that abstinent cocaine dependent individuals are more susceptible to negative emotions and cocaine craving following both stress and cocaine cue presentations.

It has been hypothesized that the emergence of negative affective states (i.e. dysphoria, irritability, anhedonia) plays an important role in relapse. For example, feelings of stress and irritability can lead to initial drug use**,** but discontinuation of drug use can lead to increased feelings of negative mood states, which in turn lead to relapse (Koob and Le Moal 1997). In fact, the Diagnostic Statistical Manual, fifth edition (DSM–V) (2013) recognizes that repeated drug use results in the devaluation of social and recreational activities, such as the failure to finish major obligations at work or school, as the addict continues to seek and use drugs regardless of negative consequences. Further, addicts typically report feelings of negative affect, particularly when exposed to drug cues during abstinence that often lead to drug craving and relapse (Koob and Volkow 2010, Nyland and Grigson 2013). Negative affective states have also been observed in animals after a natural reward is paired with the delayed access to self-administered drug (Wheeler et. al. 2008, Wheeler et. al 2011, Grigson and Twining 2002). After multiple pairings of a drug with a natural reward (i.e. saccharin), rats either avoid (Grigson and Twining 2002) or exhibit aversive responses (Wheeler et. al. 2008) to a cue that predicts drug delivery. These

findings highlight the importance of investigating factors controlling natural reward devaluation and the emergence of negative affective states in addiction.

Drug-Induced Devaluation of Natural Rewards

An animal model was developed in the Carelli laboratory to study natural reward devaluation by cocaine and the associated emergence of negative affective states in rats (Wheeler et. al. 2008, Wheeler et. al. 2011). This model is based on the finding that rats exhibit stereotyped oromotor responses to palatable and unpalatable taste stimuli when these stimuli are infused directly into the oral cavity. The oromotor responses correspond to the hedonic valence of the taste stimuli. Importantly, these oral facial responses, termed taste reactivity, reflect not only innate taste preferences but also conditioned changes in affect (Grill and Norgren 1978, Wheeler et. al. 2008). Rats exhibit appetitive taste reactivity (i.e., licks, lateral tongue protrusions) during infusion of a sweet tastant such as saccharin, and aversive taste reactivity during intraoral infusion of a bitter tastant, such as quinine. In an initial study using this approach (Wheeler et. al. 2008), a sweet taste cue (saccharin) was intraorally delivered in discrete intervals (i.e., 30, 3.5 s infusions, given every minute across 30 minutes). Immediately after the tastant infusion phase, a lever was inserted in the chamber and animals could press the lever to obtain an intravenous infusion of cocaine (i.e. during a 2 hr self-administration phase). Thus, the discrete taste cue signaled impending but delayed cocaine availability. Wheeler et al (2008) hypothesized that this ‘‘drug waiting’’ period during the infusion of the tastant allowed a strong association to develop between the taste and *delayed* drug, and enabled the emergence and expression of a negative affective state as measured by taste reactivity.

To test this possibility, members of the Carelli laboratory used videotape analysis to detect and quantify facial responses during intraoral tastant delivery, and examined EMG activity

of the anterior digastric muscle, a jaw muscle coupled to licking (Roitman et al. 2005, Roitman et al. 2010). They showed that rats initially exhibit appetitive taste reactivity during intraoral infusion of the sweet tastant. However, repeated pairing of a sweet tastant with delayed administration of cocaine results in an aversive state that is reflected in behavior. That is, animals exhibit aversive taste reactivity to the sweet which corresponds to aversive movements (gapes) evident in EMG recordings. Critically, this negative affective state increases motivation to consume cocaine. Specifically, aversive taste reactivity (gapes) was significantly correlated with cocaine loading responses (presses during the first 5 min of the session) and latency to the first press during self-administration. Rats that exhibited the most gapes showed the greatest number of load up responses and the fastest latency to initiate responding for cocaine once self-administration was available. Importantly, this aversive state was reflected in a shift in the activity of distinct populations of neurons and dopamine release events in the NAc, a brain region important for processing reward related information (Wheeler et. al. 2011, Wheeler et. al. 2008). Collectively, these findings suggest that cocaine-conditioned taste cues elicit a cocaine-need state that is aversive, is encoded by a distinct subset of NAc cells and rapid dopamine signaling, and promotes cocaine seeking, even following 1 month abstinence from the drug (Carelli and West 2014).

Cocaine Dose-Response

The great majority of self-administration studies with rodents have used a fixed ratio 1 (FR-1) schedule of reinforcement. The FR-1 schedule of reinforcement is useful for examining patterns of abuse liability such as the rate of drug intake (Arnold and Roberts 1997). Intravenous infusions of varying cocaine doses have been shown to be effective reinforcers of lever pressing behavior in experimental animals (Pickens and Thompson, 1971). In fact, reports show an

inverse relationship between response rate and magnitude of cocaine reinforcement (dose per infusion) (Pickens and Thompson, 1968a, Woods and Schuster, 1968). Decreasing the dose of cocaine increases the amount of operant responding for the drug.

Drug self-administration procedures typically involve a single unit dose of a drug infused through a catheter line contingent upon the subject performing an operant response (i.e., lever press). Increasing the concentration of the drug alters behavioral responding and is often described by an ascending dose response curve (Zimmer et. al. 2011). When given a choice, animals have shown that higher doses, to a point, are typically preferred (Lynch et al. 1998, Ward et al. 2005, Zimmer et al. 2011) over lower doses.

During self-administration of psychostimulants, however, the response rate for the drug varies inversely by dose (Pickens. et. al. 1968) such that as the drug concentration increases animals will decrease their operant responding for the drug (Pickens and Thompson 1968) (Pickens, R. et. al. 1978). For example Martin et. al. (1996) examined a cocaine dose effect relationship within a single session. Specifically, these authors examined cocaine maintained responding within a 3 hour session in which three doses (0.5, 1.0, and 2.0mg/kg) of cocaine were available in random order. The authors reported that as the dose increased the animals decreased their operant responding. In a different study (Winger et. al. 1989) a similar procedure was developed for monkeys using varying cocaine doses (0.003, 0.01, 0.03 mg/kg/infusion). This procedure involved a different dose of cocaine made available for self-administration in each of four 25-min periods with each period separated by a 10 min time out during which no programed stimuli were present. These authors reported that response rate was an increasing function of dose and the dose response relationships were independent of the order in which the doses were given. Other research has shown that cocaine presented in a descending order of doses within a

session produces a dose response curve that is similar to that seen in rats given multiple doses across sessions over days (Martin et al. 1996, Emmett-Oglesby et. al. 1993).

Environmental Enrichment

An important research area with clinical relevance focuses on the effects of environmental enrichment (EE) as a means to prevent continued drug use. EE in rodents involves exposure of animals to surrounding stimuli (i.e. toys, access to a running wheel, social interaction, and larger spaces) used to enhance sensory, cognitive, and motor behaviors (Grimm et al. 2008, Solinas et al. 2008). EE has been shown to reduce the reinforcing effects of psychostimulants such as amphetamines and cocaine (Bardo et al. 2001, Chauvet et al. 2009, Solinas et al. 2009, Puhl et al. 2012). In addition, investigators have suggested that EE influences brain development by way of neuroanatomical and neurochemical changes in the reward circuit (Stairs and Bardo 2009, Puhl et al. 2012). For instance, Solinas and colleagues (2009) reported that mice reared in an EE exhibited lower levels of the early immediate gene zif-268 expression in the NAc. Given this evidence, investigators have hypothesized that exposure to EE promotes brain development in animals that may prevent drug abuse vulnerability.

Even though environmental enrichment has been shown to protect against drug addiction, it seems that the dose of the drug and time spent in an enriched environment plays a significant role in EE effectiveness. For instance, Bardo et. al. (2001) compared environmental condition (EE), social condition (SC), and isolated condition (IC) rats self-administering amphetamine at 2 doses (0.03 and 0.1mg/kg/infusion). These authors reported that EE exhibited the greatest attenuation in amphetamine self-administration, but only at the lower dose (0.03mg/kg/infusion). In another report (Green et al. 2002), the authors examined amphetamine dose response curve (0, 0.006, 0.01, 0.02, 0.06, or 0.2mg/kg/infusion) for EE versus IC rats. They reported that the EE

rats as compared to the IC rats earned fewer infusions at the 0.006mg/kg, and 0.02mg/kg doses under fixed ratio-1 and progressive ratio schedules of reinforcement, respectively. At the other doses there were no significant differences in behavioral responding between the groups. Further, Gipson et. al. (2011) examined if EE exposure during development would protect against escalation (i.e. increase in drug intake across a 6h session) of cocaine self-administration at two doses (0.1 and 0.5 mg/kg/infusion). They reported EE prevented the escalation of cocaine self-administration only at the lower dose. These findings suggest an enriched environment may have protective effects, but only at lower doses of psychostimulants.

The duration of exposure to environmental enrichment prior to or following drug administration appears to be important in the protective effects of EE against the effects of drugs of abuse. Solinas et. al. (2008) demonstrated that prolonged exposure in the enriched environment following cocaine administration reduced sensitization to cocaine. For instance, when mice were injected with 15mg/kg dose of cocaine, they developed behavioral sensitization to cocaine by exhibiting increased locomotor activity. This was followed by abstinence in which mice were placed into their respective environmental conditions (standard or enriched). After 1, 7, and 30 days since their last cocaine injection, mice were tested for behavioral sensitization to cocaine by administering an intraperitoneal injection of 10mg/kg dose of cocaine. After 1 day, all mice regardless of environmental condition exhibited sensitization. However, 30 days after the last injection of cocaine, enriched mice showed the greatest reduction in sensitization as compared to the mice housed in standard conditions. Thiel et. al. (2011) examined if enrichment can protect against the effects of cocaine when enrichment is no longer available and reported that rats exposed to an enriched environment as compared to controls exhibited less responding during extinction and cue-induced reinstatement during the time they were exposed to an

enriched environment. These results indicate enrichment is helpful in attenuating reinstatement, but this effect may be brief with no extended benefit over time.

Results from these studies indicate that environmental enrichment can provide protection against repeated drug use, drug sensitization, and reinstatement. Nevertheless, more research is needed to discover at which point EE might be most effective. In particular, there is very little known about whether EE might alter other aspects of reward, such as the devaluation of natural rewards. Devaluation of natural rewards appears to be a consequence of delayed access to drug administration (Grigson et. al. 2002, Wheeler et al. 2008, Wheeler et al. 2011, Puhl et al. 2012). Puhl et. al. (2012) reported that EE exposure during adulthood reduced the incentive value for cocaine, but was unable to prevent the devaluation of a natural reward that predicted cocaine self-administration. There could be a number of factors why devaluation of a natural reward continued to occur. In the Puhl studies, rats were exposed to EE during adulthood. Perhaps, EE would have been more effective if exposure took place during childhood or adolescence. Also, Puhl et al (2012) only examined one dose of cocaine, 0.167mg/kg/infusion, perhaps the effect would be seen at other doses or with other types of enrichment (i.e. exposure only to a running wheel instead of only to novel objects).

Goals of the Dissertation

As mentioned in the background material above, members of the Carelli laboratory have shown that rats initially exhibit appetitive taste reactivity during intraoral infusion of a sweet tastant. However, after repeated pairings of a sweet tastant with cocaine, the natural reward is devalued and rats exhibit aversive taste reactivity (gapes) during its infusion (Wheeler et. al. 2008). The primary goal of this work is to extend the current knowledge of this preclinical model of devaluation of natural rewards by cocaine and to examine other variables that may

influence this process. Aim 1 of this dissertation will examine if the development of the negative affective state and motivation to consume the drug in this preclinical model is cocaine dose-dependent. Additional studies will manipulate aspects of the rats' environment (specifically by providing access to a running wheel in their home cages), either following (Aim 2) or prior to (Aim 3) taste-drug pairings to determine if exercise alters, or attenuates, the development of the negative affective state and motivation to consume the drug that normally occurs in this model. Each of these factors is considered in more detail below.

Specific Aims

Aim 1. To examine the effects of cocaine dose on natural reward devaluation by cocaine. In the Carelli laboratory, an animal model was developed to study natural reward devaluation by cocaine and the associated emergence of negative affective states in rats (Wheeler et. al. 2011; Wheeler et. al. 2009; Wheeler et. al. 2008). In this model a sweet taste cue (saccharin) is intraorally delivered in discrete intervals (i.e., 30, 3.5 s infusions) every minute across a 30 minute period. Immediately after the tastant infusion phase, a lever is inserted in the chamber and animals can press it for intravenous cocaine (i.e. during a 2 h self-administration phase). Normally, rats exhibit appetitive taste reactivity (i.e., licks) during intraoral infusion of a sweet tastant such as saccharin, and aversive taste reactivity (i.e., gapes) during intraoral infusion of a bitter tastant such as quinine. As expected, in our model, rats elicit appetitive taste reactivity on the first day of saccharin-cocaine pairings (i.e., naïve situation). However, after repeated taste-drug pairings rats elicit aversive taste reactivity during infusion of the same concentration of saccharin. This aversive state is correlated with increased motivation to consume cocaine once the self-administration phase begins, indicated by increased drug 'loading' behavior and decreased latency to press the lever for drug. However, in all previous studies using our model,

only one unit dose of cocaine (0.33mg/inf, or approximately 1 mg/kg) was examined. Given the differences in behavioral responding for intravenous cocaine that normally occurs across dose (Carelli and Deadwyler 1996), the objective of the present aim is to determine if the development of the negative affective state (gapes) in our model systematically varies as a function of cocaine dose during the self-administration phase. I predict that the development of aversive taste reactivity in our model will be dose-dependent. That is, I hypothesize that rats will elicit the least aversive responses to the saccharin tastant paired with the lowest cocaine dose and the greatest aversive responses to the saccharin tastant paired with the highest cocaine dose during the self-administration phase.

Aim 2. To examine if exercise (i.e. voluntary wheel running) <u>following</u> exposure to the preclinical model alters the development of negative affect and natural reward devaluation. Physical activity or exercise is a form of environmental enrichment that has been shown to reduce drug-seeking and the reinforcing effects of psychostimulants (Smith and Lynch 2011). For example, Smith et al (2008) examined if long-term voluntary exercise, an aspect of environmental enrichment, would reduce the reinforcing efficacy of cocaine self-administration. Specifically, rats exposed to a running wheel had significantly lower breakpoints (maximum number of infusions before animal stops behaviorally responding) than the sedentary group. This finding was observed across two doses (0.3 and 1.0 mg/kg/infusion) of cocaine. Likewise, Thanos and colleagues (2013), examined long-term (6 weeks) forced exercise (placed on treadmill for 1 or 2 hrs daily) in rats on cocaine- and cue-induced reinstatement. They reported that rats exposed to forced exercise exhibited a significant reduction in cue-induced reinstatement and behavioral sensitization. These findings indicate that exercise, voluntary or forced, reduces the reinforcing effects of cocaine and may be protective against drug addiction.

In aim 2, I will examine if access to a running wheel in the rats' home cage following repeated saccharin-cocaine pairings can reverse or attenuate the negative affective state (and increased motivation for drug) established in the preclinical model.

Aim 3. To examine if voluntary wheel running in the animals' home cage prior to taste-drug training will prevent the development of negative affect and natural reward devaluation in our model. A number of interesting studies have examined the effects of exercise on drug addiction using animal models. In those studies, exposure to a running wheel reduced drug seeking behavior in all phases of the addiction cycle including acquisition, maintenance, and relapse (Lynch et al. 2010, Smith et al. 2011, Smith and Witte 2012). In addition, Cosgrove and colleagues (2002) reported that wheel running serves as an alternative non-drug reinforcer that reduces cocaine seeking. Thus animals exposed to a running wheel are believed to have enhanced sensory, cognitive, and motor behaviors (Smith and Lynch 2011). In contrast an isolated environment, in which the rat is individually housed with no access to a wheel, induces stress, particularly if the rats are water or food restricted (Smith and Lynch 2011). In support, Smith and colleagues (2012) reported that a history of continuous exercise reduced the positive rewarding effects of cocaine, decreased cocaine self-administration (when exposure to a running wheel was not initiated until following drug exposure), and decreased locomotor effects of cocaine. Thus, wheel running may provide protection against cocaine seeking behavior. In aim 3, I will examine if access to a running wheel in the animal's home cage *prior* to taste-drug training in our model will alter subsequent aversive taste reactivity and cocaine-seeking behavior.

CHAPTER 2

EXAMINATION OF COCAINE DOSE IN A PRECLINICAL MODEL OF NATURAL REWARD DEVALUATION BY COCAINE

Introduction

Cocaine addiction is characterized by cycles of drug use, abstinence, and resumption of drug taking (relapse). Embedded in the addiction cycle is the development of negative affective states (i.e. dysphoria, irritability, anhedonia) as well as the devaluation of natural rewards (Koob et al. 1998, Koob and Volkow 2010). During prolonged drug abstinence, cocaine addicts typically report increased feelings of negative affect, particularly when exposed to drug cues. This aversive condition often leads to craving and relapse, with the later believed to 'correct' for this negative emotional state (Risinger et al. 2005, Grigson 2008, Koob and Volkow 2010, Nyland and Grigson 2013).

A preclinical model was developed in our laboratory to study natural reward devaluation by cocaine and the associated emergence of negative affective states in rats (Wheeler et al. 2008, Wheeler et al. 2011), built upon work by Grigson and colleagues (Grigson, 1997, Grigson and Twining, 2002). The model used here is based on the finding that rats exhibit stereotyped oromotor responses to palatable and unpalatable taste stimuli infused directly into the oral cavity that correspond to the hedonic valence of the stimulus. Importantly, oral facial responses, termed taste reactivity, reflect not only innate taste preferences but also conditioned changes in affect

(Grill and Norgren 1978, Wheeler et al. 2008). Rats exhibit appetitive taste reactivity (i.e., licks, lateral tongue protrusion) during intraoral infusion of a sweet such as saccharin and aversive taste reactivity (i.e., gapes) during infusion of a bitter tastant (e.g., quinine). In this model, the sweet (saccharin) is intraorally delivered in discrete 3.5s intervals (once a minute over 45min, phase 1) and is immediately followed by access to cocaine self-administration for 2h (phase 2). We showed that the initially palatable saccharin solution became unpalatable as the tastant became associated with impending, but delayed, opportunity to self-administer cocaine. It was hypothesized that this ‘‘drug waiting’’ period, when the tastant was infused, allowed for a strong association to develop between the taste and delayed drug access, and enabled the emergence and expression of a negative affective state as measured by taste reactivity (Wheeler et al. 2008, Wheeler et al. 2011, Carelli and West 2014). Further, rats that exhibited the most aversive responses in phase 1 were the most motivated to consume cocaine in phase 2 once it was available. Thus, this negative affective state that is reflected by aversive taste activity in phase 1 appears to be ‘corrected’ by cocaine consumption during self-administration in phase 2.

Here, we build upon that work and examine the negative affective state and increased motivation to consume cocaine as a function of cocaine dose during self-administration. We hypothesize that the negative affective state that develops in this model is cocaine dose-dependent (i.e., the number of aversive gapes will increase with increasing dose of drug).

Methods

Subjects: Male, Sprague-Dawley rats (Harlan), aged 90-120 days and weighing approximately 275-350 grams were used (n=24). Animals were housed individually and maintained on a standard 12:12h light-dark cycle (lights on at 7:00 a.m.). During training and testing, animals were restricted to no less than 85% of their preoperative body weight by limiting

water access (30 ml/day). Food was available ad libitum. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (2011), and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgeries: Rats were anesthetized with a ketamine hydrochloride (100mg/kg) and xylazine (20mg/kg) mixture and surgically implanted with intra-oral cannulae and an intravenous catheter in a single surgery, as described previously (Roitman et al. 2008, Wheeler et al. 2008). Intravenous catheters for self-administration were purchased from a commercial source (Access Technologies, Skokie, IL) and inserted into the jugular vein using established procedures (Carelli and Deadwyler 1996, Wheeler et al. 2008).

Experimental Design: Training and test sessions were conducted in a Plexiglas chamber (Med Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle. Figure 2.1A shows the experimental design. Initially, mildly water-deprived (30ml/day) rats were trained to press a lever for water; they underwent surgical procedures as described above. One week later, rats received 14 daily conditioning sessions during which the behavioral task was conducted in two phases/day: 1) intra-oral tastant infusions and 2) cocaine self-administration. In phase 1, rats received discrete intraoral saccharin infusions, delivered in 3.5s intervals (0.15% saccharin solution, 200 μl/infusion). A total of 45 tastant infusions (trials; 1 trial/min) were delivered per session. Immediately thereafter, the intravenous catheter line was attached and phase 2 (cocaine self-administration) was initiated, as described previously (Wheeler et al. 2011). Briefly, rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule of reinforcement (2h). Each lever press resulted in intravenous infusion of cocaine (over 6s), termination of the cue light positioned above the lever, and onset of a tone

(67db, 1 kHz) and house light (25W) stimulus. Lever presses during the 20s post response period had no programmed consequences. Three doses of cocaine were examined (0.16, 0.33 and 0.66 mg/inf) in a between-subjects design. The same flavor of 0.15% saccharin was used in phase 1 across all three cocaine doses (groups). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse.

