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A Tale of Two Addiction Theories:  
the effects of cocaine exposure on cue-induced motivation and action control

A dissertation submitted in partial satisfaction of the requirements  
for the degree Doctor of Philosophy in Neuroscience

by

Kimberly Hathaway LeBlanc

2012



## ABSTRACT OF THE DISSERTATION

A Tale of Two Addiction Theories:  
the effects of cocaine exposure on cue-induced motivation and action control

by

Kimberly Hathaway LeBlanc

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2012

Professor Nigel T. Maidment, Chair

Cocaine addiction affects approximately 1.4 million Americans, costing the government billions of dollars and the addicted individual their life. Addiction is characterized by a continued desire to use a drug despite decreased enjoyment from taking it and a desire to abstain. Multiple theories attempt to explain how prolonged drug use induces the chronic brain changes that result in addiction. The incentive sensitization theory suggests that repeated exposure to drugs of abuse alters the neural circuitry that is involved in incentive motivation, the process that allows drugs and their associated stimuli to more strongly encourage drug-seeking behavior. Alternatively, the habit learning theory suggests that drugs of abuse pathologically subvert the reward-learning circuitry, leading to compulsive drug seeking triggered by drug-associated stimuli. Since both theories are based on the idea that drugs of abuse affect the dopaminergic system and alter normal reward processing, it has been proposed that drug exposure may also affect behavior for natural rewards.

To understand the mechanisms by which cocaine use modifies reward-seeking behaviors, I have conducted experiments to investigate both addiction theories using cocaine

and food rewards as the outcome. I have found that cocaine-paired cues promote cocaine-seeking and taking actions via a Pavlovian motivational mechanism. I have also explored the importance of the contingency of drug delivery and found that cocaine has a general effect on incentive motivation, with both self-administered and experimenter-delivered cocaine enhancing the ability of food-paired cues to motivate food-seeking behavior. This result was not obtained with animals that passively received cocaine infusions, suggesting a role of predictability in the effects of cocaine on incentive motivation.

I have also found support for the habit learning theory, demonstrating that cocaine treatment can encourage habitual control of action selection for food rewards, even when feedback is given. However, experimenter-delivered cocaine does not prevent animals from learning to perform a behavior in a goal-directed fashion. Our results lend support to both theories, suggesting that drug induced changes to the dopaminergic systems may be affecting both incentive motivation and reward learning. These results have important implications for our comprehension of the neural processes involved in addiction.

This dissertation of Kimberly Hathaway LeBlanc is approved.

Sean B. Ostlund

Christopher Evans

David Jentsch

Aaron Blaisdell

Nigel T. Maidment, Committee Chair

University of California, Los Angeles

2012

## TABLE OF CONTENTS

LIST OF FIGURES.....	vii
LIST OF TABLES.....	viii
ACKNOWLEDGEMENTS.....	ix
VITA.....	xi
<b>Chapter 1: General Introduction.....</b>	<b>1</b>
1.1 Drug Abuse and Addiction.....	2
1.2 Reward Learning and Incentive Motivation.....	3
1.3 Dopamine and Incentive Motivation.....	8
1.4 Theories of Drug Addiction.....	10
1.5 Methods and Attributes of Drug Administration.....	13
1.6 Specific Aims.....	15
<b>Chapter 2: Pavlovian-to-Instrumental Transfer in Cocaine Seeking Rats.....</b>	<b>18</b>
2.1 Introduction.....	19
2.2 Materials and Methods.....	21
2.3 Results.....	26
2.4 Discussion.....	29
<b>Chapter 3: Repeated Cocaine Exposure Facilitates the Expression of Incentive Motivation and Induces Habit Formation.....</b>	<b>40</b>
3.1 Introduction.....	41
3.2 Materials and Methods.....	42
3.3 Results.....	46
3.4 Discussion.....	49
<b>Chapter 4: Repeated Cocaine Exposure Does Not Prevent Goal-Directed Learning But Alters How the Behavior is Performed.....</b>	<b>58</b>
4.1 Introduction.....	59
4.2 Materials and Methods.....	60
4.3 Results.....	62
4.4 Discussion.....	65
<b>Chapter 5: Prolonged Experience With Self-administered Cocaine, But Not Yoked Cocaine, Enhances Pavlovian-to-Instrumental Transfer For Food Rewards.....</b>	<b>73</b>
5.1 Introduction.....	74
5.2 Materials and Methods.....	76
5.3 Results.....	80
5.4 Discussion.....	81
<b>Chapter 6: General Discussion.....</b>	<b>88</b>
6.1 Cocaine and Incentive Sensitization.....	89
6.2 Cocaine and Control of Action Selection.....	96
6.3 Implications for Addiction.....	100
<b>References.....</b>	<b>106</b>