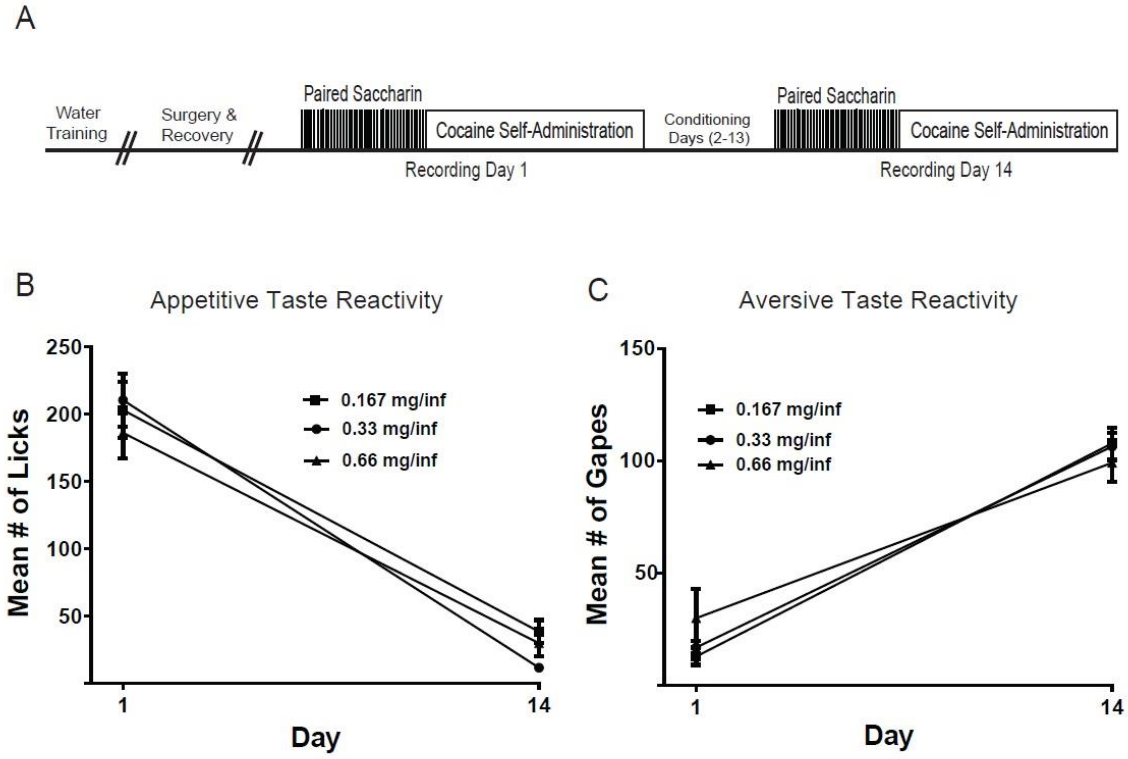

Figure 2.1. Schematic diagram of task timeline and taste reactivity across doses. (A) Schematic diagram of task. See text for details on task procedure. Mean number of licks (B) and gapes (C) during saccharin infusions (phase 1) on the first and last days of the taste-drug pairings as a function of cocaine dose. Note that the animals exhibited a significant decline in appetitive taste reactivity and a significant increase in aversive taste reactivity across days that were not cocaine dose-dependent.

Taste Reactivity: Taste reactivity was analyzed in a frame by frame analysis using digital video recorded on days 1 and 14. Appetitive and aversive taste reactivity were counted using the procedure developed by Grill and Norgren (1978). Briefly, mouth movements expressed in the

6s following infusion onset that matched a "triangle" shape for a duration exceeding 90ms were counted as aversive. Instances in which the tongue protruded and crossed the midline were counted as appetitive.

Cocaine Self-Administration: Cocaine self-administration was examined across training days. On the last day of training (day 14), the average number of lever presses, latency to the first lever press, and average number of cocaine load-up presses were examined across cocaine doses using separate one-way ANOVAs. Load-up behavior was defined as rapid lever pressing in the beginning of the session in which the average inter-press interval (INT) during load-up was less than half the average INT during the remainder of the session.

Data Analysis: Changes in the total number of lever presses during self-administration across the 14 days of training were examined as a function of cocaine dose using a 2-way mixed design ANOVA. Changes in the average number of licks (appetitive taste reactivity) and average number of gapes (aversive taste reactivity) were compared on day 1 versus day 14 as a function of dose using separate 2-way mixed design ANOVAs. Pearson product correlation coefficients examined relationships between aversive taste reactivity on day 14 to the: 1) total number of load-up presses, 2) total number of lever presses, and 3) latency to the first lever press during self-administration. Statistical analyses of all behavioral data were performed using commercially available software (Statstica, Tulsa, OK).

Results

Phase 1: Taste Reactivity

A two-way mixed design ANOVA on appetitive taste reactivity across cocaine doses revealed a significant main effect of day ($F_{1,21}$=207.08, p<0.001), but no main effect of dose ($F_{2,21}$=0.37, p>0.05) and no significant day X dose interaction ($F_{2,21}$=1.248, p>0.05). The results

indicate that appetitive taste reactivity was lower on day 14 compared to day 1 across all rats independent of cocaine dose (Figure 2.1B). A two-way mixed design ANOVA on aversive taste reactivity revealed a significant main effect of day ($F_{1,21}$=135.42, p<0.001), but no significant main effect of dose ($F_{2,21}$=1.901, p>0.05) and no significant day X dose interaction ($F_{2,21}$=0.0682, p>0.05). The results indicate that aversive taste reactivity increased for all rats across training days (on day 14 compared to day 1), independent of cocaine dose (Figure 2.1C).

Phase 2: Cocaine self-administration

All rats acquired self-administration across the 14 training days. A two-way mixed design ANOVA on cocaine self-administration revealed a significant main effect of day ($F_{13,20}$=2.6202, p<0.01), and main effect of dose ($F_{2,20}$=48.2084, p<0.001) but no significant dose X day interaction ($F_{26,260}$=1.00, p>0.05). Next, we examined aspects of self-administration behavior on day 14 across the three cocaine doses. First, we determined if the total number of lever presses during the self-administration phase varied as a function of cocaine dose. A one-way ANOVA revealed a significant main effect of cocaine dose ($F_{2,21}$= 21.6180 p<0.05) on lever pressing for cocaine. Post hoc tukey tests revealed that rats pressed the lever significantly less as the dose of cocaine increased (Figure 2.2A). Second, a one-way ANOVA revealed a non-significant trend toward a decrease in latency to the first press as a function of cocaine dose ($F_{2,21}$= 1.50, p > 0.05, Figure 2.2B). Finally, a one-way ANOVA revealed a significant main effect of cocaine dose ($F_{2,21}$ = 8.287, p<0.05) on load-up behavior (Figure 2.2C). Post hoc tukey tests revealed that as cocaine dose increased, the number of load-up presses decreased.

Our prior studies show that animals that exhibited the most aversive taste reactivity on the last day of training displayed the highest levels of load-up presses for cocaine, once available (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). To determine if this

finding is cocaine dose-dependent, Pearson correlation coefficients were conducted. The results revealed that the number of load-up presses was correlated with the number of aversive responses (gapes) when all doses were combined (r^2=0.55, p<0.05, Figure 2.2D) and for each individual cocaine dose (0.167 mg/inf: r^2=0.88, p<0.05; 0.33 mg/inf: r^2= 0.91, p<0.05; 0.66 mg/inf: r^2= 0.63, p<0.05). There were no significant correlations between aversive responses and mean number of lever presses (all doses combined, r^2=0.07, p>0.05; 0.167 mg/inf r^2=0.11; 0.33 mg/inf r^2=0.028; 0.66 mg/inf r^2=0.0006), or latency to first press (all doses combined r^2=0.10, p>0.05; 0.167 mg/inf r^2=0.12; 0.33 mg/inf r^2=0.000035; 0.66 mg/inf r^2=0.31).

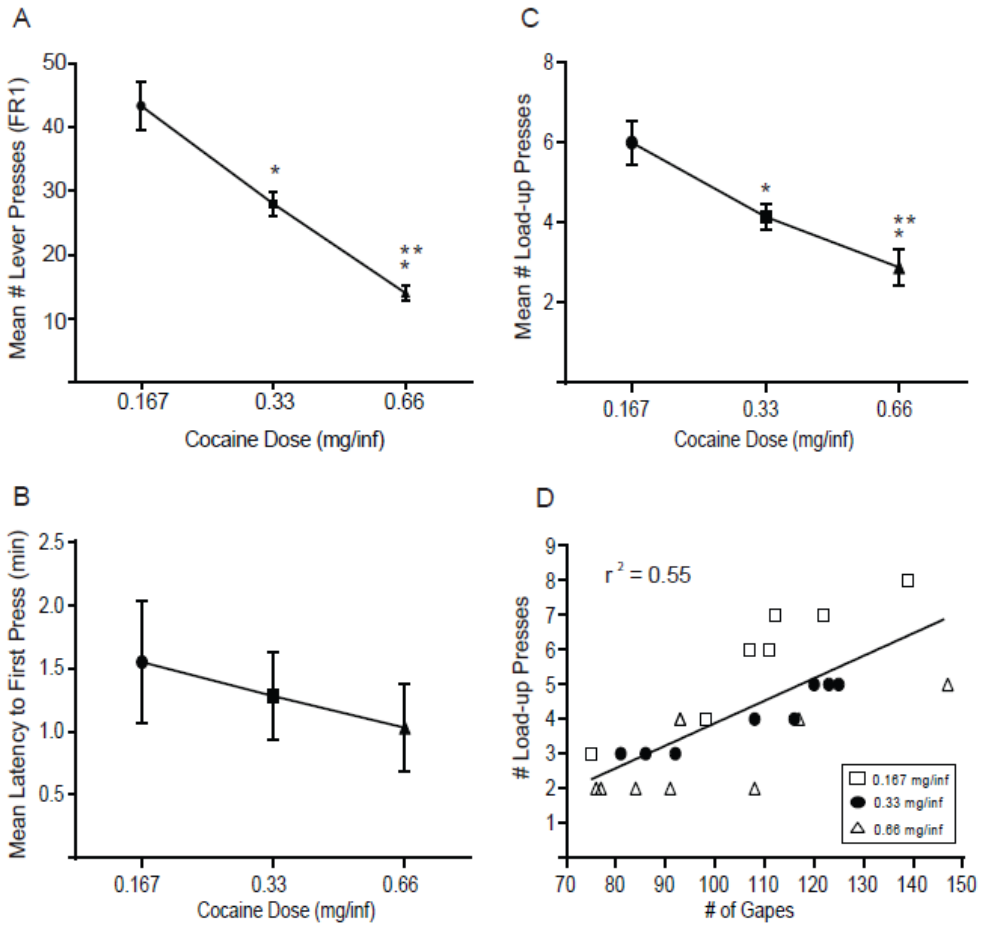

Figure 2.2. Correlations between self-administration behaviors, cocaine dose and aversive taste reactivity. Mean number of lever presses (A), mean latency to the first press (B) and mean

number of load-up presses (C) as a function of cocaine dose on day 14. (D) Mean number of load-up presses is significantly correlated with aversive taste reactivity (gapes) across all cocaine doses (r^2 = 0.74) and for all individual doses (see text for details). *p< 0.05 compared to 0.167mg/inf; **p< 0.05 compared to 0.167 and 0.33mg/inf.

Discussion

The main objective of the present study was to determine if the negative affective state that develops in the preclinical model of natural reward devaluation by cocaine is dose-dependent. The current findings replicate earlier work by showing that rats exhibited a shift from appetitive to aversive taste reactivity as the tastant came to predict impending but delayed cocaine availability (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). Here, we extend those findings and show that once this aversive state develops (day 14), it does not vary as a function of cocaine dose during the self-administration phase, consistent with Cason and Grigson (2013).

An important feature of this preclinical model is that rats exhibiting the most aversive responses are also the most motivated to consume cocaine once available (Wheeler et al. 2008, Wheeler et al. 2011). That is, we previously reported that rats that exhibited the most gapes during phase 1 showed the greatest number of load-up presses and were fastest to press the lever for cocaine once self-administration was available in phase 2. It is well known that load-up behavior during the start of cocaine self-administration sessions is cocaine dose-dependent (Carelli and Deadwyler 1996). However, here we extend those findings and show that in this preclinical model positive correlations exist between load-up presses and aversive taste reactivity across all doses tested. This finding supports the view that rats are more motivated to consume cocaine when they experience a negative aversive state across all doses tested. A possible explanation for this finding may be that the rapid rate of responding at the start of the self-

administration phase in phase 2 (i.e., load-up behavior) reflects the animals attempt to achieve an optimal level of drug in their system to overcome the aversive state that develops in phase 1. Indeed, prior studies have shown that the rate of self-administration responding is linked to achievement and maintenance of an optimal level of drug (Pettit and Justice 1989, Pettit and Justice 1991), perhaps reflective of a hedonic set point (Koob and Caine 1999, Koob and Volkow 2010). Although the emergence of negative affect in this preclinical model is not cocaine dose-dependent, the present findings also show that load-up behavior may reflect a correction of this aversive state that is observed across cocaine dose.

Drug users identify negative affect as a main reason for continued drug use and relapse (Sinha et al. 2000, Baker et al. 2004). Indeed, the emergence of negative affect and natural reward devaluation related to repeated drug use is considered a key aspect in models of drug addiction (Koob et al. 1998, Grigson and Twining 2002, Grigson 2008, Koob and Volkow 2010). As such, the current preclinical model may be a useful tool to examine possible behavioral interventions to reduce the emergence of this negative affective state, as well as neurobiological mechanisms that may underlie it.

CHAPTER 3

EFFECTS OF VOLUNTARY WHEEL RUNNING ON AN ESTABLISHED COCAINE-INDUCED NEGATIVE AFFECTIVE STATE IN A RODENT MODEL

Introduction

Cocaine addiction is characterized by cycles of drug use, abstinence, and resumption of drug taking (relapse). Embedded in the addiction cycle is the development of negative affective states (i.e. dysphoria, irritability, anhedonia) as well as the devaluation of natural rewards (Koob et al. 1998; Koob and Volkow 2010). During prolonged drug abstinence, cocaine addicts typically report increased feelings of negative affect, particularly when exposed to drug cues. This aversive condition often leads to craving and relapse, with the later believed to 'correct' for this negative emotional state (Risinger et al. 2005; Grigson 2008; Koob and Volkow 2010; Nyland and Grigson 2013).

An animal model was developed in the Carelli laboratory to study natural reward devaluation by cocaine and the associated emergence of negative affective states in rats (Wheeler et al. 2008, Wheeler et al. 2011). This model is based on the finding that rats exhibit stereotyped oromotor responses to palatable and unpalatable taste stimuli when infused directly into the oral cavity that correspond to the hedonic valence of the stimulus. Importantly, oral facial responses, termed taste reactivity, reflect not only innate taste preferences but also conditioned changes in affect (Grill and Norgren 1978, Wheeler et al. 2008). Rats exhibit appetitive taste reactivity (i.e.,

licks, lateral tongue protrusion) during infusion of a sweet tastant such as saccharin and aversive taste reactivity (i.e., gapes) during intraoral infusion of a bitter tastant, such as quinine. In the initial study by the Carelli laboratory using this approach (Wheeler et al. 2008), a sweet taste cue (saccharin) was intraorally delivered in discrete intervals (i.e., 30, 3.5 s infusions, given every minute across 30 minutes). Immediately after the tastant infusion phase, a lever was inserted in the chamber and animals could press it for intravenous cocaine (i.e. during a 2 hr self-administration phase). Thus, the discrete taste cue signaled impending but delayed cocaine availability. The Carelli laboratory hypothesized that this ‘‘drug waiting’’ period when the tastant was infused allowed for a strong association to develop between the taste and *delayed* drug, and enabled the emergence and expression of a negative affective state as measured by taste reactivity.

An important research area with clinical relevance focuses on the effects of physical activity, specifically exercise, as a means to prevent continued drug use (Greenwood et al. 2011, Lynch et al. 2013). Exercise has been shown to reduce the reinforcing effects of psychostimulants such as amphetamines and cocaine (Smith et al. 2008, Smith and Lynch 2011, Lynch et al. 2013). For instance, Smith et. al. (2011), reported that rats with access to a running wheel for 6 weeks self-administered significantly less cocaine and showed less escalation of cocaine intake over time as compared to sedentary rats. There are similar reports of an attenuation of methamphetamine (Engelmann et al. 2014) and alcohol consumption (Hammer et al. 2010, Brager and Hammer 2012) in rats that had access to a running wheel for an extended period.

Interestingly, several recent studies have examined if the effects of exercise during abstinence from chronic drug administration prevents subsequent drug craving (Lynch et al.

2010, Sanchez et al. 2013, Peterson et al. 2014, Peterson et al. 2014). For instance, Lynch et. al. (2010) using a reinstatement model, examined if 2h/day access to a running wheel during a 14 day abstinence period would reduce cocaine seeking in Sprague-Dawley rats. The authors reported that aerobic exercise signficantly reduced lever presses during extinction and reduced cocaine seeking in response to cocaine-associated cues. Similar results were reported for the effects of voluntary wheel running on nicotine seeking (Sanchez et al. 2013). Furthermore, Peterson et. al. (2014) reported that even though 2h/day access to a running wheel was effective in reducing cocaine seeking, 6h/day and 24h/day access during abstinence almost abolished subsequent cocaine seeking in male Sprague Dawley rats. This suggests that exercise not only has the potential to reduce drug seeking, it appears to do so in an access-dependent manner (i.e., the more access to the running wheel the higher attenuation in drug seeking). Further, Thanos and colleagues examined long-term (6 weeks) forced exercise (placed on treadmill for 1 or 2h daily) in rats on cocaine cue-induced reinstatement and sensitization (Thanos et al. 2013). The authors reported that rats exposed to forced exercise exhibited a significant reduction in cue-induced reinstatement and behavioral sensitization. These findings indicate that exercise, voluntary or forced, reduces the reinforcing effects of cocaine and may be protective against drug addiction.

The objective of this Aim is to examine if access to a running wheel in the animal's home cage ***following*** taste-drug training in our preclinical model will alter subsequent aversive taste reactivity and cocaine-seeking behavior. We hypothesize that after multiple taste-drug pairings, rats exposed to a wheel in their home cage during 7 weeks of abstinence will exhibit less aversive and more appetitive taste reactivity as compared to rats with no exposure to a running wheel. In addition, the Exercise rats exhibiting the highest wheel running will show less aversive

taste reactivity and less motivation to seek cocaine. These findings would indicate that exercise can reverse or attenuate the negative affective state that develops in our preclinical model.

Methods

Subjects: Male, Sprague-Dawley rats (Harlan), aged 90-120 days and weighing approximately 300-375 grams were used (*n*=18). Animals were housed individually in standard polycarbonate cages (19 x 10.5 x 8 in) and maintained on a 12:12h light-dark cycle (lights on at 7:00 a.m.). During training and testing, animals were restricted to no less than 85% of their preoperative body weight by limiting water access (30 ml/day). Food was available ad libitum. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (2011), and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgeries: Rats were anesthetized with a ketamine hydrochloride (100mg/kg) and xylazine (20mg/kg) mixture and surgically implanted with intra-oral cannulae and an intravenous catheter in a single surgery, as described previously (Roitman et al. 2008, Wheeler et al. 2008). Intravenous catheters for self-administration were purchased from a commercial source (Access Technologies, Skokie, IL) and inserted into the jugular vein using established procedures (Carelli and Deadwyler 1996, Wheeler et al. 2008).