## LIST OF FIGURES

Figure 2-1. Acquisition of the seeking-taking chain, shown as average lever presses over the session for the last five sessions of training.....	36
Figure 2-2. Results of the first Pavlovian-to-instrumental transfer test.....	37
Figure 2-3. Results of the second Pavlovian-to-instrumental transfer test.....	38
Figure 2-4. Probability of transitioning from the seeking to the taking lever vs. transitioning from the taking to the seeking lever.....	39
Figure 3-1. Instrumental training on the active and inactive lever.....	54
Figure 3-2. Pavlovian training.....	55
Figure 3-3. PIT test results .....	56
Figure 3-4. Habit learning test results.....	57
Figure 4-1. Activity and magazine entries during cocaine sensitization.....	69
Figure 4-2. Instrumental training days 1-4.....	70
Figure 4-3. Devaluation tests 1 and 2.....	71
Figure 4-4. Instrumental training days 5-12.....	72
Figure 5-1. Pavlovian training and cocaine self-administration.....	86
Figure 5-2. PIT test results and correlation with self-administration.....	87



## LIST OF TABLES

Table 2-1. Test 1 Pre-CS baselines and extinction responding.....	34
Table 2-2. Test 2 Pre-CS baselines.....	35
Table 5-1. Pre-CS baseline lever pressing.....	85

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## VITA

### Education

BS, Neuroscience and Behavioral Biology    Emory University    magna cum laude

### Publications

**LeBlanc, KH**, Maidment, NT, Ostlund, SB (2012). Prolonged experience with self-administered cocaine, but not yoked cocaine, enhances Pavlovian-to-instrumental transfer for food rewards – in preparation

**LeBlanc, KH**, Maidment, NT, Ostlund, SB (2012). Cocaine sensitization facilitates Pavlovian-to-instrumental transfer and promotes habitual control *Neuropsychopharmacology* – submitted

**LeBlanc, KH**, Ostlund, SB, Maidment, NT (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behavioral Neuroscience*. Epub ahead of print (PMID: 22866668)

**Hathaway, KA** (2007). Investigating the co-localization of enkephalin and serotonin receptors in the bed nucleus of the stria terminalis using fluorescent in situ hybridization. Emory University honor's thesis.

### Presentations

**LeBlanc, KH**, Maidment, NT, Ostlund, SB (October 2012). Repeated exposure to self-administered, but not passive, cocaine enhances Pavlovian-to-Instrumental transfer for food reward, Society for Neuroscience, New Orleans, LA - nanosymposium

**LeBlanc, KH**, Maidment, NT, Ostlund, SB (August 2012). Cocaine self-administration enhances cue-motivated behavior, Charles Drew University 7<sup>th</sup> Drug Abuse Research Symposium, Los Angeles, CA. – oral presentation

**LeBlanc, KH**, Ostlund, SB, Maidment, NT (November 2011). Cocaine sensitization facilitates Pavlovian-instrumental transfer and promotes habitual control, Society for Neuroscience, Washington D.C.