Experimental Design: Training and test sessions were conducted in a Plexiglas chamber (Med Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle. Figure 3.1 shows the experimental design. Initially, mildly water-deprived (30ml/day) rats were trained to press a lever for water then underwent surgical procedures as described above. One

week later, rats received 14 daily conditioning sessions during which the behavioral task was conducted in two phases/day: 1) intra-oral tastant infusions and 2) cocaine self-administration. In phase 1, rats received discrete intraoral saccharin infusions, delivered in 3.5s intervals (0.15% saccharin solution, 200 µl/infusion). A total of 45 tastant infusions (trials; 1 trial/min) were

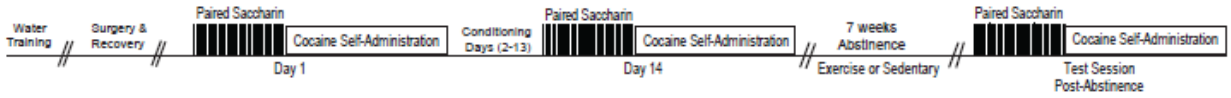

Figure 3.1. Experimental Timeline. Schematic diagram of task. See text for details on task procedure.

delivered. Immediately thereafter, the intravenous catheter line was attached and phase 2 (cocaine self-administration) was initiated, as described previously (Wheeler et al. 2011). Briefly, rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule of reinforcement (2h). Each lever press resulted in intravenous infusion of cocaine (over 6s), termination of the cue light positioned above the lever, and onset of a tone (67db, 1 kHz) and house light (25W) stimulus. Lever presses during the 20s post response period had no programmed consequences. Animals were trained over 14 conditioning days. Immediately following the last conditioning day, animals were divided into two groups and a 7 week abstinence period was imposed. Half the rats were assigned to the Exercise group (EX, n=9) and were housed in the colony room within a cage containing a running wheel (Coulbourn, Whitehall, PA, USA). During the abstinence period, EX rats had continuous access to the wheel. The other half of the animals were assigned to the Sedentary group (SED, n=9) and housed in standard cages with no running wheels. Immediately following the 7 week abstinence period, all

rats were placed back in the experimental chamber for a single test session. The test session was conducted in two phases as described above: 1) intra-oral tastant infusions (45, 3.5 s infusions per minute; over 45 mins) and 2) cocaine (0.33 mg/inf) self-administration (2 hrs). Taste reactivity was recorded: 1) on the first (day 1) and last (day 14) training days before the 7 week abstinence period and 2) during the one test session after abstinence. Self-administration behavior was recorded before, and if catheters remained patent, following the 7 week abstinence period. Wheel revolutions were recorded daily by the experimenter from a LCD counter (placed on the side of each wheel). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse.

Data Analysis:

Taste Reactivity: Taste reactivity was analyzed in a frame by frame analysis using digital video recordings. Appetitive and aversive taste reactivity were counted using the procedure developed by Grill and Norgren (1978). Briefly, mouth movements expressed in the 6s following infusion onset that matched a "triangle" shape for a duration exceeding 90 ms were counted as aversive. Instances in which the tongue protruded and crossed the midline were counted as appetitive. Prior to abstinence, appetitive and aversive taste reactivity were examined across all rats using paired t-tests on days 1 and 14. To determine if there were differences in appetitive and aversive taste reactivity prior to impending group assignment (EX or SED) a 2-way mixed design ANOVA was completed, followed by Tukey post hoc tests. Following abstinence, 2-way mixed design ANOVAs were performed to examine differences between groups in appetitive and aversive taste reactivity across days (i.e. day 14 versus the single test day following abstinence).

Cocaine Self-Administration: Behavioral measures of cocaine self-administration included the average number of lever presses, latency to the first lever press, and average number of cocaine load-up presses. Load-up behavior was defined as rapid lever pressing in the beginning of the session in which the average inter-press interval (INT) during load-up was less than half the average INT during the remainder of the session. Cocaine self-administration was examined during the two week training period prior to abstinence as follows. Changes in the total number of lever presses during self-administration across the 14 days of training were examined across all animals using a one-way repeated measures ANOVA. On the last day of training (day 14), Pearson product correlation coefficients were calculated to examine relationships between aversive taste reactivity and number of load-up presses across all animals, as well as those destined for the EX or SED groups.

Cocaine self-administration was examined during the test session conducted immediately after the 7 week abstinence period as follows. First, we compared between groups using unpaired t-tests the mean number of lever presses and load-up presses on the test session following abstinence. Pearson product correlation coefficients examined relationships across groups between aversive taste reactivity during the test session following abstinence to # load-up presses.

Wheel Running: A one-way ANOVA was used to examine differences in the average number of wheel rotations across weeks. In addition, Pearson product correlation coefficients examined relationships between number of wheel rotations for week 7 to the behavioral data exhibited during the test session following abstinence including: 1) aversive taste reactivity, 2) load-up presses, and 3) total number of lever presses.

Statistical analyses of all behavioral data were performed using commercially available software (Statstica, Tulsa, OK and GraphPad Prism, La Jolla, CA).

Results

Pre-Abstinence

Phase 1: Taste Reactivity

Prior to abstinence, we examined the development of appetitive and aversive taste reactivity for all animals across day 1 versus day 14 of training. A paired t-test revealed that appetitive taste reactivity was significantly lower on day 14 as compared to day 1 across all rats (t_{17} = 14.68, $p<0.0001$, Figure 3.2A). A separate paired t-test revealed that aversive taste reactivity was significantly higher on day 14, compared to day 1 (t_{17} = 8.29, $p<0.0001$, Figure 3.2B) across all rats. Next, rats were divided into their impending group assignment (EX and SED) and we examined if there were pre-existing differences in appetitive and aversive taste reactivity as a function of group. A two-way mixed design ANOVA on appetitive taste reactivity across groups revealed a significant main effect of day ($F_{1,16}$=222.73, $p<0.0001$), no main effect of group ($F_{1,16}$=0.96, $p>0.05$) and no significant day X group interaction ($F_{1,16}$=1.77, $p>0.05$), (Figure 3.2C). Likewise, a two-way mixed design ANOVA on aversive taste reactivity revealed a significant main effect of day ($F_{1,16}$=65.11, $p<0.0001$), but no significant main effect of group ($F_{1,16}$=1.04, $p>0.05$) and no significant day X group interaction ($F_{1,16}$=0.094, $p>0.05$), (Figure 3.2D). These findings indicate similar appetitive and aversive taste reactivity prior to abstinence regardless of impending group assignment (EX or SED).

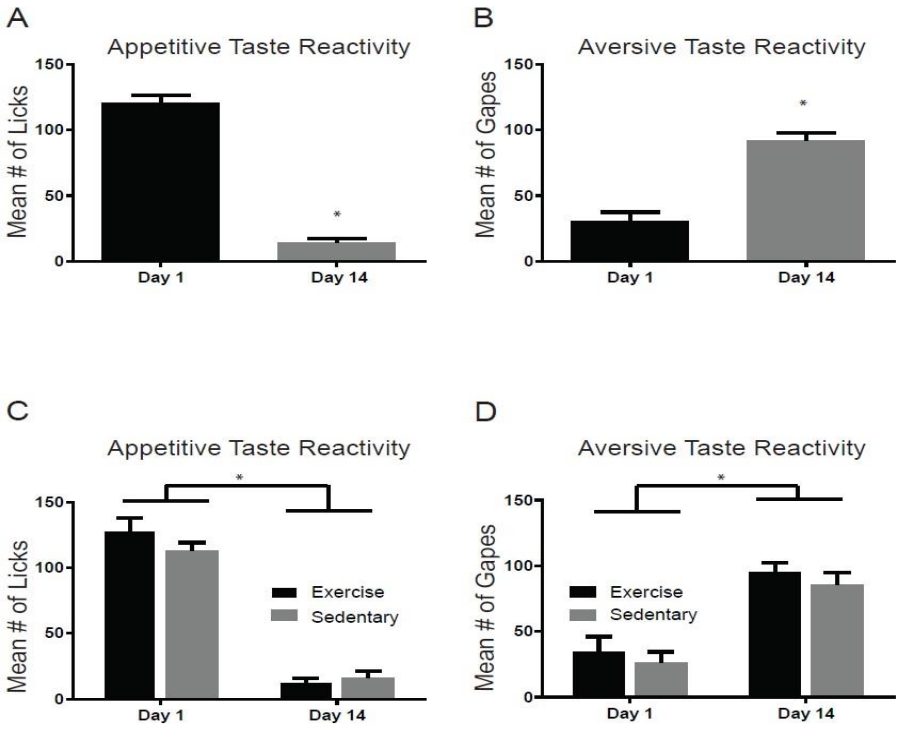

Figure 3.2. Taste Reactivity Prior to Abstinence. Development of negative affective state prior to abstinence. Mean number of licks (A) and gapes (B) during saccharin infusions (phase 1) on the first and last days of the taste-drug pairings prior to abstinence across all rats. Mean number of licks (C) and gapes (D) separated by impending group assignment (Exercise vs Sedentary), prior to abstinence. * $p < 0.05$.

Phase 2: Cocaine self-administration

Prior to abstinence, all rats acquired self-administration across the 14 training days. Figure 3.3 shows lever press responding across all rats. A one-way ANOVA on cocaine self-administration revealed a significant main effect of day ($F_{14,18}$=20.58, p<0.0001). Post-hoc Tukey tests revealed that the total number of lever presses on day 1 was significantly lower than on days 5, 7, 8, 9, 10, 12, 13, and 14. In addition, lever pressing on day 2 was significantly lower than that observed on days 8, 10, 14.

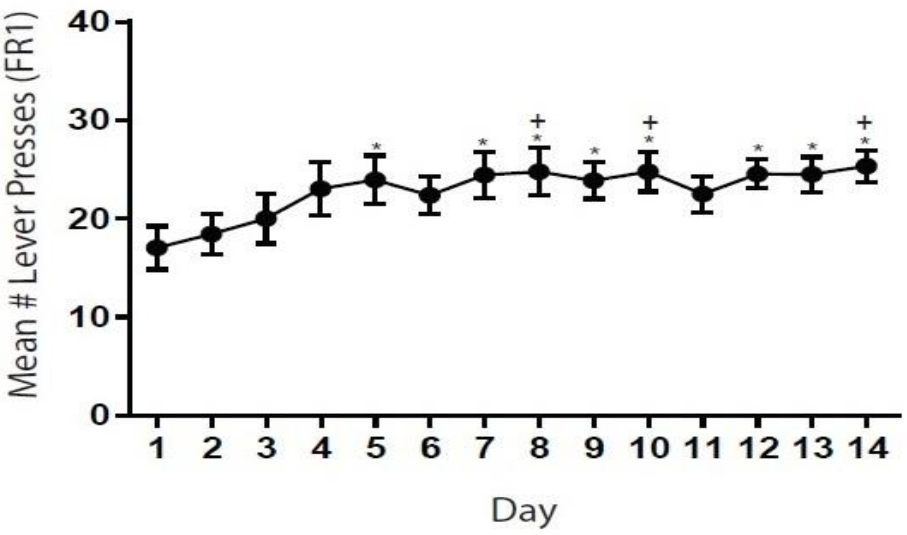

Figure 3.3. Cocaine Self Administration Prior to Abstinence. Total number of lever presses for intravenous cocaine (0.33 mg/inf) during cocaine self-administration phase across training. * $p <$ 0.05 for day 1 compared to days 5, 7, 8, 9, 10, 12, 13, and 14. + $p < 0.05$ for day 2 compared to days 8, 10, and 14.

Next, we examined aspects of self-administration behavior on day 14 (last day before abstinence). First, we determined if the total number of lever presses during the self-administration phase varied as a function of impending group assignment. A t-test revealed no significant effect of group (t_{16}= 1.87 p>0.05) on lever pressing for cocaine (data not shown). Likewise, no significant difference in latency to the first press as a function of group was observed (t_{16}= 1.04, p>0.05; data not shown). Finally, a t-test revealed no significant main effect of group (t_{16} = 1.30, p>0.05) on load-up behavior (data not shown). These findings indicate that there were no pre-existing differences in self-administration behavior on day 14 between rats that were then divided into EX and SED groups.

Our prior studies show that animals that exhibited the most aversive taste reactivity on the last day of training displayed the highest levels of load-up presses for cocaine, once available (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). Pearson correlation

coefficients were conducted here to determine if animals in the present study showed similar motivation to seek cocaine following 14 days of training in our preclinical model. The results revealed that the total number of load-up presses was significantly (positively) correlated with the number of aversive responses (gapes) across all rats (r^2=0.431, p<0.01, Figure 3.4A). That is, rats that exhibited the most gapes on Day 14, showed the largest number of load-up responses indicating higher motivation for the drug once the lever was available. Further, this relationship held before abstinence for animals destined for the EX (r^2=0.774, p<0.01, Figure 3.4B) or SED (r^2= 0.692, p<0.01, Figure 3.4C) groups.

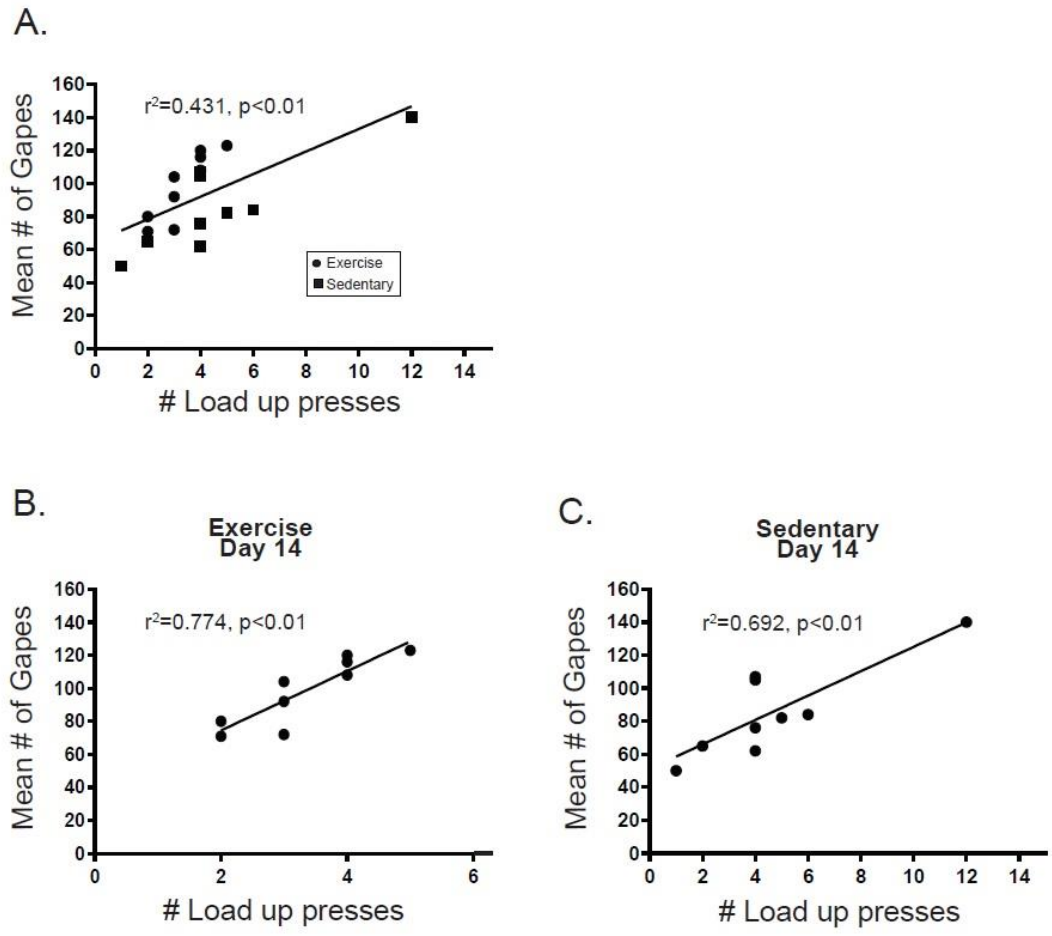

Figure 3.4. Motivation to seek cocaine prior to abstinence. Aversive taste reactivity (gapes) is correlated with increased motivation to consume cocaine prior to abstinence. Aversive taste reactivity is positively correlated with load-up presses across all rats (A), and for rats destined for the EX (B) and SED (C) groups.

Abstinence: Wheel Running

Wheel running steadily increased from week 1 through week 7 for the EX group (Figure 3.5). A one-way ANOVA revealed a significant main effect of wheel running across the 7 week period ($F_{6,8}$= 13.81, p<0.0001). Post-hoc Tukey test revealed that average wheel running for weeks 5, 6 and 7 were significantly higher than wheel running during week 1 (p <0.05). During the 7th week abstinence period, rats with exposure to the running wheel (EX) ran an average of 5,734 rev/week (6,305 meters/week), with a range across rats from 991 rev/week (1,090 m/week) to 19,957 rev/week (21,946 m/week).

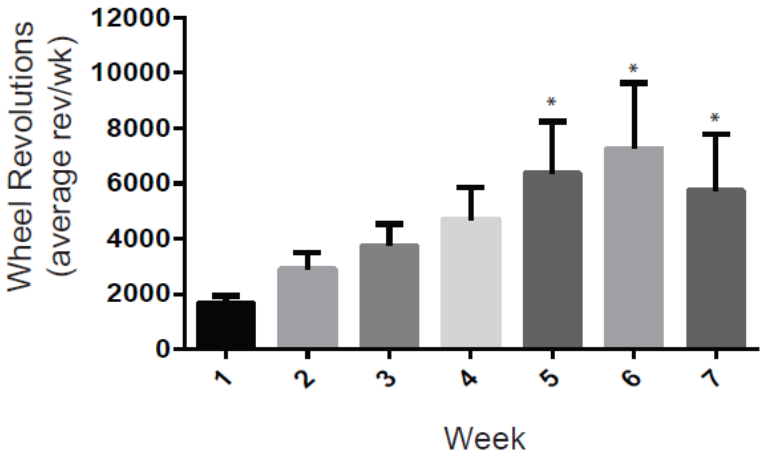

Figure 3.5. Wheel Running Activity. Wheel running across 7 weeks of abstinence. Average number of wheel revolutions per week significantly increased during weeks 5, 6 and 7, compared to week 1. *$p < 0.05$.

Post-Abstinence

Taste Reactivity

Next, we determined if access to the running wheel altered the negative affective state observed in our preclinical model. First, taste reactivity measures prior to abstinence (day 14) were compared to those observed during the test day immediately following abstinence across

groups. A two-way mixed design ANOVA on appetitive taste reactivity across groups revealed a significant main effect of day ($F_{1,16}$= 7.97, p<0.05), no significant main effect of group ($F_{1,16}$= 2.79, p>0.05), but a significant day X group interaction ($F_{1,16}$= 7.31, p<0.05). A post-hoc Tukey test revealed that appetitive taste reactivity for the EX group on the test day following abstinence was higher than SED on the same test day post-abstinence as well as EX and SED on day 14 prior to abstinence (Figure 3.6A). These findings indicate that a significant increase in appetitive taste reactivity at test in animals with access to a running wheel during abstinence. However, a two-way mixed design ANOVA on aversive taste reactivity revealed no significant main effect of day ($F_{1,16}$=0.02, p>0.05), no significant main effect of group ($F_{1,16}$=0.39, p>0.05), and no significant day X group interaction ($F_{1,16}$=0.38, p>0.05), (Figure 3.6B). The results indicate that aversive taste reactivity did not decrease following abstinence for the EX group, inconsistent with a potential reversal of the negative affective state by exercise.

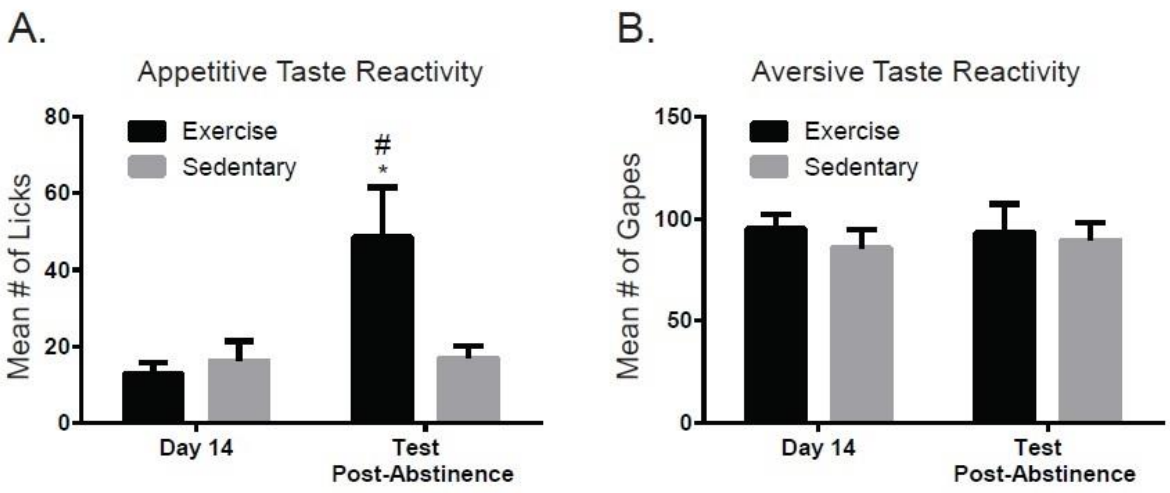

Figure 3.6. Taste Reactivity Post-Abstinence. Appetitive and aversive taste reactivity on day 14 before abstinence compared to the test session following 7 weeks of abstinence as a function of group. (A) Appetitive taste reactivity significantly increased for the EX group on the test day following abstinence compared to day 14 of training (*p < 0.05). # p < 0.05 compared to SED on test day. (B) Aversive taste reactivity did not significantly change on test day post abstinence compared to day 14 of training for both groups.