**LeBlanc, KH**, Ostlund, SB, Wassum, KM, Balleine, BW, Maidment, NT. (November 2010). Involvement of basolateral amygdala endogenous opioids in cocaine incentive learning, Society for Neuroscience, San Diego, CA.

**Hathaway, KA**, Hazra, R, Jasnow, AM, Ressler, KJ, Rainnie, DG. (November 2007). Localization of Distinct Serotonin Receptor Subtypes to Neuropeptide Containing Neurons in the Bed Nucleus of the Stria Terminalis using dual fluorescent in situ hybridization, Society for Neuroscience, San Diego, CA

#### **Honors and Awards**

Brain Research Institute Travel Award (2011)

Translational Neuroscience of Drug Abuse (T32) trainee (2008-2010)

Achievement Rewards for College Scientists Fellowship (2007-2010)

Phi Beta Kappa (2007)

Dean's Achievement Scholar (2005-2007)

**Chapter 1**  
**General Introduction**

The usage of psychoactive substances, both legal and illicit, is commonplace in the United States and throughout the world. For the majority of individuals, some substances can be used responsibly, and even those who choose to use drugs with a high abuse potential can maintain control over their usage and still manage to be functional members of society. However, a certain percentage of individuals who try a substance become dependent on it, and their drug consumption transitions from casual use to abuse and addiction. Importantly, how this transition to addiction occurs remains unknown, and understanding the processes involved in the formation and persistence of drug abuse is critical to our ability to prevent and develop treatments for addiction.

### **1.1 Drug Abuse and Addiction**

Drug abuse is a serious issue in the United States, resulting in negative consequences not just for the individual, but for society as well. Estimates of the total overall costs of substance abuse in the United States, including productivity and health- and crime-related costs, exceed \$600 billion annually, with approximately \$181 billion for illicit drugs (ONDCP, 2004). More than half of the estimated costs of drug abuse are associated with drug-related crime. Twenty percent of crime-related costs were accounted for in lost productivity of victims and incarcerated perpetrators of drug-related crime. Cocaine addiction affects a significant percentage of Americans, with nearly 1.4 million Americans meeting the *DSM-IV* criteria for dependence or abuse of cocaine (in any form) in 2008 (Administration, 2009). These individuals continue to use cocaine even when it puts their lives in jeopardy. In 2009, almost one million emergency room visits involved an illicit drug, either alone or in combination with other types of drugs, and cocaine was responsible for nearly half of them (422,896) (Administration, 2010). Finally, cocaine addiction is a serious financial drain on our society and individuals. Between 1988 and 1995, Americans spent \$49 billion on illegal drugs, with the vast majority - \$31 billion - spent on cocaine, more than 4 times the amount spent on the next illegal drug (Rhodes et al.,

1995). It is evident that advancing our knowledge of cocaine addiction can have a significant effect on the cost of cocaine abuse to both individuals and to society, reducing the financial burden on our hospitals, penitentiaries, and tax payers.

The DSM IV defines substance dependence as a maladaptive pattern of substance use leading to clinically significant impairment or distress, often resulting in tolerance (a need for markedly increased amounts of the substance to achieve intoxication or the desired effect) and withdrawal. Critically, substance dependence results in a loss of control over drug-related behaviors, with abusers using more of the drug than intended, being incapable of abstaining despite a strong desire to stop using, or despite knowing that their use has resulted in severe negative consequences to themselves or others. In addition, the ability of the drug or its associated cues to motivate drug-seeking behavior may be enhanced, resulting in a great deal of effort spent to obtain and use the drug, increased drug usage, and sacrificing other priorities in deference to drug usage. In order to comprehend how drug abuse results in a loss of control of behavior and an aberration of motivation we must first understand normal reward processing.