Relationship between Wheel Running, Self-Administration, and Negative Affective State

Prior studies show that exercise reduces the reinforcing effects of cocaine (Smith et. al. 2008, Smith et. al. 2011, Lynch et. al 2010, and Smith and Lynch 2012). Specifically, animals that exhibited the least cocaine seeking behavior, showed the most wheel running. Pearson correlation coefficients were conducted here to determine if the EX rats in our study also showed a reduction in cocaine seeking on test day. The results revealed that the total number of lever presses after abstinence was negatively correlated with wheel running (r^2=0.734, p<0.01, Figure 3.7A). That is, the more the EX rats ran, the less drug they self-administered cocaine.

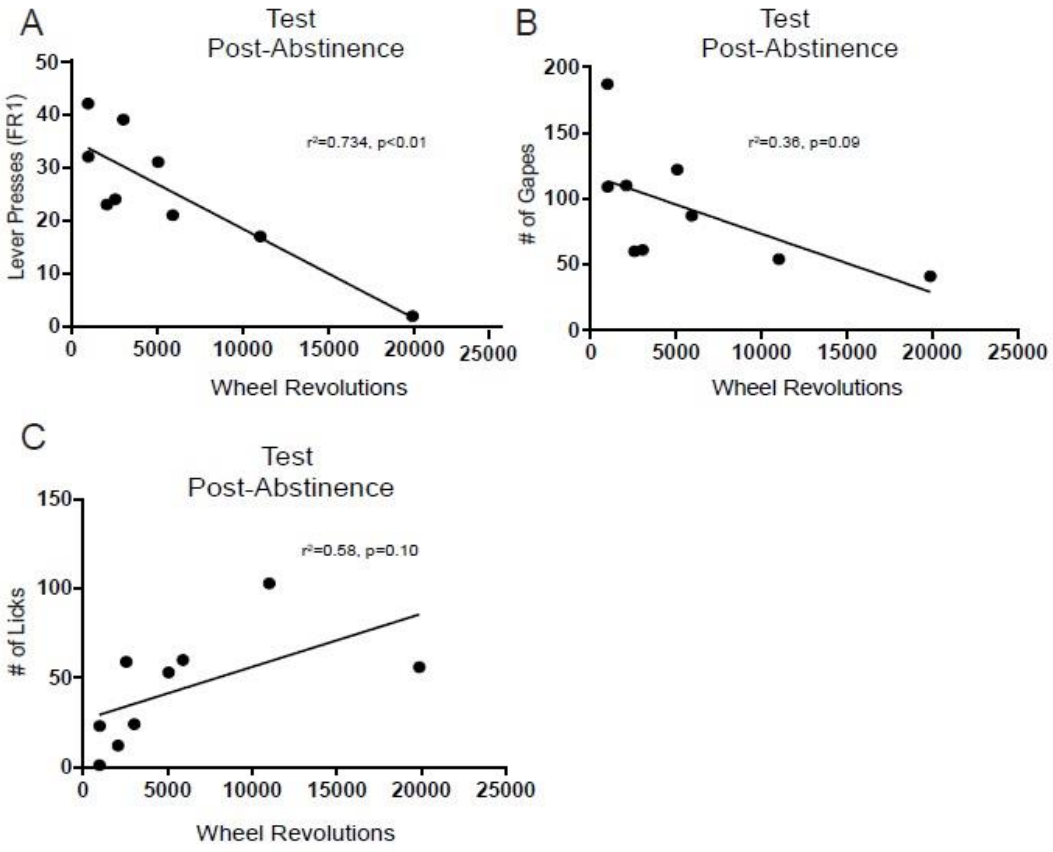

Figure 3.7. Relationship between Wheel running and Cocaine Self-Administration and Taste Reactivity. Lever pressing responses for cocaine self-administration during the single test session following abstinence was negatively correlated with wheel revolutions during week 7 of

abstinence (A). A trend toward a significant correlation between number of wheel revolutions and the mean # of gapes (B) and # of licks (C) was also observed.

To further examine if wheel running experience had a protective effect on the development of negative affect in our preclinical model, additional analyses were completed. First, we examined if the number of wheel revolutions during week 7 was correlated with either appetitive or aversive taste reactivity during the test session following abstinence. Although there was trend toward significance, wheel running was not correlated with aversive responses (r^2=0.36, p=0.09, Fig 3.7B). Likewise, although appetitive taste reactivity appeared to increase with increased wheel running, the two were not significantly correlated (r^2= 0.58, p= 0.10, Fig 3.7C). Next, we determined if the total number of presses and load up presses on the test day after abstinence varied as a function of group. A t-test revealed no main effect of group (t_{16}= 1.26, p>0.05) on mean number of lever presses for cocaine (Fig 3.8A). However, EX rats exhibited significantly less load-up presses than sedentary rats (t_{16}= 2.39, p<0.05, Figure 3.8B) on the test day after abstinence.

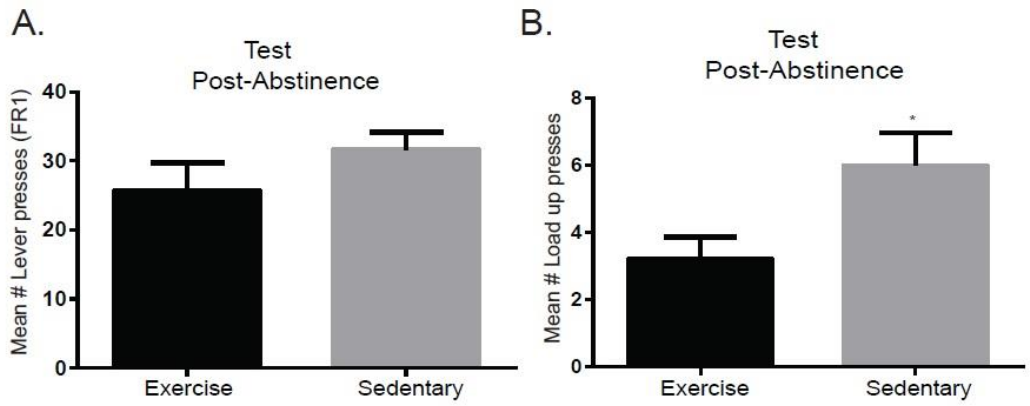

Figure 3.8. Cocaine Self-administration Post-Abstinence. During the single post abstinence test session, EX and SED rats exhibited similar numbers of lever pressing for cocaine (A). However, rats in the SED group showed more load-up responses for drug compared to the EX group (B) ($p < 0.05$).

As noted above, the results revealed that prior to abstinence rats that exhibited the most aversive taste reactivity on the last taste-drug pairing displayed the highest level of load-up presses for cocaine, regardless of impending group assignment (Figure. 3.4). Here, Pearson correlation coefficients were conducted to determine if these animals continued to show similar motivation to seek cocaine or if exposure to wheel running in the EX group had a protective effect (that is, the expression of the negative affective state was attenuated following abstinence). The results revealed that the number of load-up presses at test was correlated with the number of aversive responses (gapes) for each group (EX: r^2=0.949, p<0.0001, Figure 3.9A; SED: r^2= 0.791, p<0.01, Figure 3.9B). These findings indicate that even though the EX rats showed less load-up behavior following abstinence (Fig 3.8B), load-up behavior remained significantly correlated with aversive gapes, suggesting that exercise did not completely alter the enhanced motivation for cocaine following abstinence evident in our preclinical model.

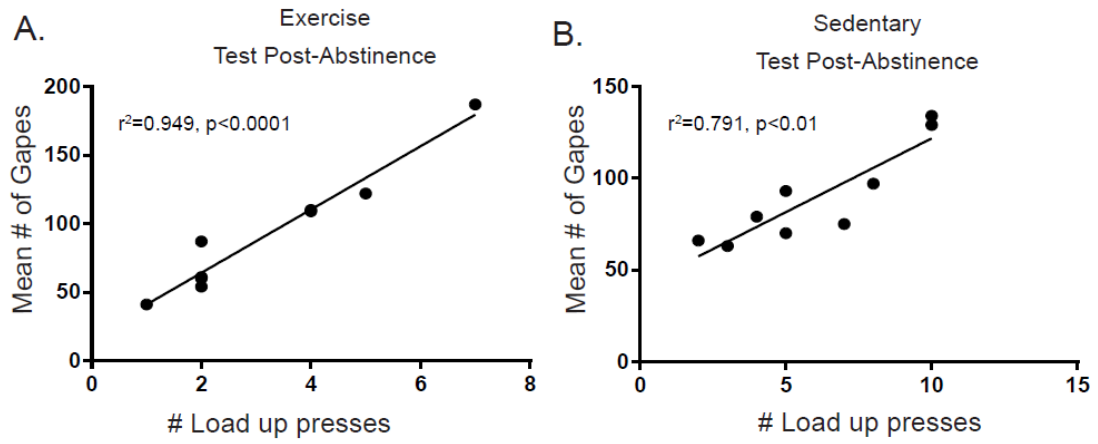

Figure 3.9. Motivation to seek Cocaine Post-Abstinence. During the single post abstinence test session, mean # of gapes was significantly correlated with # load-up presses for the EX (A) and SED (B) rats.

Discussion

The main objective of the present study was to determine if access to a running wheel during abstinence would reverse or reduce the negative affective state that develops in our preclinical model of natural reward devaluation by cocaine. Although our data indicate a trend toward a protective effect, these results were not strong. Exercise rats exhibited a significant increase in appetitive taste reactivity following abstinence and this increase was significantly higher than the SED rats (Fig 3.6). In addition, EX rats exhibited significantly lower load-up behavior following abstinence as compared to SED rats (Fig 3.8) indicating access to a running wheel reduces the motivation to seek cocaine. However, the same EX rats did not show a significant reduction in aversive taste reactivity (Fig.3.6), and continued to exhibit a positive correlation between the aversive state (i.e. gapes) and load-up presses. These findings indicate exercise has a subtle effect in attenuating the negative affective state after it has developed in our preclinical model.

Negative Affective State Develops Prior to Drug Abstinence

The pre-abstinence results in the present study replicate earlier work by showing that rats exhibited a shift from appetitive to aversive taste reactivity as the saccharin tastant came to predict impending but delayed cocaine availability (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). Another important feature of the model is that rats that exhibited the most aversive responses were also the most motivated to consume cocaine once available (Wheeler et al. 2008, Wheeler et al. 2011). That is, we previously reported that rats that exhibited the most gapes during phase 1 showed the greatest number of load-up presses in phase 2. Similar results were found here, in that regardless of impending group assignment, there was a positive correlation between the number of gapes and the number of load-up presses on the last day of training (day 14). Collectively, these findings replicate earlier work and show the development

of a negative affective state and increased motivation to consume cocaine in our preclinical model.

Effects of voluntary wheel running on cocaine seeking and taste reactivity

Voluntary wheel running in rodents can serve as an alternative non-drug reinforcer, and with extended use reduces the reinforcing effects of drugs (Smith et. al. 2008, Smith et. al. 2012, Zlebnik et. al. 2010). Smith et. al. (2012) reported that wheel running prior to cocaine reinstatement reduced drug-seeking during extinction, and drug-primed and cue-induced reinstatement. In addition, Smith and colleagues (2008) reported that the more the rats used the wheel the less motivated they were to seek cocaine. Here, we replicated this finding by showing that the EX rats that exhibited the most wheel running behavior during abstinence lever pressed the least for cocaine, and were less motivated to seek cocaine once it was available, compared to SED rats. Further, following exposure to the running wheel, EX rats significantly increased appetitive responses to the saccharin taste and exhibited less load-up responding than SED rats. In addition, there was a trend toward a significant correlation between wheel running, more appetitive and less aversive taste reactivity responses during the test session. However, access to a running wheel did not alter the relationship between the aversive state and load-up presses. Collectively, these results indicate that exposure to a running wheel during an extended abstinence period (7 weeks) has slight protective effects in the expression of the negative affective state and motivation to consume the drug.

Why wheel running exposure did not reverse negative affect: Possible explanations

One possible explanation as to why we did not observe a strong reversal or attenuation of the negative affective state in the present study may be related to a phenomenon known in the literature as ‘incubation of craving’. Preclinical studies have reported that abstinence from

cocaine self-administration induces an 'incubation of craving' effect in which rats show increased responding during extinction following 1 month abstinence from cocaine (Lu et. al. 2004, Pickens et. al. 2011). Indeed, research from our laboratory confirms an incubation of craving effect in that rats given one month abstinence from cocaine exhibit increased lever pressing during extinction compared to rats only given 1 day abstinence from the drug (Hollander and Carelli 2005, Hollander and Carelli 2007). Further, recent research in our laboratory also reveals an incubation of the negative affective state in our preclinical model (Carelli and West 2013). Here, a significant increase in aversive responses correlated to more drug seeking (during extinction) following 1 month abstinence. Given this 'incubation' effect then, it may be the case that the lack of a strong effect of EX in the current study may be related to an underlying incubation of craving effect evident during abstinence. That is, exercise may have not been able to reverse the negative affective state here because it was incubating (increasing) and thereby 'competing' with any protective effect that may have occurred with exercise.

A second possible reason as to why we did not observe a strong attenuation or reversal in the negative affective state by exposure to the running wheel may be related to reports showing that exercise itself diminishes the reward value of the saccharin cue. Investigators have reported that environmental enrichment (i.e. social housing, novel objects, and larger spaces) can cause a reduction in sucrose consumption (Brenes and Fornaguera 2008), attenuate cue induced reinstatement of sucrose seeking (Grimm et. al. 2008), and reduce responding for other nondrug rewards such as novel environmental stimuli (Cain et. al. 2006). For instance, rats reared in an environmentally enriched condition consumed less sucrose than their littermates reared in an isolated condition (Brenes and Fornaguera 2008). In an interesting study, Puhl and colleagues

(Puhl et al. 2012) examined if Sprague Dawley rats exposed to environmental enrichment (i.e. social housing and novel objects) showed altered avoidance of a saccharin cue that was paired with the opportunity to self-administer cocaine. These investigators reported that environmentally enriched animals continued to avoid the saccharin cue even though the same rats failed to acquire cocaine self-administration at a high rate, and had higher latencies to the first press as compared to controls. As such, the lack of a strong reduction or reversal of the negative affective state in the present study may be because the saccharin cue itself became devalued (and therefore difficult to reverse) as a direct consequence of exercise, independent of its pairing with cocaine.

Finally, it is possible that EX rats in our study continued to exhibit drug-induced devaluation of the taste cue following abstinence because they were in a state of withdrawal. Withdrawal symptoms in humans are initiated early in drug use and their magnitude increases as their addiction gets worse. Aversive drug-withdrawal symptoms are typically associated with increases in addicts' reported desires and intended drug consumption (Baker et. al. 1987, O'Brien 1976, Wikler 1980). Further, addicts report that negative affect is the motive for drug seeking and relapse (Wetter et. al. 1994, Baker et. al. 2004). Indeed, relapse to cocaine seeking behavior can be motivated by drug-priming, environmental cues, and negative affect (Thanos et al. 2013). It is possible that EX rats continue to exhibit drug-induced devaluation of the taste cue following abstinence, because the drug-associated cue continued to elicit a negative affective state related to drug withdrawal.

Concluding remarks

Drug users identify negative affect as a main reason for continued drug use and relapse (Sinha et al. 2000; Baker et al. 2004). Indeed, the emergence of negative affect and natural

reward devaluation related to repeated drug use is considered a key aspect in models of drug addiction (Koob et al. 1998, Grigson and Twining 2002, Grigson 2008, Koob and Volkow 2010). For instance, there is a potential reduction in dopamine function during abstinence (Orsini et. al. 2001, Koob and Volkow 2010, Lynch et. al. 2013) so drug dependent individuals may have a reduced capacity to experience positive mood states during early recovery. However, aerobic exercise has the potential to serve as a treatment for drug seeking, and attenuate or reverse this negative emotional state. For example, Bock et. al. (1999) reported that women attempting to quit smoking self-reported that exercise reduced their negative mood states and withdrawal symptoms. Thus exercise may offer addicts the ability to experience positive affective states without using drugs. Likewise, other investigators (Lynch et al. 2010) reported that in rats, 2h/day of exercise during abstinence reduced extinction responding by 35% and reduced cue-induced reinstatement responding by 50%. Exercise had a promising use in reducing negative affective state exhibited using this preclinical model of devaluation. In the current aim exercise attenuated motivation for cocaine, however exercise did not attenuate or reduce negative affect. Given these findings, an additional study was conducted (next chapter of this dissertation) to determine if exercise experience *prior to* exposure to our preclinical model would alter/attenuate the *development* of the negative affective state and increased motivation for the drug that occurs following repeated taste-drug pairings.

CHAPTER 4

EFFECTS OF VOLUNTARY WHEEL RUNNING ON THE DEVELOPMENT OF COCAINE-INDUCED NEGATIVE AFFECT IN A RODENT MODEL

Introduction

Research has shown that regular exercise (i.e. physical activity) can reduce drug-seeking behavior in humans (Field et. al. 2001) and animals (Smith et. al. 2012, Lynch et. al. 2013). Although there is no evidence of a causal effect of exercise on the rates of initiation of drug use, there are studies with adolescents that report a negative association between drug taking and physical activity. Epidemiological studies report teens who exercise regularly are less likely to use drugs (Field et al., 2001, Kirkcaldy et al., 2002, Ströhle et al., 2007 and Iannotti et al., 2009) and have fewer risk factors that are associated with the development of addictive disorders (Collingwood et al., 1991 and Collingwood et al., 2000) as compared to their peers who do not exercise regularly. In addition, Bock et. al. 1999 investigated the acute effects of physical activity on negative affect and craving for nicotine. These investigators found that exercising at least 3 times/week, with each session lasting between 30-40 minutes, attenuated cravings for cigarettes and negative affect as compared to the control group (no exercise).

Preclinical studies in animal models have examined physical activity as a means to alter drug-seeking behavior. Researchers have reported that exercise reduces animals' drug intake in procedures that model different stages of addiction. For example, access to a running wheel decreases the maintenance of operant behavior in animals with well-established self-administration histories (Cosgrove et al., 2002 and Smith et al., 2008) and reduces drug-primed

and cue-induced reinstatement (Cosgrove et. al. 2002). There have been limited studies that have focused on the ability of physical activity to influence the establishment of regular patterns of cocaine self-administration (i.e. acquisition) in animals. Smith et. al. (2011) tested male Long-Evan rats following 6 weeks of either access (Exercise) or no access (Sedentary) to voluntary wheel running on acquisition to cocaine seeking. After 6 weeks, rats were implanted with intravenous catheters and placed in an operant chamber for 2hours/day for 15 consecutive days. Exercise rats lever pressed less for cocaine across training days as compared to the sedentary rats. Other investigators (Lacy et. al. 2014) examined physical activity on acquisition of self-administration of cocaine, heroin, and combinations of both drugs. They reported that access to a running wheel reduced the acquisition of drug seeking across heroin, cocaine, and cocaine/heroin combinations. These findings indicate access to a running wheel reduces the acquisition to drug seeking. As such, these reports suggest that physical activity may be used as a preventative treatment for substance abuse (Smith and Lynch 2012).

In the previous chapter we examined if access to a running wheel in the animal's home cage *following* taste-drug training in our preclinical model alters subsequent aversive taste reactivity and cocaine-seeking behavior. Although our data indicate a trend toward a protective effect against the established negative affective state, these results were not strong. However, exercise during abstinence had some protective effect against the motivation to consume cocaine when rats were reintroduced to the paradigm. An interesting question we considered here is whether exposure to the running wheel *before* taste-drug training would have a protective effect on the later development of the negative affective state. Given these findings the objective of this Aim was to examine if access to a running wheel in the animal's home cage *prior* to taste-drug training in our preclinical model will alter subsequent aversive taste reactivity and cocaine-

seeking behavior. We predict that exposure to a running wheel in the rats' home cage prior to taste-drug training will prevent, or at least attenuate, the development of aversive taste reactivity to the sweet taste that predicts impending, but delayed cocaine self-administration. In addition, we predict a relationship between wheel running, and aversive taste reactivity and lever presses for intravenous cocaine. That is, we hypothesize that the more the animals wheel run, the less they will exhibit aversive responses and the less they will lever press for cocaine.

Methods

Subjects: Male, Sprague-Dawley rats (Harlan), aged 60-90 days at arrival to facility, weighed approximately 320-450 grams by the start of the experiment (*n*=19). Animals were housed individually and maintained on a standard 12:12hr light-dark cycle (lights on at 7:00 a.m.). During training and testing, animals were restricted to no less than 85% of their preoperative body weight by limiting water access (30 ml/day). Food was available ad libitum. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (2011), and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgeries: Rats were anesthetized with a ketamine hydrochloride (100mg/kg) and xylazine (20mg/kg) mixture and surgically implanted with intra-oral cannulae and an intravenous catheter in a single surgery, as described previously (Roitman et al. 2008, Wheeler et. al. 2008). Intravenous catheters for self-administration were purchased from a commercial source (Access Technologies, Skokie, IL) and inserted into the jugular vein using established procedures (Carelli and Deadwyler 1996, Wheeler et. al. 2008).