## **1.2 Reward Learning and Incentive Motivation**

The question “what motivates us to perform an action?” has been the subject of philosophic debate for millennia, and numerous philosophical and psychological theories have been proposed to answer this question. Generally, it is believed that a reward or incentive will motivate behavior to obtain that incentive (i.e. incentive motivation), whereas a punishment or negative outcome will motivate behavior to avoid it. Thorndike’s Law of Effect (1911) suggests that the presentation of a reward shortly after the performance of an action strengthens or reinforces an association between the stimuli present when the response was performed and the response itself, allowing these stimuli to become capable of eliciting the response. Hull further refined this theory into the drive-reduction theory (Hull, 1943), being one of the first to try to account for how incentive processes influence habitual behavior. These theories do not



acknowledge that animals can learn about the consequences of their actions; instead, they theorize that it is the stimuli associated with the reward that elicit the consummatory response.

### *Reward-associated stimuli*

Since the famous experiment of Pavlov (Pavlov, 1927) it has been known that a neutral stimulus (the conditioned stimulus, CS) that is repeatedly paired with an outcome (the unconditioned stimulus, US) comes to elicit a behavioral response (conditioned response, CR) to either obtain or avoid the outcome or prepare for its delivery. The CS can be a discrete cue, such as a light or tone, or a more diffuse cue, such as the environment in which the conditioning occurs. The elicited response may either be directed towards the outcome, or towards the conditioned stimulus. CS-directed responses can interfere with the retrieval and consumption of the outcome (Breland and Breland, 1961, 1966; Timberlake et al., 1982), creating a response that must be suppressed if the reward is to be obtained. In addition, the CS might be activating behavior through a reflex (Pavlov, 1927; Sokolov, 1963) or habit mechanism ((Hull, 1930; Skinner, 1935)) or through an alteration in motivation (Rescorla and Solomon, 1967; Trapold and Overmier, 1972). Whatever the mechanism, stimuli paired with natural rewards (Pavlov, 1927; Rescorla, 1988; Pfaus et al., 2001) and drug rewards (Siegel, 1979; Hinson and Poulos, 1981; Poulos et al., 1981) are able to motivate behavior. In fact, even after the instrumental response to obtain the outcome has been extinguished by withholding the outcome after the response is performed, presentation of the cue can reinstate the outcome seeking behavior (Weiss et al., 2001; Shaham et al., 2003). Many have thought that this Pavlovian form of learning can explain all motivated behaviors (Thorndike, 1911; Hull, 1943; Donahoe et al., 1997), but studies have provided evidence that another form of learning, known as instrumental or operant learning, can also occur (Konorski, 1948).

### *Instrumental Actions*

There are two competing strategies for instrumental action selection. The first theory proposed that actions performed to acquire a reward are learned through association with a stimulus that predicts the reward, known as an S-R process. While this type of action selection strategy does occur, it cannot explain all instrumental actions. S-R processes are sensitive to only the contiguous pairing of action and reinforcer rather than to the causal relationship between these events. Since there is no understanding of a causal relationship between the action and the outcome, it would be impossible to evaluate different courses of action in terms of the relevance of the outcomes to current needs and motivational states (Balleine and Dickinson, 1998). In the next two sections, I describe evidence demonstrating that under normal conditions, animals are not always subject to these constraints, but that performance of instrumental behavior can fall into this S-R category when certain conditions are met.

### *Goal-Directed Actions*

We typically regard our actions as purposeful and explicitly selected and performed because of our knowledge of their beneficial consequences. In other words, we perform actions to obtain specific outcomes, motivated by the outcome's value (Tolman, 1932). These actions are called goal-directed, and the association formed is called action-outcome (A-O) encoding. It has been demonstrated that animals are sensitive to the causal relation between response and reward even when the contiguous pairings between them are kept constant, and that enhancing the probability of a reward in the absence of the response depresses instrumental performance (Hammond, 1980). Thus, animals are sensitive to the contingency between their action and the reward, which is counter to the predicted result if an S-R association was responsible for all instrumental behavior. Not only is instrumental behavior responsive to changes in the contingency between the action and the outcome, it is also sensitive to changes to the value of the outcome. Studies have shown that decreasing the value of an outcome by pairing it with