Experimental Design: At arrival to the animal facility rats were randomly assigned to either the Exercise (EX) or Sedentary (SED) groups. Rats in the EX group (n=10) were singly housed in the colony room within a cage containing a running wheel (Coulbourn, Whitehall, PA, USA). The EX rats were given continuous access to the running wheel in their home cages for 7 weeks. The SED rats (n=9) were singly housed in standard polycarbonate cages (19 x 10.5 x 8 in) with no running wheel access for the 7 week period. Following the seven weeks, EX rats were placed into home cages similar to the housing of sedentary rats while in the laboratory (approx. 7 hrs per day), but remained in cages with running wheels when placed back into the colony room each night. Training and test sessions were conducted in a Plexiglas chamber (Med Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle. Figure 4.1 shows the experimental design. Initially, mildly water-deprived (30ml/day) rats were trained to press a lever for water then underwent surgical procedures as described above. One week later, rats received 14 daily conditioning sessions as described in chapters 2 and 3. Briefly, the behavioral task was conducted in two phases/day: 1) intra-oral tastant infusions and 2) cocaine self-administration. In phase 1, rats received discrete intraoral saccharin infusions, delivered in 3.5s intervals (0.15% saccharin solution, 200 µl/infusion). A total of 45 tastant infusions (trials; 1 trial/min) were delivered. Immediately thereafter, the intravenous catheter line was attached and phase 2 (cocaine self-administration) was initiated. Here, rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule of reinforcement (2hrs). Each lever press resulted in intravenous infusion of cocaine (over 6s), termination of the cue light positioned above the lever, and onset of a tone (67db, 1 kHz) and house light (25W) stimulus. Lever presses during the 20s post response period had no programmed consequences. Taste reactivity was recorded on days 1, 7 and 14 of the 14 training sessions. Self-administration behavior was recorded daily. Wheel

revolutions (i.e. Exercise) were recorded daily by the experimenter from a LCD counter (placed on the side of the wheel). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse.

Figure 4.1. Experimental Timeline. Schematic diagram of task. See text for details on task procedure.

Taste Reactivity: Taste reactivity was analyzed in a frame by frame analysis using digital video recorded on days 1, 7, and 14. As in prior chapters, appetitive and aversive taste reactivity were counted using the procedure developed by Grill and Norgren (1978). Briefly, mouth movements expressed in the 6s following infusion onset that matched a "triangle" shape for a duration exceeding 90ms were counted as aversive. Instances in which the tongue protruded and crossed the midline were counted as appetitive.

Cocaine Self-Administration: Cocaine self-administration including mean lever presses, mean latency to the first press, and load-up presses were recorded during each session. Load-up behavior was defined as rapid lever pressing in the beginning of the session in which the average inter-press interval (INT) during load-up was less than half the average INT during the remainder of the session.

Wheel Running: Wheel revolutions were recorded daily during the 7 week habituation period prior to the initiation to our preclinical model. In addition, wheel revolutions were recorded daily during training (i.e. weeks 8 and 9).

Data Analysis: Changes in wheel running behavior over the 9 weeks of exposure was examined using a one-way ANOVA. The average number of licks (appetitive taste reactivity) and average number of gapes (aversive taste reactivity) were examined on days 1, 7 and 14 of training on the task separated by group using separate one-way ANOVAs. To compare appetitive and aversive taste reactivity on the same test days across groups 2-way mixed design ANOVAs were completed. Changes in the mean number of lever presses during cocaine self-administration across test days 1, 7, and 14 were examined as a function of group using a 2-way mixed design ANOVA. Pearson product correlation coefficients examined relationships between the number of lever presses for intravenous cocaine on day 14 and exercise output (wheel revolutions, 7^{th} week). In addition, mean load-up presses on test days 1, 7 and 14 were examined for each group using separate one-way ANOVAs, and compared across groups using a 2-way mixed design ANOVA. Finally, pearson product correlation coefficients examined relationships between aversive taste reactivity on days 7 and 14 and the total number of load-up presses on days 7 and 14. Statistical analyses of all behavioral data were performed using commercially available software (Statstica, Tulsa, OK and GraphPad Prism, La Jolla, CA).

Results

Wheel Running

During the 7 week period of wheel exposure, EX rats gradually increased their wheel running for the first 3 weeks then exhibited stable rates of running for the following weeks (Figure 4.2). A one-way ANOVA revealed a significant main effect of wheel running across the

7 week period ($F_{8,9}$ = 10.74, p<0.0001). Post-hoc tukey test revealed that average wheel running for weeks 3, 4, 5, 6 and 7 were significantly higher than wheel running during week 1 (p <0.05). During the 7th week period, rats with exposure to the running wheel (EX) ran an average of 34,414 rev/week (37,843 meters/week), with a range across rats from 6,876 rev/week (7,561 m/week) to 52,731 rev/week (57,985 m/week). Wheel running declined significantly (p<0.05) from week 7 to weeks 8 and 9 once the rats were introduced to our preclinical model.

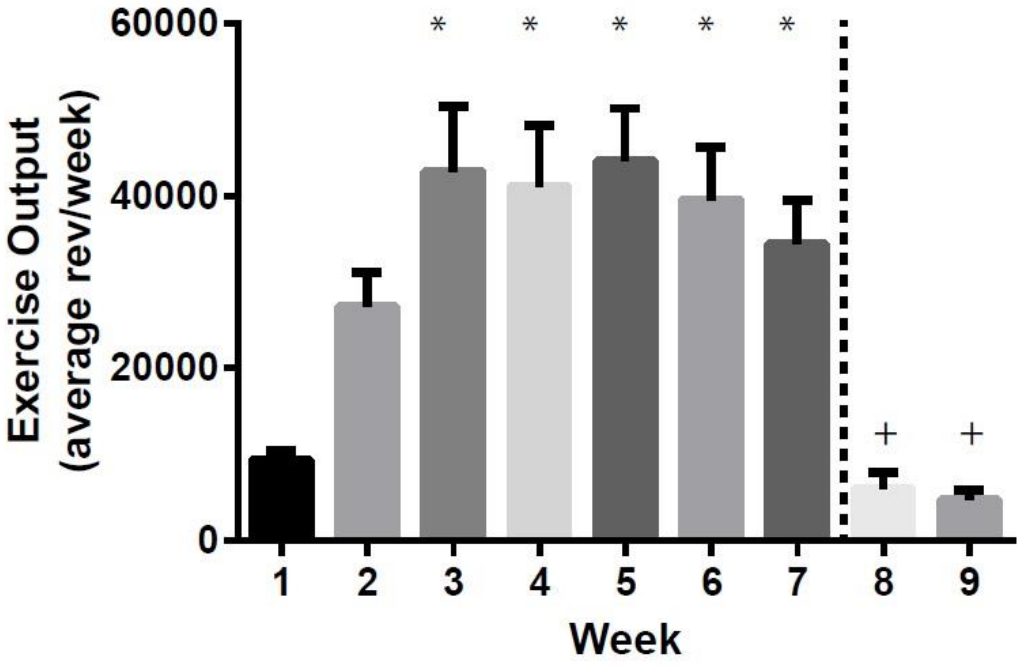

Figure 4.2. Wheel Running Activity. Wheel running across training. Average number of wheel revolutions per week significantly increased during weeks 3, 4, 5, 6 and 7, compared to week 1. In addition, mean wheel revolutions during weeks 8 and 9 were significantly less than week 7. *p < 0.05 as compared to week 1. +p<0.05 as compared to week 7.

Phase 1: Taste Reactivity

First, we examined the development of appetitive taste reactivity separated by group (i.e. EX or SED) across days 1, 7, and 14 of training. A one-way ANOVA on appetitive taste reactivity for the SED rats revealed a significant main effect of day (Figure 4.3A; $F_{2,8}$= 45.31, p<0.0001) in which appetitive taste reactivity decreased significantly from day 1 to days 7 and 14. The same was found for EX rats (Figure 4.3B; $F_{2,9}$= 32.22, p<0.0001). Further, a two-way mixed design ANOVA on appetitive taste reactivity across groups revealed a significant main effect of day ($F_{2,17}$= 68.41, p<0.0001), a significant main effect of group ($F_{2,17}$= 8.499, *p*<0.01), but no significant day X group interaction ($F_{2,17}$= 0.563, *p*>0.05), (Figure 4.4). These findings indicate that rats in both groups reduced appetitive behavior towards the saccharin tastant that predicts delayed cocaine self-administration. However, EX rats exhibited more appetitive responses compared to the sedentary rats indicating prior access to a running wheel has an effect in altering the positive affective state in our preclinical model.

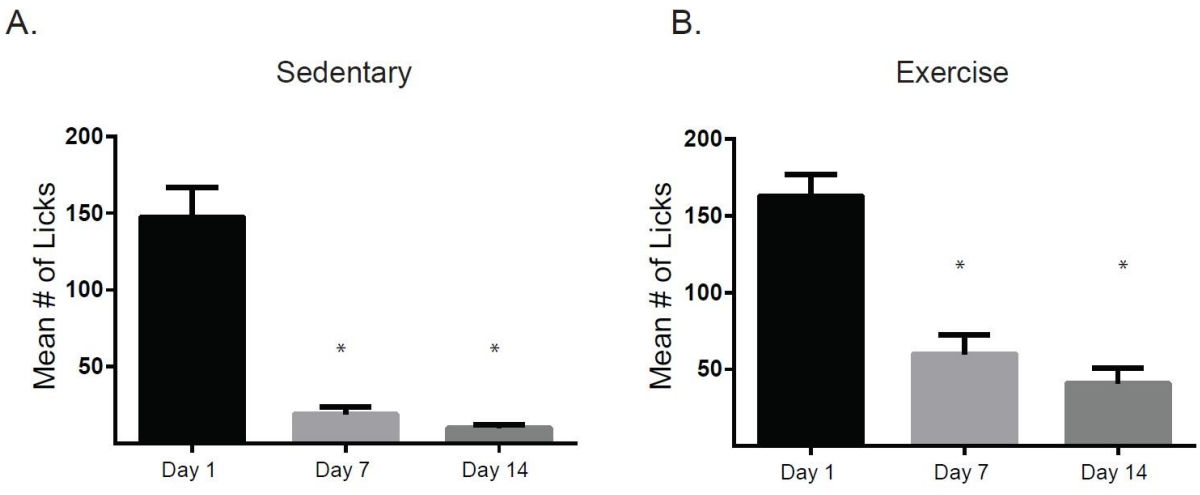

Figure 4.3. Appetitive Taste Reactivity. Appetitive taste reactivity across test days. Mean number of licks for Sedentary (A) and Exercise (B) rats during saccharin infusions (phase 1) on test days 1, 7, and 14. *p<0.0001.

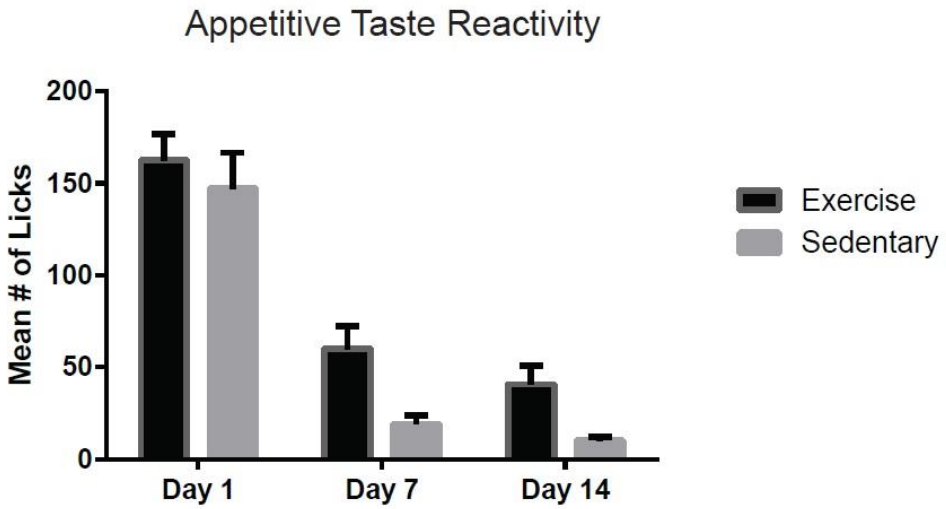

Figure 4.4. Appetitive Taste Reactivity as a function of group. Mean number of licks for Sedentary and Exercise rats across test days 1, 7, and 14 as a function of group.

Next, we examined the development of aversive taste reactivity for each group across test days (days 1, 7, and 14). A one-way ANOVA on aversive taste reactivity for the SED rats revealed a significant main effect of day (Figure 4.5A; $F_{2,8}$=66.07, $p<0.0001$) wherein aversive taste reactivity increased from day 1 to days 7 and 14. The same was found for EX rats (Figure 4.5B; $F_{2,9}$= 9.15, $p<0.01$). Further, a two-way mixed design ANOVA on aversive taste reactivity revealed a significant main effect of day ($F_{2,17}$= 40.91, $p<0.0001$), a significant main effect of group ($F_{2,17}$=12.02, $p<0.01$), and significant day X group interaction ($F_{2,17}$=3.97, $p<0.05$), (Figure 4.6A). A post-hoc tukey test revealed that aversive taste reactivity for the EX group was significantly lower than SED group on days 7 and 14. In addition, EX rats on day 7 exhibited

significantly less gapes than SED rats on day 14. These findings indicate that exercise attenuated aversive responses to the saccharin tastant that predicts delayed cocaine self-administration.

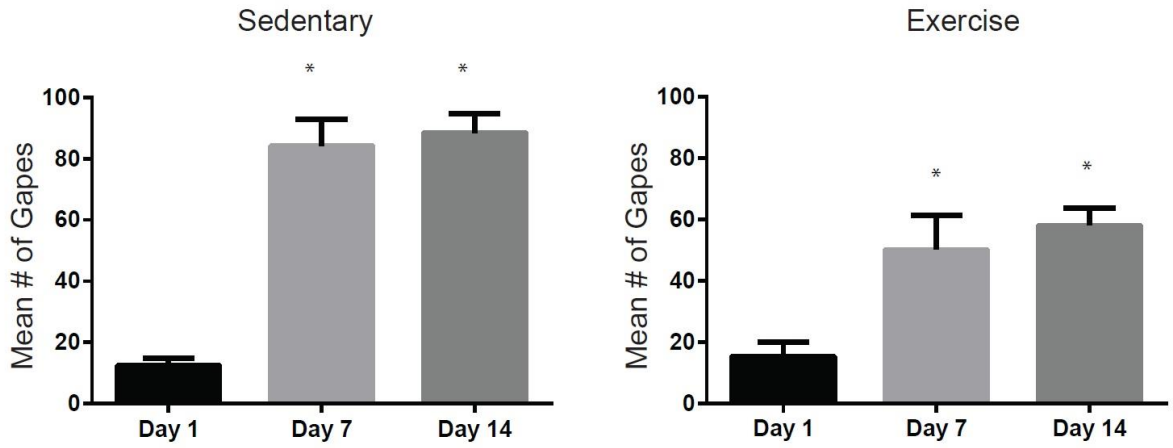

Figure 4.5. Aversive Taste Reactivity. Aversive taste reactivity across test days. Mean number of gapes for Sedentary (A) and Exercise (B) rats during saccharin infusions (phase 1) on test days 1, 7, and 14. *p<0.0001 for SED. *p<0.01 for EX.

The above findings indicate that prior exposure to exercise may blunt the development of aversive taste reactivity in our task. As such, we next examined if the number of wheel revolutions during week 7 (last week before training) was correlated with aversive taste reactivity on days 7 and 14. No relationship was observed between the amount of wheel running during week 7 and aversive taste reactivity on test day 7 (data not shown; r^2= 0.071, p>0.05). However, a significant relationship was found between the amount of wheel running during week 7 and aversive taste reactivity on day 14 (r^2= 0.403, p<0.05; Figure 4.6B). These results consider aversive responses on day 14 and the wheel activity summed for the 7th week for each rat. That is, the more running on the wheel during week 7 the less gapes exhibited on day 14. These findings indicate that although rats' exhibit aversive taste reactivity with training on the

task, prior access to a running wheel reduces aversive responding to the saccharin tastant that predicts impending cocaine self-administration.

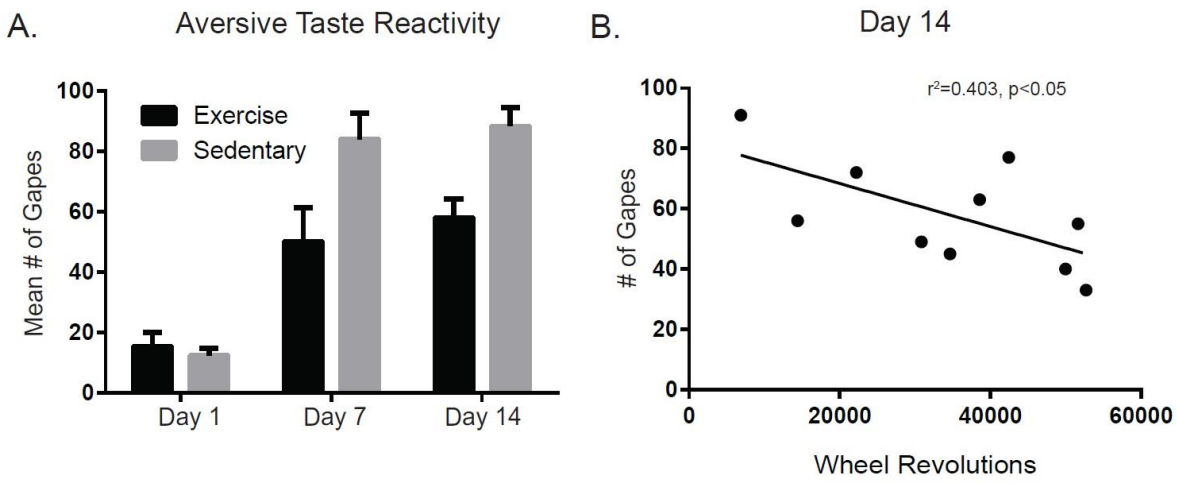

Figure 4.6. Aversive Taste Reactivity as a function of group. Mean number of gapes for Sedentary and Exercise rats across test days 1, 7, and 14 as a function of group (A). Aversive taste reactivity on day 14 was negatively correlated with wheel revolutions during week 7 (B).

Phase 2: Cocaine self-administration

All rats acquired self-administration across the 14 training days. Figure 4.7A shows lever press responding on test days (days 1, 7 & 14) for all rats. A two-way ANOVA on cocaine self-administration revealed a significant main effect of group ($F_{2,17}$= 4.85, $p<0.05$), but no main effect of day ($F_{2,17}$= 1.4003, $p>0.05$), and no interaction ($F_{2,17}$= 0.0892, $p>0.05$). The lack of a significant main effect of day may be due to water training prior to initiation of the preclinical model resulting in relatively high lever pressing rates on day 1. Next, we examined if there was a relationship between the number of wheel revolutions during week 7 and number of lever presses on day 14 (Figure 4.7B). Wheel revolutions and number of lever presses were significantly correlated such that the more running during week 7 the less number of lever presses on day 14

(r^2= 0.6062, p=0.008). These findings indicate that prior access to a wheel for 7 weeks reduces lever responding for cocaine in well-trained rats.

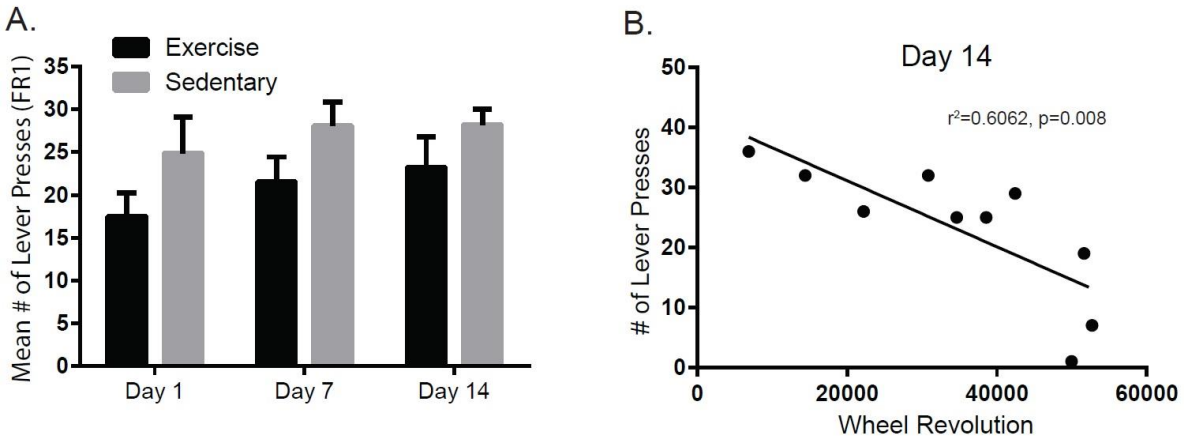

Figure 4.7. Cocaine Self-administration across test days. Mean number of lever presses for cocaine (0.33 mg/inf) during self-administration phase across test days 1, 7, and 14 (A). Lever pressing responses for cocaine self-administration for test day 14 was negatively correlated with wheel revolutions during week 7 (B).

Relationship between taste reactivity and motivation to consume cocaine

Next, we completed a number of analyses to examine if prior history of exercise significantly alters the motivation to consume cocaine, which normally becomes heightened by the development of the negative affective state, a consequence of the task (Wheeler et. al. 2008, Wheeler and Carelli 2009, Wheeler et.al. 2011). As indicated previously, motivation to consume cocaine is indicated by several variables including number of load-up presses, and latency to the first press once cocaine is available in phase 2 of the task. Here, we first determined if the total number of load-up presses during the self-administration phase varied for each group. A one-way ANOVA on load up presses for the EX rats revealed no main effect of day (Figure 4.8A; $F_{2,9}$= 0.759, p>0.05). The same was found for SED rats (Figure 4.8B; $F_{2,8}$= 0.404, p>0.05). These findings indicate that load-up behavior did not increase across training days in either group. To

compare load-up behavior across groups, we completed a two-way ANOVA that examined main

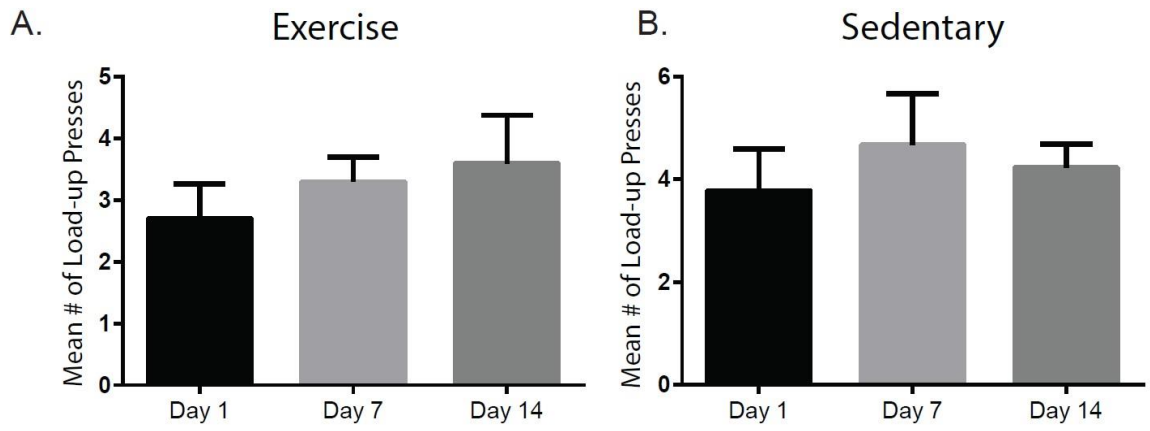

Figure 4.8. Load-up presses across test days. Mean number of load-up presses during the cocaine self-administration phase across test days for Exercise (A) and Sedentary (B) rats.

effects of group, day and an interaction between them (Figure 4.9). However, this analysis revealed no main effect of day ($F_{2,17}$=0.903, p>0.05), no main effect of group ($F_{2,17}$=2.265, p>0.05), and no interaction ($F_{2,17}$=0.189, p>0.05). These findings indicate that prior exercise experience did not significantly alter load-up behavior across days, or relative to the SED group. Next, we examined if latency to the first press varied as a function of group on the test days 1, 7, and 14. A two-way ANOVA (data not shown) revealed that there was no main effect of day ($F_{2,17}$= 1.254, p>0.05), no main effect of group ($F_{2,17}$= 0.367, p>0.05), and no interaction ($F_{2,17}$=0.187, p>0.05). Together with the load-up data, these findings indicate that access to a running wheel did not alter the motivation to self-administer cocaine, once it became available in the task.

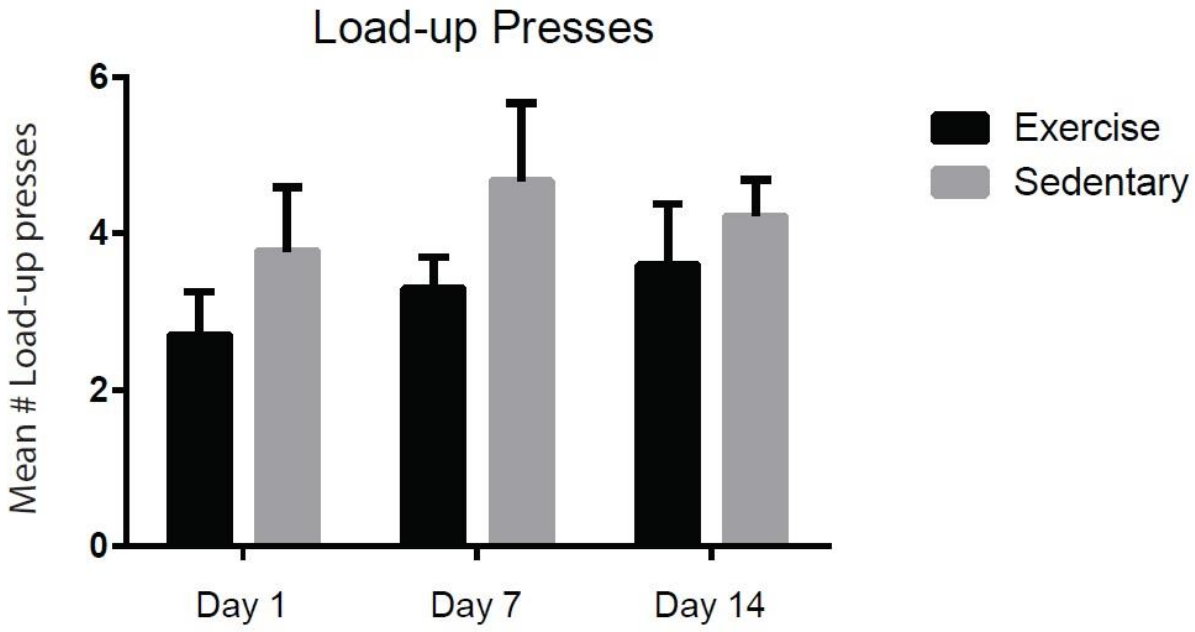

Figure 4.9. Load-up presses across test days as a function of group. Mean number of load-up presses during the cocaine self-administration phase across test days as a function of group.

Although all rats showed aversive taste reactivity, our prior studies also show that animals that exhibited the most aversive taste reactivity on the last day of training displayed the highest levels of load-up presses for cocaine, once available (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). Pearson correlation coefficients were conducted here to determine if a similar relationship holds on test days 7 and 14 for each group in the current study. The results revealed that the total number of load-up presses was significantly (positively) correlated with the number of aversive responses (gapes) on days 7 (Figure 4.10A; r^2=0.765, p=0.002) and 14 (Figure 4.10C; r^2=0.6879, p=0.0057) for the SED rats. Likewise, similar findings were observed for EX rats on day 7 (Figure 4.10B; r^2=0.4381, p=0.0371) and day 14 (Figure 4.10D; r^2=0.6138, p=0.007). That is, rats that exhibited the most gapes on days 7 and 14,

showed the largest number of load-up responses on those respective days indicating higher motivation for the drug once the lever was available.

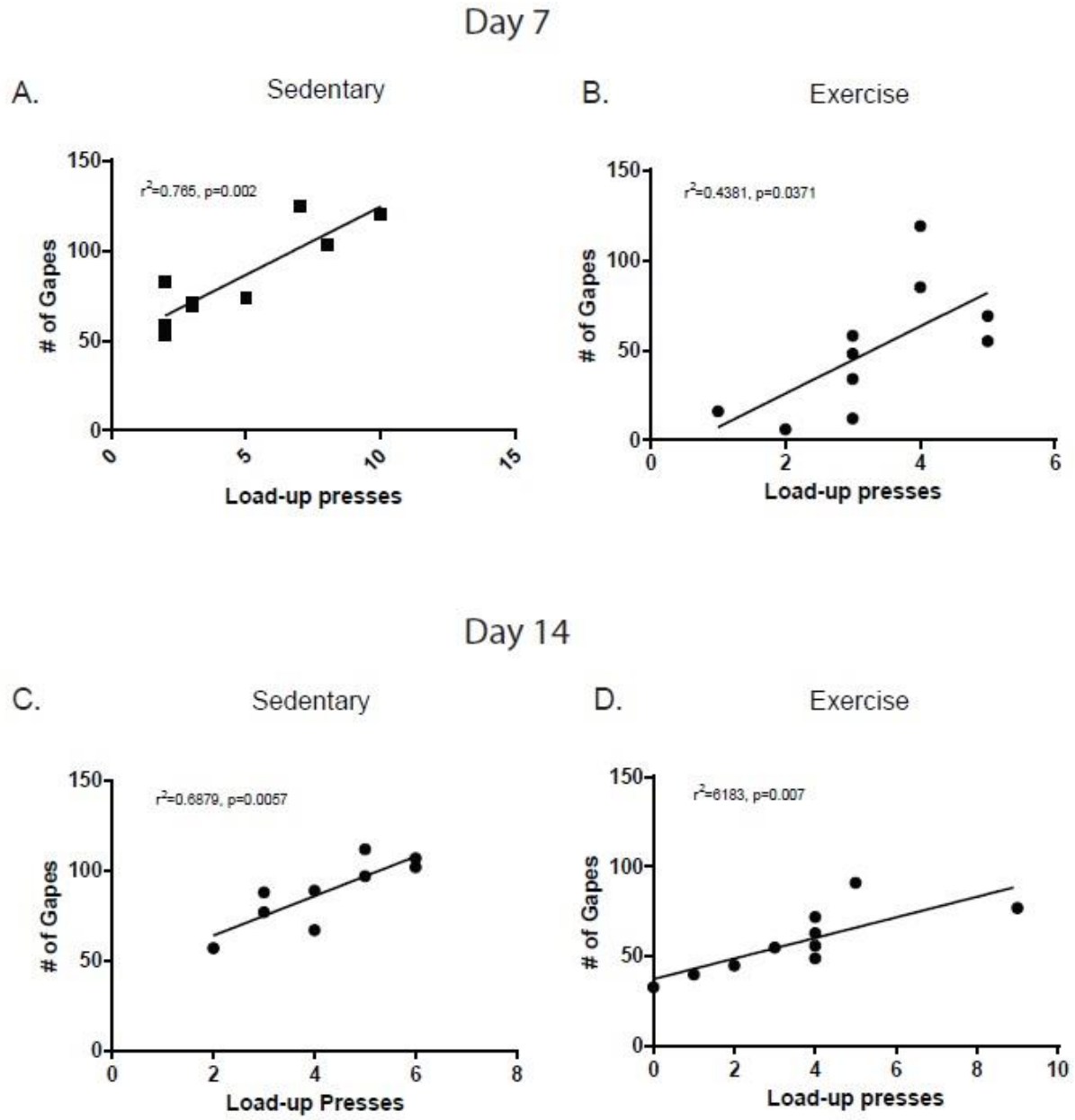

Figure 4.10. Motivation to seek cocaine across days 7 and 14. Aversive taste reactivity (gapes) is correlated with increased motivation to consume cocaine. Aversive taste reactivity was positively correlated with load-up presses on day 7 for the Sedentary (A) and Exercise rats (B). Similar findings were observed on day 14 for the Sedentary (C) and Exercise (D) rats.

Discussion

The main objective of the present study was to determine if access to a running wheel prior to training in our preclinical model would prevent or attenuate the negative affective state that develops later. Our data indicate that exercise does have a protective effect against the development of the negative affective state in this model. EX rats exhibited significantly lower aversive responses (Figure 4.6) and significantly higher appetitive responses (Figure 4.4) to the saccharin tastant as compared to SED rats following multiple taste-drug pairings. In addition, EX rats exhibited significantly lower lever presses across test days as compared to SED rats (Figure 4.7) indicating that access to a running wheel reduces the reinforcing effects of cocaine. However, the same EX rats did not show a significant reduction in load-up presses (Figure.4.9), and continued to exhibit a positive correlation between the aversive state (i.e. gapes) and load-up presses on days 7 and 14. These findings indicate that while exercise attenuated the negative affective state, the motivation to consume cocaine continued after it had developed.

Effects of voluntary wheel running on cocaine seeking and taste reactivity

Preclinical studies have reported that voluntary wheel running in rodents can decrease the self-administration of many drugs of abuse including cocaine, methamphetamine, and heroin (Smith et. al. 2008, Smith et. al. 2012, Cosgrove et. al. 2002, Miller et. al. 2012, Lacy et. al. 2014). Lacy et. al. (2014) reported that wheel running prior to cocaine and heroin acquisition reduced drug responding across both drugs and drug combinations in EX rats as compared to SED rats. In chapter 3, we reported that EX rats that exhibited the most wheel running behavior during the 7^{th} week of access lever pressed the least for cocaine following abstinence. Here, when given access to the running wheel *prior* to our preclinical model, EX rats also exhibited a similar relationship between wheel running and lever presses. EX rats that used the wheel the

most during the 7th week, lever pressed the least for cocaine on day 14 of training. Exercise rats exhibited a wide range of wheel activity over the course of 7th week. There are a couple of factors that can influence this variability including the circadian rhythm (Sherwin 1998) and water deprivation (Sherwin 1998, Bolles 1975) which has shown to decrease wheel running during acquisition. However, this variability in wheel running activity showed that the more the rat used the running wheel the less drug intake.

Puhl and colleagues (Puhl et. al. 2012) examined if adult rats exposed to an enriched environment (i.e. novel objects and social housing) would prevent devaluation of a natural reward that was paired with cocaine self-administration. These investigators reported that environmentally enriched (EE) rats were slow to take their first infusion (high latency) and self-administered less than the controls, non-enriched (i.e. isolated condition) rats. Further the EE rats tested on a progressive ratio schedule of reinforcement failed to work for cocaine suggesting exposure to the enriched environment may decrease the reinforcing value of cocaine. In this study, rats were not given intraoral infusions of the tastant as in our task, but instead, could voluntary drink the sweet from a bottle inserted into the operant box (5 min access) before drug access in the self-administration phase. Typically, rats will avoid drinking the sweet in this condition, and EE did not change this finding. The differences in devaluation of the natural reward between Puhl et. al. 2012 and the current study may be due to design. That is, in our model rats are forced to intake saccharin (intraoral deliveries) while in the Puhl study rats had a choice to avoid it. Further, unlike the presentations of novel objects or social housing, exercise itself can function as a nondrug reinforcer such that rats will perform an operant response to have access to a running wheel (Cosgrove et. al. 2002, Iverson 1993). Thus voluntary wheel running

may have higher value than other types of enrichment which assists in reducing the negative affective state.

Clinical literature suggests that exercise has anti-depressive effects (Morgan 1994, North et. al.1990) including positive changes in affective state such as increases in arousal and reduced anxiety. Bock et. al. (1999) reported that human smokers who have an exercise regimen decrease negative affect indicating that exercise may be able to alter emotional state in addicts. Here, data suggests that exercise (wheel running) affects the positive and negative affective states in rats using our preclinical model. For instance, exercise rats that exhibited more wheel running activity during the 7^{th} week of access showed less gapes. In addition, across training exercise rats exhibited more appetitive responses to the saccharin tastant suggesting that wheel running has an effect on the affective state. However, access to a running wheel did not alter the relationship between the aversive state and load-up presses indicating motivation for cocaine is still present. Collectively, these results indicate that exposure to a running wheel prior to training in our preclinical model (7 weeks) has a protective effect in the expression of the negative affective state, but not motivation to consume the drug.

Why wheel running exposure did not change the motivation to seek cocaine

A possible explanation as to why we did not observe a change in motivation to consume cocaine in the present study may be due to incentive salience. Incentive salience refers to the motivational element of reward such that a cue can become a "motivational magnet" in the environment leading to pathological or compulsive behavior (Flagel et. al. 2008). Here, we presented multiple discrete cues (saccharin tastant infusions) repeatedly in 'anticipation' of impending drug reward. Different conditioned responses emerged including changes in emotional (negative affective responses) and motivational (load-up) states. These conditioned

responses can be seen both behaviorally and neurologically. For instance, Wheeler et. al. (2011), examined the development of the negative affective state and simultaneously measured dopamine release to the taste that predicted delayed cocaine availability. Initially dopamine was reduced during tastant infusion at the beginning of training on the task. However, later in training dopamine release was elevated during tastant infusion, when cocaine delivery was imminent. These investigators demonstrated that dopamine could either elevate or diminish dopamine release depending upon the temporal relationship to cocaine availability. The result that attenuated dopamine release and aversive responses were observed in response to the 'delay,' but not the 'immediate' taste cue suggests that the mechanism of learning across this time period is fundamentally different and motivationally relevant. Thus the more immediate taste cues became a "motivational magnet" for the rats to take the drug.

Concluding remarks

Aerobic exercise has the potential to serve as a preventative treatment for drug seeking, and attenuate the negative emotional state that develops following repeated drug administration. Voluntary wheel running can attenuate the initiation of drug use (Smith and Lynch 2012), or when presented concurrently with drug, can lead to less drug intake (Miller et. al. 2012; Ehringer et. al. 2009). Here, these findings are extended and show voluntary wheel running can attenuate the negative affective state that develops across training. Together these findings suggest that exercise can serve as a non-drug alternative reinforcer that competes with the drug and attenuates vulnerability. Similar results were reported for cocaine self-administration in which wheel running attenuated the rates of acquisition under non-concurrent conditions (Smith and Pitts 2011a) suggesting that exercise may have protective effects that extend beyond its ability to function as an alternative non-drug reinforcer. Future studies are needed to examine the casual

effects between physical activity and the vulnerability to addiction and to identify important parameters that may influence this relationship. For instance, there is a wide range of physical activity each rodent will maintain. Thus it would be beneficial to examine the genetic markers to determine likelihood of subjects engaging in certain amount of activity to predict subsequent responses to drug taking.

CHAPTER 5

GENERAL DISSCUSSION

Summary of experiments

The studies described in the previous chapters were designed to extend the current knowledge of the preclinical model of devaluation of natural rewards that was developed in the Carelli laboratory, and to examine different variables that may influence this process. Taken together, the results indicate differential effects of cocaine dose and exercise on the negative affective state that develops during training on this preclinical model. With respect to cocaine dose, we found a dose-dependent effect on load-up behavior and lever pressing for intravenous cocaine. While load-up presses were correlated with aversive responses this occurred across all doses tested, indicating that rats in this model stay motivated for the drug regardless of cocaine dose. In the remaining chapters, physical activity (wheel running) reduced the reinforcing effects of cocaine in that rats in the EX group pressed less for cocaine as compared to SED rats once the drug was available. However, when rats were exposed to the running wheel (i.e., before or following training on the preclinical model) had differential effects on the negative affective state observed in this model. This suggests that timing of the exposure to physical activity is important when investigating protective effects (i.e. attenuation of negative affect versus motivation) against addiction. A brief summary of each experiment and the results of these studies are presented below.

Effects of cocaine dose on the preclinical model of natural reward devaluation

The study described in chapter two examined if the negative affective state that develops in the preclinical model of natural reward devaluation by cocaine was dose-dependent. The findings replicated earlier work by showing that rats exhibited a shift from appetitive to aversive taste reactivity as the tastant came to predict impending but delayed cocaine availability (Wheeler et al. 2008, Wheeler and Carelli 2009, Wheeler et al. 2011). However, once this aversive state developed (day 14) it did not vary as a function of cocaine dose during the self-administration phase. These findings are consistent with other findings that used a different model of natural reward devaluation by cocaine (Cason and Grigson 2013). In that study, rats could voluntary drink the sweet, as opposed to forced intraoral infusions that occurred in our study. Importantly, the development of gaping behavior in our study, and the decline (i.e., avoidance) in voluntary sweet consumption in the Cason & Grigson (2013) study was not cocaine dose-dependent.

Devaluation of the saccharin cue involves both reward and aversion, so there are multiple factors that may explain why a dose dependent effect did not occur including the nature of the drug being examined, dose of the drug, the individual, and sex of the individual (Colechio and Grigson 2014). The study in Chapter 2 of this dissertation used male rats but in a different study (Cason and Grigson 2013) investigators reported female rats exhibited a dose-dependent avoidance of the saccharin cue that predicted cocaine self-administration. That is, female rats showed increased avoidance behavior (i.e. avoided saccharin intake) at the highest dose of drug (0.66 mg/inf) as compared to the lower doses of drug (0.16 and 0.33 mg/inf). Thus, if both sexes were used in the current dissertation study, the results may have shown dose-dependent effects on the negative affective state (i.e. more gapes as the dose increased), but only in females.