illness or allowing animals to consume it until satiety significantly decreases the performance of an action paired with that 'devalued' outcome, without affecting performance on a different action that has been paired with a different outcome (Adams and Dickinson, 1981; Colwill and Rescorla, 1985; Colwill, 1986; Balleine and Dickinson, 1998). This devaluation effect can also be demonstrated with opposing actions on the same manipulandum (Dickinson et al., 1996), refuting arguments that a Pavlovian stimulus-reward approach behavior could account for devaluation sensitivity. Thus, reward-motivated behavior is said to be goal-directed and sensitive to the value of the outcome and to the contingency between the action and the outcome. However, although there is considerable evidence of goal-directed behavior, the S-R process can also control action selection.

### *Habitual Actions*

Under normal conditions, animals perform actions in a goal-directed fashion, via an A-O association. However, under certain conditions, instrumental behavior can transition from A-O governed behavior to S-R governed behavior, in which environmental stimuli that have been associated with an action that obtains a reward elicit the instrumental response. When this occurs, action selection is said to be under habitual control. Rather than motivating behavior directly, the outcome reinforces the S-R relationship. These S-R habits are insensitive to the A-O contingency and to the capacity of the value of the outcome to alter behavior, as demonstrated through outcome devaluation procedures. When animals are tested on their instrumental responding for the devalued outcome, those behaving in a goal-directed fashion will decrease their responding, while those acting habitually will not (Adams, 1982; Dickinson et al., 1983; Dickinson, 1985). Interestingly, when tested later on their reacquisition of lever pressing, animals that are insensitive to devaluation under non-rewarded conditions will quickly decrease the response for the devalued outcome once they have experienced it in the devalued state (Dickinson, 1985). Thus, habit learning does not affect the ability to evaluate the value of

an outcome; rather, it prevents this knowledge from affecting outcome-seeking behavior. Habitual food seeking is usually achieved through extended instrumental training, well beyond what is required to achieve and maintain the predetermined response requirement (Adams, 1982; Dickinson, 1985). This has led to the suggestion that, with increasing amounts of training, the contribution of A-O associations to action selection decreases, while the contribution of S-R associations to action selection increases (Dickinson et al., 1995), creating a gradual transition from goal-directed to habitual behavior.

### *Pavlovian-to-instrumental transfer (PIT)*

PIT is a paradigm that allows us to investigate the ability of Pavlovian motivational processes to govern instrumental behavior. It has been shown that conditioned stimuli (CS) that have been imbued with incentive salience can enhance appetitively-motivated operant responding as measured by PIT (Robinson and Berridge, 2008). In this paradigm, a conditioned stimulus (an auditory or visual cue, CS+) is paired with an outcome over multiple sessions, while another stimulus (CS-) is paired with no outcome. This Pavlovian training is followed by instrumental training, in which a response (e.g., lever press) is reinforced with the same outcome. Importantly, the CS+ is not delivered during the instrumental training phase of the experiment, ensuring that subjects will not learn any direct associations between this cue and the instrumental response. When tested in the absence of outcome delivery, presentation of the CS+ (compared to the CS-) increases instrumental responding, demonstrating the incentive motivational properties of the independently trained cue on responding (Rescorla, 1994a). However, there is the potential, as mentioned previously, for a CS to invoke a CS-directed response that could compete with its ability to instigate the instrumental goal-motivated behavior (Breland and Breland, 1961, 1966; Timberlake et al., 1982). This response competition can be eliminated by slightly extinguishing the CS-US relationship (Holmes et al., 2010). The potential for response competition will be important for our findings in Chapter 2.