An important feature of our preclinical model is that rats exhibiting the most aversive responses are also the most motivated to consume cocaine once available (Wheeler et al. 2008, Wheeler et al. 2011). That is, rats that exhibited the most gapes during phase 1 showed the greatest number of load-up presses and were fastest to press the lever for cocaine once self-administration was available in phase 2. Interestingly, this aversive responding to the taste cue can occur after only one taste drug pairing for some rats while with others it takes multiple taste-drug pairings to start exhibiting aversive responding (Colechio et. al. 2014) indicating individual differences across rats. As such, prior to examining cocaine dose-dependent effects in this study, it would have been interesting to separate rats into different groups based on when they started exhibiting aversive responding (i.e. 2^{nd}, 4^{th}, or 7^{th} taste-drug pairing). For example, rats exhibiting aversive responses on the 2^{nd} taste-drug pairing as compared to rats exhibiting similar responses following the 7^{th} pairing may indicate that the early responders have higher reward value to the drug as compared to the late responders. I predict that the early responders would show a cocaine dose-dependent effect since they exhibited early anticipation for the drug.

Previous studies have shown that load-up behavior during the start of cocaine self-administration sessions is dose-dependent (Carelli and Deadwyler 1996). Here, those findings were extended and we showed that in our preclinical model positive correlations exist between load-up presses and aversive taste reactivity across all doses tested. This finding supports the view that rats are more motivated to consume cocaine when they experience a negative aversive state across all doses tested. A possible explanation for this finding may be that the rapid rate of responding at the start of the self-administration phase in phase 2 (i.e., load-up behavior) reflects the animals attempt to achieve a peak level of drug in their system to overcome the aversive state that developed during intra-oral saccharin infusions (phase 1). Although the emergence of

negative affect in this preclinical model is not cocaine dose-dependent, the present findings showed that load-up behavior may reflect a correction of this aversive state that was observed across cocaine dose.

Effects of voluntary wheel running on an established cocaine-induced negative affective state

The study described in Chapter 3 investigated if voluntary wheel running after training on the preclinical model (i.e., during forced abstinence) would reverse or attenuate the negative affective state when tested on the task later. Although the results indicated a trend toward a protective effect, these results were not strong. EX rats exhibited a significant increase in appetitive taste reactivity following abstinence and this increase was significantly higher than the SED rats. In addition, EX rats exhibited significantly lower load-up behavior following abstinence as compared to SED rats indicating access to a running wheel reduced the motivation to seek cocaine. However, the same EX rats did not exhibit a significant reduction in aversive taste reactivity, and continued to exhibit a positive correlation between the aversive state (i.e. gapes) and load-up presses. These findings indicate exercise has a subtle effect in altering the affective state and even though motivation is attenuated it remains evident.

Investigators have reported exposure to exercise during abstinence reduces cue-induced and cocaine primed reinstatement only following extinction (Lynch et. al 2010, Smith et. al. 2012). Thanos et. al. 2013 did not include extinction and reported that forced exercise during abstinence only reduced cue-induced but not cocaine primed reinstatement. This suggests that extinction is an important component that may need to be included when examining the effects of exercise exposure during abstinence on drug seeking behavior. In chapter 3, extinction was not included in our design. Thus, if rats had extinction as part of our design a change in aversive state may have occurred following abstinence. Collectively, the current findings indicate that

exposure to a running wheel during an extended abstinence period (7 weeks) has slight protective effects in the expression of the affective state and the motivation to consume the drug.

Voluntary wheel running prior to acquisition of our preclinical model of natural reward devaluation

The study described in Chapter 4 examined if access to a running wheel prior to training in our preclinical model would prevent or attenuate the negative affective state that typically develops later. Our data indicated that exercise did have a protective effect against the negative affective state and reduced lever press responding across test days. However, the motivation to seek cocaine remained. When access to a running wheel is given prior to acquisition of drug seeking, findings indicate wheel running reduces the levels of cocaine and heroin self-administration under short access conditions (Smith et. al. 2008, Smith and Pitts 2011, Smith and Witte 2012). In addition, a history of exercise prevents the development of a preference for a drug-associated context (Lett et. al. 2002). This result has been shown in other studies involving enriched environments such that social housing, novel objects, and running wheels in the home cages prevents subsequent development of a preference for a drug-associated environment in a conditioned place preference experiment (El-Rawas et. al. 2009, Solinas et. al. 2008). Even forced running on a treadmill attenuates preference for a drug associated context (Chen et. al 2008, Thanos et. al. 2010) suggesting that exercise, voluntary or forced, has the ability to reduce the reinforcing effects of drugs.

Together, these findings indicate that exercise may alter the reinforcing effects of drugs of abuse. The findings from this chapter indicate exercise attenuates (but does not eliminate) the negative affective state which has not been reported in other preclinical studies. However, the negative affective state that remains is strong enough for the motivation to consume cocaine to

continue after it has developed in our preclinical model. One potential next step would be to determine if we can predict, based on wheel activity, if animals prior exposure to the preclinical model will exhibit aversive responding to the saccharin cue sooner or later in training. I would predict that rats that showed the most wheel running activity during the last week of wheel running will take longer to exhibit aversive responding during training indicating exercise provides an early indication of vulnerability to, or resilience from, drug and protects against the onset of anticipation for the drug.

General Discussion and relevance of findings

Although the implications of each study are discussed individually following each original data chapter, these findings also have further implications for how drug-seeking and taking behavior is altered as a function of cocaine dose and exposure to physical activity. As such, the findings of this dissertation provide insights into the role of withdrawal/negative affective and potential treatment for cocaine addiction. These topics are discussed below.

Negative Affective State/Withdrawal

As conceptualized by Koob and colleagues, there are three stages of the addiction cycle including 'binge/intoxication', 'withdrawal/negative affect' and 'preoccupation/anticipation (craving)' (Koob and Simon 2009, Koob and Le Moal 2002, Koob and Volkow 2010). This cycle of addiction provides aspects of both impulsivity and compulsion in which impulsivity begins at the early stages of addiction and impulsivity combined with compulsivity dominates at the later stages (Koob and Volkow 2010). Impulsivity is characterized as increasing arousal and tension before committing an impulsive act which is associated with positive reinforcement (American Psychiatric Association 2013, Koob and Volkow 2010). While compulsive behavior is characterized by anxiety and stress before doing a compulsive repetitive act which is

associated with negative reinforcement mechanisms. Anxiety and stress play keys roles in addiction and encompasses the stage of negative affect. Thus here, I will consider my dissertation research with respect to the 'withdrawal/negative affect' stage.

Despite negative consequences of addiction including decreases in health, economic, and social activities, addicted individuals use drugs frequently (Baker et. al. 2004) and if interrupted, whether forced or voluntary, will likely relapse (Koob and Volkow 2010). Why do addicts continue to take drugs in the face of these negative consequences? According to early models of drug motivation (Wikler, 1948), addicts abuse substances to escape or avoid aversive states such as withdrawal, stress, or other forms of negative affect. Negative affect can lead individuals to relapse and initiate drug seeking behavior (Koob and Volkow 2010). Interestingly, findings in this dissertation suggest how important negative affect is on motivation of drug seeking behavior in animals regardless of the environmental condition or cocaine dose. Very few preclinical studies have investigated the effect exercise has on withdrawal symptoms however one study examined the effects of environmental enrichment (i.e. social housing, and novel objects) has on affective state in rodents (Puhl et. al 2012). These investigators found that exposure to social housing and novel objects did not alter avoidance behavior to the saccharin cue that predicted access to cocaine. This dissertation, as far as the author knows, is the only preclinical study to examine the effects physical activity (i.e. wheel running) has specifically on the development of negative affect using taste reactivity. Previous studies have shown that voluntary exercise in rodents during ethanol withdrawal protects against seizures, a symptom of ethanol withdrawal (Devaud et. al. 2012, McCulley et. al. 2012). In addition, exercise attenuates thermal sensitivity observed in mice during withdrawal (Balter and Dykstra 2012), and reduces anxiety like behaviors in morphine-dependent rats (Miladi-Gorj et. al. 2012). Clinical studies have shown

that exercise has the potential to reduce stress, anxiety, and depression (Bock et. al. 1999, Morgan 1994, North et. al.1990).

We have shown in chapters 3 and 4 that voluntary wheel running can increase positive affect (e.g., licks during sweet infusion) as compared to animals with no exposure to the wheel. However, the negative affective state did develop and was correlated with load-up presses indicating animals were still motivated to self-administer cocaine. In a prior study conducted in the Carelli lab (Wheeler et. al. 2011), investigators reported that a taste cue can increase or diminish dopamine concentrations in the nucleus accumbens (a key component of the brain reward system) depending on the temporal (i.e. delayed versus immediate) relationship between the cue and the drug. One potential follow up study could be to examine dopamine release in rats exposed to running wheels to determine if voluntary wheel running (either before or after training) alters dopamine release within this preclinical model. I predict that exercise rats would show higher dopamine release to the saccharin cue during the delay as compared to the sedentary rats suggesting that exercise can have a broad impact on conditioned behavior, altering affect and drug intake, reflected in enhanced dopamine release in the nucleus accumbens.

Potential treatment for cocaine addiction

Even though there are few clinical studies that have examined if physical activity can be used as an effective prevention or intervention strategy for addiction, the idea that it may be effective is not new. Epidemiological studies have reported negative correlational relationships between duration (i.e. exercise 2 months versus 6 months) and type (i.e. sports or aerobic exercise) of physical activity and drug use (Correia et. al. 2005, Roessler 2010). For instance, Roessler (2010) examined if physical activity can be used as an intervention to change addicts' behavior. These investigators reported that the longer the addicts were physically active (6

months versus 2 months) the less alcohol and drug intake they consumed with less feelings of withdrawal symptoms. This suggests that addicts in recovery may use exercise as a replacement for drug intake or to assist in maintaining abstinence.

More clinical trials are necessary to determine whether physical activity may attenuate developing addiction as well as maintain abstinence once addiction has developed. However, preclinical studies can prove useful in determining casual relationships between exercise and vulnerability to drug addiction. For instance, there are individual differences in preference of physical activity and this may determine if the individual will engage in exercise and the intensity of that physical activity (i.e. CrossFit versus Aerobic). The intensity or levels of exercise varies in humans such that some forms of exercise can cause positive affective states in some individuals while negative states in others (Ekkekakis et. al. 2011). It would therefore be interesting to determine individual differences in rodent baseline exercise levels, and to determine if those differences are correlated with initial drug consumption rates and relapse in animals models. Thus more research is necessary to determine the conditions that would be most efficacious in preventing acquisition of drug consumption and reducing withdrawal symptoms to prevent drug relapse.

Future Directions

The majority of animal models of human drug addiction, including our model, incorporate drug self-administration procedures that typically lack choices for alternative reinforcers (Wheeler et. al. 2008, Ahmed et. al. 2013, Grigson 2008). Generally speaking, drug self-administration procedures are used to examine pharmacological, environmental, and biological determinants of drug seeking and taking behavior (Banks and Negus 2012). Even though this procedure has been informative, an important concern that has been recently raised in

the literature is if this approach truly models drug addiction in humans. Importantly, nearly all rats trained to lever press for intravenous cocaine develop this behavior over 1-2 week period. However, epidemiological research in humans has shown that only a minority of drug users actually become addicted. For example, some statistics indicate that only 17% of cocaine users become addicted to cocaine in their lifetime and only 6% become addicted within first 2 years following acquisition (Ahmed et. al. 2013). Preclinical studies have demonstrated that having concurrent access to a nondrug reinforcer (e.g., food, exercise) and cocaine influences self-administration behavior (Ahmed 2005, Campbell and Carroll 2000). For instance, when rats are given a choice between drinking a 0.2% saccharin solution or self-administering intravenous cocaine, 94% of rats chose the nondrug reinforcer (Ahmed et. al. 2013). It has been proposed by those authors that the small percentage of rats that chose to self-administer cocaine when alternative reinforcers are available, may better model human drug addiction since only a small percentage of humans that try cocaine actually become addicted to the drug (Ahmed et. al. 2013, Campbell and Carroll 2000). For example, according to the National Comorbidity Survey (Wagner and Anthony 2002) for Americans between the ages of 15-54 years who tried cocaine at least once, only 1 in 6 people became addicted (approx. 16-17%). With these caveats in mind, one potential next step will be to determine if the negative affective state induced in our model will alter subsequent choice for the drug versus a natural reward (exercise), using a modified version of a discrete-trials choice procedure developed by Lenoir et. al. (2013). Briefly, all rats will learn that one flavored saccharin solution is paired with impending cocaine self-administration, while a different flavored saccharin solution is associated with access to a running wheel. On the test day, rats will again be exposed to the intraoral tastant, then given a choice between self-administering cocaine versus exercise. In this model most rats will normally

choose the natural reinforcer when given a choice between it and cocaine. However, this choice may shift to cocaine once animals are experiencing a negative affective state associated with waiting for the drug in our model suggesting the importance of negative affect in drug seeking and taking.

A great majority of studies have used a fixed ratio schedule of reinforcement to measure the rewarding effects of a drug (Arnold and Roberts 1997). Further, using this schedule has been useful in determining patterns of drug intake and screen for drugs with abuse liability. However, the use of progressive ratio schedule has been proposed to be more appropriate to examine effort. Under this schedule, to earn a reward the operant response requirements increase after each reward delivery. Typically animals increase responding for higher doses of drugs (Pickens and Thompson, 1968a, Woods and Schuster, 1968) indicating the reward value is highest with the highest drug dose. In the current preclinical model of natural reward devaluation, we have only used a fixed ratio schedule to examine patterns of responding during cocaine self-administration. Thus I am suggesting repeating our dose response study from chapter 2, and add a progressive ratio schedule during phase 2, cocaine self-administration. Then examine if there is a dose dependent effect. I predict that animals that have highest responding for each drug dose will show the most aversive responses. This predicted result would suggest effort to obtain a drug is related to the negative affective state that develops in this model.

In this dissertation, I used taste reactivity to measure the affective state in rodents. Even though this technique is a great measurement for examining appetitive and aversive behavior across species, there are issues with analyzing the data. Depending on video recording length and the number of animals that have been recorded, it may take hours to measure appetitive versus aversive responses. In addition with human error, there may be variability from one

experimenter to the next on the number of appetitive and aversive responses quantified. Thus, it is important to find alternative methods to examine affective state. Ultrasonic vocalization (USV) is a technique that can be used to measure the affective state in rodents (USVs) (Knutson et. al. 2002, Barker et. al. 2014). Ultrasonic vocalizations can serve as a technique to measure affect in rodents because a bimodal frequency distribution has been segmented into two frequency ranges. One frequency ranges from 18 to 33 kHz (22 kHz range) that has been associated with negative/aversive outcomes while the second ranging from 38 to 80 kHz (50 kHz range) is associated with positive/appetitive outcomes (Barker et. al. 2014, Knutson et. al. 2002, Burgdorf et. al. 2011). USV expression does not rely on training thus it can be viewed as a spontaneous measure of affective state (Browning et. al. 2011). For instance Browning et. al. (2011) monitored spontaneous USVs throughout cocaine self-administration and reinstatement to examine the changes in affective state across the different phases. These investigators reported that over the course of the experiment there was an increase in appetitive USVs during acquisition then it significantly decrease during extinction. In addition, appetitive USVs were associated with how rapidly the animals acquired cocaine self-administration. As such, I believe that it would be informative to determine if the negative affective state induced in our model will be reflected in differential USVs associated with negative outcomes. This would be beneficial because this would be a different measure to examine negative affect beyond taste reactivity.

Concluding remarks

Exercise has the potential to be used as a treatment in human drug addicts by altering their affective state and thereby attenuating drug seeking behavior. The findings of this dissertation add to addiction literature by demonstrating that the negative affective state observed in our preclinical model is attenuated (but not reversed) by physical activity. Thus, additional

studies are necessary to examine other aspects of our preclinical model that can investigate individual differences (i.e. onset of aversive responses), differences between sexes, examine choice between a natural reinforcer and drug, alternative methods to examine affective state, and examine effort for the drug.

REFERENCES

Abuse, N. I. o. D. (2010). "Cocaine: Abuse and Addiction." NIH Pub Number: 10-4166: 8.

Ahmed, S.H. (2010). "Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction." Neuroscience and Biobehavioral Reviews 35:172-184

Amato, L., S. Minozzi, P. P. Pani, R. Solimini, S. Vecchi, P. Zuccaro and M. Davoli (2011). "Dopamine agonists for the treatment of cocaine dependence." Cochrane Database of Systematic Reviews (12).

Association, A. P. (2013). Diagnostic and statistical manual of mental disorders. Washington D.C. 5th edition

Baker, T. B., Morse, E., Sherman, J.E. (1987). "The Motivation to Use Drugs - a Psychobiological Analysis of Urges." Nebraska Symposium on Motivation **34**: 257-323.

Baker, T. B., M. E. Piper, D. E. McCarthy, M. R. Majeskie and M. C. Fiore (2004). "Addiction motivation reformulated: An affective processing model of negative reinforcement." Psychological Review **111**(1): 33-51.

Balter, R.E. and Dykstra, L.A. (2012). "The effect of environmental factors on morphine withdrawal in C57BL/6J mice: running wheel access and group housing." Psychopharmacology 224 (1)**:** 91–100.

Bardo, M. T., J. E. Klebaur, J. M. Valone and C. Deaton (2001). "Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats." Psychopharmacology (Berl) **155**(3): 278-284.

Barker, D.J., Bercovicz1, D., Servilio, L.C., Simmons, S.J., Ma, S., Root, D.H., Anthony P. Pawlak, A.P., West, M.O. (2014). "Rat ultrasonic vocalizations demonstrate that the motivation to contextually reinstate cocaine-seeking behavior does not necessarily involve a hedonic response." Addiction Biology 19(5): 781-790.

Bock, B.C., Marcus, B.H., King, T.K., Borrelli, B., Roberts, M.R. (1999). "Exercise effects on withdrawal and mood among women attempting smoking cessation." Addictive Behaviors **24**(3) 399-410.

Bolles, R. C. 1975. Theory of Motivation. 2nd edn. New York: Harper & Row

Brager, A. J. and Hammer, S.B. (2012). "Impact of wheel running on chronic ethanol intake in aged Syrian hamsters." Physiol Behav **107**(3): 418-423.

Brenes J.C. and Fornaguera J. (2008). "Effects of environmental enrichment and social isolation

on sucrose consumption and preference: associations with depressive-like behavior and ventral striatum dopamine". Neurosci Lett **436**: 278–282.

Browning J.R., Browning D.A., Maxwell A.O., Dong Y., Jansen H.T., Panksepp J., Sorg B.A. (2011). "Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats." Neuropharmacology **61**:268–275.

Burgdorf J., Panksepp J., Moskal J.R. (2011). "Frequency-modulated 50-kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect." Neurosci Biobehav Rev **35**:1831–1836.

Cain M.E., Green T.A., Bardo M.T. (2006). "Environmental enrichment decreases responding for visual novelty." Behav Process **73**:360–366.

Carelli, R. M. and Deadwyler, S.A. (1994). "A comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats." J Neurosci **14**(12): 7735-7746.

Carelli, R. M. and Deadwyler, S.A. (1996). "Dose-dependent transitions in nucleus accumbens cell firing and behavioral responding during cocaine self-administration sessions in rats." J Pharmacol Exp Ther **277**(1): 385-393.

Carelli, R. M., Ijames, S.G., Crumling, A.J. (2000). "Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus "natural" (water and food) reward." J Neurosci **20**(11): 4255-4266.

Carelli, R. M. and West, E.A. (2014). "When a good taste turns bad: Neural mechanisms underlying the emergence of negative affect and associated natural reward devaluation by cocaine." Neuropharmacology **76**: 360-369.

Cason, A.M. and Grigson, P.S. (2013). "Prior access to a sweet is more protective against cocaine self-administration in female rats than in male rats." Physio Behav **112–113**: 96–103.

Chauvet, C., Lardeux, V., Goldberg, S.R., Jaber, M., Solinas, M. (2009). "Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine." Neuropsychopharmacology **34**(13): 2767-2778.

Colechio, E.M. and Grigson, P.S. (2014). "Conditioned Aversion for a Cocaine-Predictive Cue is Associated with Cocaine seeking and taking in rats." IntJ Comp Psychol **27** (3): 488-500.

Colechio, E.M., Imperio, C.G., and Grigson, P.S. (2014). "Once is too much: conditioned aversion develops immediately and predicts future cocaine self-administration behavior in rats." Behav Neuro **128** (2) 207-216.

Collingwood, T.R., Reynolds, R., Kohl, H.W., Smith, W., Sloan ,S (1991). “Physical fitness effects on substance abuse risk factors and use patterns.” J Drug Educ **21**:73–84.

Collingwood, T.R., Sunderlin, J., Reynolds, R., Kohl, H.W. (2000). “3rd Physical training as a substance abuse prevention intervention for youth.” J Drug Educ **30**:435–51.

Correia, C.J., Benson, T.A., Carey, K.B. (2005). “Decreased substance use following increases in alternative behaviors: a preliminary investigation.” Addict. Behav.**30** (1), 19–27.

Cosgrove, K. P., Hunter, R.G., Carroll, M.E. (2002). "Wheel-running attenuates intravenous cocaine self-administration in rats: sex differences." Pharmacol Biochem Behav **73**(3): 663-671.

Devaud, L.L., Walls, S.A., McCulley 3rd, W.D., Rosenwasser, A.M., (2012). “Voluntary wheel running attenuates ethanol withdrawal-induced increases in seizure susceptibility in male and female rats.” Pharmacol. Biochem. Behav **103** (1):18–25.

Di Chiara, G. and Bassareo, V. (2007). “Reward system and addiction: what dopamine does and doesn’t do.” Current Opinion in Pharmacology **7**(1):69-76.

Ehringer, M.A., Hoft, N.R., Zunhammer, M. (2009). “Reduced alcohol consumption in mice with access to a running wheel.” Alcohol **43** (6): 443–452.

Ekkekakis, P., Parfitt, G., Petruzzello, S.J. (2011). “The pleasure and displeasure people feel when they exercise at different intensities: decennial update and progress towards a tripartite rationale for exercise intensity prescription.” Sports Med **41** (8), 641–671.

El-Rawas, R., Thiriet, N., Lardeux, V., Jaber, M., Solinas, M., (2009). “Environmental enrichment decreases the rewarding but not the activating effects of heroin.” Psychopharmacology **203** (3): 561–570.

Engelmann, A. J., Aparicio, M.B. et al. (2014). "Chronic wheel running reduces maladaptive patterns of methamphetamine intake: regulation by attenuation of methamphetamine-induced neuronal nitric oxide synthase." Brain Struct Funct **219**(2): 657-672.

Field T., Diego M., Sanders C.E. (2001). “Exercise is positively related to adolescents’ relationships and academics.” Adolescence. **36**:105–10.

Flagel, S.B., Watson, S. J., Akil, H., Robinson, T.E. (2008). **“**Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization.**”** Behavioural Brain Research **186** (1): 48-56.

Fox, H. and R. Sinha (2014). "The Role of Guanfacine as a Therapeutic Agent to Address Stress-Related Pathophysiology in Cocaine-Dependent Individuals." Emerging Targets & Therapeutics in the Treatment of Psychostimulant Abuse **69**: 217-265.

Fox, H. C., K.-I. A. Hong, K. Siedlarz and R. Sinha (2008). "Enhanced sensitivity to stress and drug/alcohol craving in abstinent cocaine-dependent individuals compared to social drinkers." Neuropsychopharmacology **33**(4): 796-805.

Ghitza, U. E., A. T. Fabbricatore, V. F. Prokopenko and M. O. West (2004). "Differences between accumbens core and shell neurons exhibiting phasic firing patterns related to drug-seeking behavior during a discriminative-stimulus task." J Neurophysiol **92**(3): 1608-1614.

Gipson, C. D., J. S. Beckmann, S. El-Maraghi, J. A. Marusich and M. T. Bardo (2011). "Effect of environmental enrichment on escalation of cocaine self-administration in rats." Psychopharmacology (Berl) **214**(2): 557-566.

Goeders, N. E. (2002). "Stress and cocaine addiction." J Pharmacol Exp Ther **301**(3): 785-789.

Green, J.L. (2012). "Nucleus Accumbens Neurons Differentially Encode Information about Aversive Cues that Predict Cocaine Availability and Cocaine Self-Administration Following Extended Taste-Drug Pairings" (Masters Thesis). ProQuest, UMI Dissertations Publishing.

Green, T. A., B. J. Gehrke and M. T. Bardo (2002). "Environmental enrichment decreases intravenous amphetamine self-administration in rats: dose-response functions for fixed- and progressive-ratio schedules." Psychopharmacology (Berl) **162**(4): 373-378.

Greenwood, B. N., T. E. Foley, et al. (2011). "Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway." Behav Brain Res **217**(2): 354-362.

Grigson, P.S. and Twining, R.C. (2002). "Cocaine-induced suppression of saccharin intake: a model of drug-induced devaluation of natural rewards." Behav Neurosci **116**(2): 321-333.

Grigson, P. S. (1997). "Conditioned taste aversions and drugs of abuse: A reinterpretation". Behav Neurosci **111:** 129–136.

Grigson, P. S. (2008). "Reward Comparison: The Achilles' heel and hope for addiction." Drug Discov Today Dis Models **5**(4): 227-233.

Grill, H. J. and R. Norgren (1978). "The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats." Brain Res **143**(2): 263-279.

Grimm J.W., Osincup D., Wells B., Manaois M., Fyall A., Buse C., Harkness J.H. (2008). "Environmental enrichment attenuates cue-induced reinstatement of sucrose seeking in rats." Behav Pharmacol **19**(8): 777-785.

Guide for the Care and Use of Laboratory Animals: Eighth Edition. (2011). The National

Academies Press.

Hammer, S. B., Ruby, C.L., Brager, A.J., Prosser, R.A., Glass, J.D. (2010). "Environmental modulation of alcohol intake in hamsters: effects of wheel running and constant light exposure." Alcohol Clin Exp Res **34**(9): 1651-1658.

Harris, D. S., Reus, V.I., Wolkowitz, O.M., Mendelson, J.E., Jones, R.T. (2005). "Repeated psychological stress testing in stimulant-dependent patients." Progress in Neuro-Psychopharmacology and Biological Psychiatry **29**(5): 669-677.

Hollander, J. A. and Carelli, R.M. (2007). "Cocaine-associated stimuli increase cocaine seeking and activate accumbens core neurons after abstinence." J Neurosci **27**(13):3535-3539.

Hollander, J. A. and Carelli, R.M. (2005). "Abstinence from cocaine self-administration heightens neural encoding of goal-directed behaviors in the accumbens." Neuropsychopharmacology **30**(8):1464-1474.

Ito, R. and Hayen, A. (2011). "Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing." J Neurosci **31**(16): 6001-6007.

Iannotti R.J., Kogan M.D., Janssen I., Boyce W.F. (2009). "Patterns of adolescent physical activity, screen-based media use, and positive and negative health indicators in the U.S. and Canada." J Adolesc Health. **44**:493–9.

Iversen I.H. (1993). "Techniques for establishing schedules with wheel running as reinforcement in rats." J Exp Annal Behav **60**:219– 38.

Kavanagh, D. J., Andrade, J., May, J. (2005). "Imaginary relish and exquisite torture: The elaborated intrusion theory of desire." Psychological Review **112**(2): 446-467.

Kelley, A.E., Berridge, K.C. (2002). "The neuroscience of natural rewards: relevance to addictive drugs." Journal of Neuroscience 22 (9):3306-3311

Kirkcaldy B.D., Shephard R.J., Siefen R.G.(2002). "The relationship between physical activity and self-image and problem behaviour among adolescents." Soc Psychiatry Psychiatr Epidemiol. **37**:544–50.

Knutson B, Burgdorf J, Panksepp J. (2002). "Ultrasonic vocalizations as indices of affective states in rats." Psychol Bull 128: 961–977.

Koob, G. F. (2008). "A role for brain stress systems in addiction." Neuron **59**(1): 11-34.

Koob, G. F. and Caine, S.B. (1999). "Cocaine addiction therapy--are we partially there?" Nat Med **5**(9): 993-995.

Koob, G. F. and Le Moal, M. (1997). "Drug abuse: hedonic homeostatic dysregulation." Science

278(5335): 52-58.

Koob, G. F. and Le Moal, M. (2002). "Neurobiology of drug addiction." Stages and pathways of drug involvement: examining the gateway hypothesis. Cambridge University Press, New York: 337-361.

Koob, G. F. and Le Moal, M. (2008). "Addiction and the brain antireward system." Annu Rev Psychol **59**: 29-53.

Koob, G. F., Sanna, P.P., Bloom, F.E. (1998). "Neuroscience of addiction." Neuron **21**(3): 467-476.

Koob, G. F., Sanna, P.P., Bloom F.E. (1998). "Neuroscience of addiction." Neuron **21**(3): 467-476.

Koob, G.F., and Simon, E.J. (2009). "The Neurobiology of Addiction: Where We Have Been and Where We Are Going." J Drug Issues. **39**(1):115-132.

Koob, G. F. and Volkow, N.D. (2010). "Neurocircuitry of addiction." Neuropsychopharmacology **35**(1): 217-238.

Lacy R.T., Strickland J.C., Brophy M.K., Witte M.A., Smith M.A. (2014). "Exercise decreases s speedball self-administration." Life Sci. **114**(2):86-92.

Lett, B.T., Grant, V.L., Koh, M.T., Flynn, G. (2002). "Prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine." Pharmacol. Biochem. Behav **72**(1-2): 101-105.

Lu, L., Grimm, J.W., Hope, B.T., Shaham, Y. (2004) "Incubation of cocaine craving after withdrawal: a review of preclinical data." Neuropharmacology, **47**:.214–226.

Lynch, W. J., Peterson, A.B., et al. (2013). "Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis." Neurosci Biobehav Rev **37**(8): 1622-1644.

Lynch, W. J., LaBounty, L. P., Carroll, M. E. (1998). "A novel paradigm to investigate regulation of drug intake in rats self-administering cocaine or heroin intravenously." Exp Clin Psychopharmacol **6**(1): 22-31.

Lynch, W. J., Peterson, A.B., Sanchez, V., Abel, J., Smith, M. A. (2013). "Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis." Neurosci Biobehav Rev **37**(8): 1622-1644.

Lynch, W. J., Piehl, K. B., Acosta, G., Peterson, A.B, Hernby, S. E. (2010). "Aerobic exercise attenuates reinstatement of cocaine-seeking behavior and associated neuroadaptations in the prefrontal cortex." Biol Psychiatry **68**(8): 774-777.

Martin, T. J., L. E. Walker, G. M. Sizemore, J. E. Smith and S. I. Dworkin (1996). "Within-session determination of dose-response curves for heroin self-administration in rats: Comparison with between-session determination and effects of naltrexone." Drug Alcohol Depend **41**(2): 93-100.

McCulley 3rd, W.D., Walls, S.A., Khurana, R.C., Rosenwasser, A.M., Devaud, L.L., (2012). "Running wheel activity protects against increased seizure susceptibility in ethanol withdrawn male rats." Pharmacol. Biochem. Behav **100** (3), 485–489.

Miladi-Gorji, H., Rashidy-Pour, A., Fathollahi, Y. (2012). "Anxiety profile in morphine dependent and withdrawn rats: effect of voluntary exercise." Physiol. Behav **105** (2), 195–202.

Miller, M.L., Vaillancourt, B.D., Wright Jr., M.J., Aarde, S.M., Vandewater, S.A., Creehan, K.M., Taffe, M.A., (2012). "Reciprocal inhibitory effects of intravenous dmethamphetamine self-administration and wheel activity in rats." Drug Alcohol Depend. **121** (1–2), 90–96.

Mogenson, G. J., D. L. Jones and C. Y. Yim (1980). "From motivation to action: functional interface between the limbic system and the motor system." Prog Neurobiol **14**(2-3): 69-97.

Morgan, W. P. (1994). "Physical activity, fitness, and depression." In C. Bouchard, R. J. Shephard, & T. Stephens (Eds.), Physical activity, fitness, and health: International proceedings and consensus statement. Champaign, IL: Human Kinetics.

Nestler, E.J. (2005). "The neurobiology of cocaine addiction." Sci Pract Perspect 3(1):4-10

North, C. T., McCullagh, P., Vu Tran, Z. (1990). "Effect of exercise on depression." Exercise and Sports Science Reviews **37:** 379–415

Nyland, J. E. and P. S. Grigson (2013). "A drug-paired taste cue elicits withdrawal and predicts cocaine self-administration." Behav Brain Res **240**: 87-90.

O'Brien, C. P. (1976). "Experimental analysis of conditioning factors in human narcotic addiction." Pharmacological Reviews, 27: 533–543.

Official Foundation for a Drug-Free World, Cocaine Addiction, Substance Abuse, Alcohol (2015). "Drug free world: substance & alcohol abuse, education & prevention." N.P., n.d. Web 13 Jan. 2015.

Pecina, S., Smith, K.S., Berridge, K.C. (2006). "Hedonics hot spots in the brain." The Neuroscientist. **12**(6): 500-511.

Peterson, A. B., Abel, J.M., Lynch, W.J. (2014). "Dose-dependent effects of wheel running on

cocaine-seeking and prefrontal cortex Bdnf exon IV expression in rats." Psychopharmacology (Berl) **231**(7): 1305-1314.

Peterson, A. B., Hivick,D.P., Lynch, W.J. (2014). "Dose-dependent effectiveness of wheel running to attenuate cocaine-seeking: impact of sex and estrous cycle in rats." Psychopharmacology (Berl) **231**(13): 2661-2670.

Pettit, H. O. and Justice, Jr., J.B. (1989). "Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis." Pharmacol Biochem Behav **34**(4): 899-904.

Pettit, H. O. and Justice, Jr., J.B. (1991). "Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens." Brain Res **539**(1): 94-102.

Phillips, P.E., Stuber, G.D., Heien, M.L.A.V., Wightman, R.M., Carelli, R.M. (2003). "Subsecond dopamine release promotes cocaine seeking." Nature 422:614-618.

Pickens, C.L., Airavaara, M., et. al. (2011) Neurobiology of the incubation of drug craving Trends Neurosci., 34: 411–420

Pickens, R. and Thompson, T. (1968). "Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size." J Pharmacol Exp Ther **161**(1): 122-129.

Puhl, M. D., Blum, J.S, Acosta-Torres, S., Grigson, P.S. (2012). "Environmental enrichment protects against the acquisition of cocaine self-administration in adult male rats, but does not eliminate avoidance of a drug-associated saccharin cue." Behav Pharmacol **23**(1): 43-53.

Risinger, R. C., Salmeron, B.J., Ross, T.J., Amen, S.L., Sanfilipo, M., Hoffmann, R.G., Bloom, A.S., Garavan, H., Stein, E.A. (2005). "Neural correlates of high and craving during cocaine self-administration using BOLD fMRI." Neuroimage **26**(4): 1097-1108.

Robbins, S.J., Ehrman, R.N., Childress, A.R., Cornish, J.W., O'Brien, C.P. (2000). "Mood state and recent cocaine use are not associated with levels of cocaine cue reactivity." Drug and Alcohol Dependence. **59**: 33-42.

Robbins, T.W. and Everitt, B.J. (1996). "Neurobehavioral mechanisms of reward and motivation." Current Opinion in Neurobiology 6(2):228-236.

Roessler, K.K. (2010). "Exercise treatment for drug abuse: a Danish pilot study." Scand. J. Public Health **38** (6), 664–669.

Roitman, M. F., Wheeler, R. A., Carelli, R. M. (2005). "Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output." Neuron **45**(4): 587-597.

Roitman, M. F., Wheeler, R. A, Wightman, R.M., Carelli, R. M. (2008). "Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli." Nat Neurosci **11**(12): 1376-1377.

Roitman, M. F., Wheeler, R. A, Tiesinga, P.H., Roitman, J. D., Carelli, R. M. (2010). "Hedonic and nucleus accumbens neural responses to a natural reward are regulated by aversive conditioning." Learn Mem **17**(11): 539-546.

Sanchez, V., Moore, C.F., Brunzell, D.H., Lynch, W.J. (2013). "Effect of wheel-running during abstinence on subsequent nicotine-seeking in rats." Psychopharmacology (Berl) **227**(3): 403-411.

Schultz, W. (1998). "Predictive reward signal of dopamine neurons." Journal of neurophysiology 80 (1): 1-27

Sherwin, C.M. (1998). Voluntary wheel running: a review and novel interpretation. Animal Behavior 56: 11-27.

Sinha, R., Fuse, T., Aubin, L.R., O'Malley, S.S. (2000). "Psychological stress, drug-related cues and cocaine craving." Psychopharmacology (Berl) **152**(2): 140-148.

Smith, M. A. and Lynch, W.J. (2012). "Exercise as a potential treatment for drug abuse: evidence from preclinical studies." Front Psychiatry **2(**82):1-10.

Smith, M. A., Schmidt, K.T., Iordanou. J.C., Mustroph, M.L. (2008). "Aerobic exercise decreases the positive-reinforcing effects of cocaine." Drug Alcohol Depend **98**(1-2): 129-135.

Smith, M. A., Pennock, M. M., Walker, K.L., Lang, K.C. (2012). "Access to a running wheel decreases cocaine-primed and cue-induced reinstatement in male and female rats." Drug and Alcohol Dependence 121: 54-61.

Smith, M.A., and Pitts, E.G. (2011a). "Access to a running wheel inhibits the acquisition of cocaine self- administration." Pharmacol. Biochem.Behav **100:** 237–243.

Smith, M. A., Walker, K.L., Cole, K.T., Lang, K.C. (2011). "The effects of aerobic exercise on cocaine self-administration in male and female rats." Psychopharmacology (Berl) **218**(2): 357-369.

Smith, M. A. and M. A. Witte (2012). "The effects of exercise on cocaine self-administration, food-maintained responding, and locomotor activity in female rats: importance of the temporal relationship between physical activity and initial drug exposure." Exp Clin Psychopharmacol **20**(6): 437-446.

Sofuoglu, M. (2010). "Cognitive enhancement as a pharmacotherapy target for stimulant

addiction." Addiction **105**(1): 38-48.

Solinas, M., Chauvet, C., Thiriet, N., El Rawas, R. and Jaber, M. (2008). "Reversal of cocaine addiction by environmental enrichment." Proc Natl Acad Sci U S A **105**(44): 17145-17150.

Solinas, M., Thiriet, N., El Rawas, R., Lardeux, V., and Jaber, M. (2009). "Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine." Neuropsychopharmacology **34**(5): 1102-1111.

Stairs, D. J. and Bardo, M.T. (2009). "Neurobehavioral effects of environmental enrichment and drug abuse vulnerability." Pharmacol Biochem Behav **92**(3): 377-382.

Ströhle A., Höfler M., Pfister H., Müller A.G., Hoyer J., Wittchen H.U., et al (2007). "Physical activity and prevalence and incidence of mental disorders in adolescents and young adults." Psychol Med **37**:1657–66.

Thanos, P. K., J. Stamos, L. S. Robison, G. Heyman, A. Tucci, G. J. Wang, J. K. Robinson, B. J. Anderson and N. D. Volkow (2013). "Daily treadmill exercise attenuates cocaine cue-induced reinstatement and cocaine induced locomotor response but increases cocaine-primed reinstatement." Behav Brain Res **239**: 8-14.

Thiel, K. J., B. Engelhardt, L. E. Hood, N. A. Peartree and J. L. Neisewander (2011). "The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats." Pharmacol Biochem Behav **97**(3): 595-602.

Tiffany, S. T. (1990). "A Cognitive Model of Drug Urges and Drug-Use Behavior - Role of Automatic and Nonautomatic Processes." Psychological Review **97**(2): 147-168.

Tiffany, S. T. (2010). "Drug Craving And Affect." Substance Abuse and Emotion. J. D. Kassel. Washington, D.C., American Psychological Association**:** 83-108.

Volkow, N. D. (2010). "Cocaine: Abuse and addiction." National Institute on Drug Abuse.

Ward, S. J., D. Morgan and D. C. Roberts (2005). "Comparison of the reinforcing effects of cocaine and cocaine/heroin combinations under progressive ratio and choice schedules in rats." Neuropsychopharmacology **30**(2): 286-295.

Wetter, D. W., Smith, S. S., Kenford SL, Jorenby DE, Fiore MC, Hurt RD, Offord KP, Baker TB (1994). "Smoking outcome expectancies: Factor structure, predictive validity, and discriminant validity." Journal of Abnormal Psychology 103: 801–811.

Wheeler, R. A., Aragona, B.J., Fuhrmann, K.A., Jones, J.L., Day, J.J., Cacciapaglia, F., R. M. Wightman, R.M., Carelli, R.M. (2011). "Cocaine cues drive opposing context-dependent shifts in reward processing and emotional state." Biol Psychiatry **69**(11): 1067-1074.

Wheeler, R. A. and R. M. Carelli (2009). "Dissecting motivational circuitry to understand substance abuse." Neuropharmacology **56 Suppl 1**: 149-159.

Wheeler, R. A., R. C. Twining, J. L. Jones, J. M. Slater, P. S. Grigson and R. M. Carelli (2008). "Behavioral and electrophysiological indices of negative affect predict cocaine self-administration." Neuron **57**(5): 774-785.

Wikler, A. (1980). Opioid dependence: Mechanisms and treatment. New York: Plenum.

Wise, R.A. (2006). Role of brain dopamine in food reward and reinforcement. Philos Trans R Soc Lond B Biol Sci 361(1471) 1149-1158.

Zimmer, B. A., C. V. Dobrin and D. C. Roberts (2011). "Brain-cocaine concentrations determine the dose self-administered by rats on a novel behaviorally dependent dosing schedule." Neuropsychopharmacology **36**(13): 2741-2749.

Zlebnik, N. E., Anker, J.J., et. al. (2010). "Reduction of extinction and reinstatement of cocaine seeking by wheel running in female rats." Psychopharmacology 209:113-125.