The PIT procedure has a number of advantages in determining the mechanism by which a cue enhances an instrumental behavior. The PIT procedure rules out other potential mechanisms by which the cue could be governing behavior, such as conditioned reinforcement, in which a stimulus reinforces a behavior after it has been associated with a primary reinforcer, or conditioned habits, in which the stimulus comes to evoke the response through a direct association with it. PIT does not produce conditioned response reinforcement because no contingency exists between the instrumental response and cue presentation. In addition, conditioned habits evoked by stimulus-response associations cannot facilitate responding because the instrumental response has never before been performed in the presence of the CS+. Thus, PIT provides a behavioral measure of Pavlovian incentive motivation. Interestingly, PIT has been shown to involve dopamine in the nucleus accumbens (NAcc) (Dickinson et al., 2000), which will be discussed in the next section.

### **1.3 Dopamine and Incentive Motivation**

There have been a multitude of studies linking dopamine (DA) signaling with reward and incentive motivation. Intracranial self-stimulation studies have found that regions rich in dopaminergic cell bodies produce strong self-stimulation, with rats pressing the lever frequently to electrically stimulate dopaminergic brain regions (Olds and Milner, 1954; Corbett and Wise, 1980; Wise, 1981), suggesting that stimulation of these dopaminergic regions is reinforcing. In line with these results, DA receptor antagonists (Franklin and McCoy, 1979; Mogenson et al., 1979) and lesions to dopaminergic regions (Olds, 1975; Strecker et al., 1982) reduce self-stimulation behavior, without altering motor functions. Recent research has clarified the role of DA in reward-motivated behaviors, which may vary with the temporal dynamics of release. Fast, phasic DA release has been shown to signal reward prediction errors, in which DA is released in response to outcome delivery and to stimuli in the environment that predict the reward (Schultz et al., 1997), whereas slower DA signaling is associated with motivated

behaviors more generally (Schultz, 2002) and incentive motivation (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Bassareo et al., 2002; Berridge, 2007). Though other neurotransmitter systems may also be involved (Koob, 1992; Bardo, 1998), and though DA can also signal dysphoric stimuli (Abercrombie et al., 1989; Saal et al., 2003), many reward-seeking behaviors for a variety of reinforcers have been demonstrated to depend on DA, including natural rewards and drugs of abuse (Wise and Rompre, 1989; Berridge and Robinson, 1998). I will examine the role of dopamine in food reward and drug reward in the following sections.

### *Dopamine and food reward*

Dopamine has been shown to be involved in the motivation to obtain food rewards, since DA receptor antagonists blunt food-seeking behavior in hungry animals without affecting motor behavior generally (Wise et al., 1978; Wise and Schwartz, 1981). Though DA is critically involved in processing the reinforcement of food rewards, the exact role of DA in food-motivated behavior has been the topic of much debate. Dopamine may reflect the value of the food, since DA levels increase in response to food, and the amount of increase is related to the incentive value of the food (Smith and Schneider, 1988; Martel and Fantino, 1996). However, DA does not seem to be involved in the palatability of food rewards or the value of actions that obtain food rewards. Studies have suggested that opioids are involved in processing food palatability (Berridge and Robinson, 1998; Berridge, 2009) and learning about the value of an action that produces food rewards (Wassum et al., 2009), while DA is involved in Pavlovian incentive learning (Wassum et al., 2011; Ostlund and Maidment, 2012), meaning that DA is important for evaluating the value of cues associated with food rewards. Ultimately, the DA that is released in response to rewards is likely involved in learning about stimuli that predict the delivery of the reward. It is this involvement of DA in reward learning and processing that may be coopted by drugs of abuse.

### *Dopamine and drug reward*

It is not surprising that psychostimulants such as amphetamine and cocaine can increase DA neurotransmission in the NAcc (Hernandez and Hoebel, 1988; White and Kalivas, 1998), since their primary mechanism of action is either blocking the dopamine transporter (cocaine) or increasing DA efflux directly (amphetamine). Interestingly, all major classes of drugs of abuse have been shown to result in an increase in DA transmission (Wise, 1984; Rowell et al., 1987; Di Chiara and Imperato, 1988; Carboni et al., 1989). It has also been found that drug-related cues can induce changes in DA release as well (Bradberry et al., 2000; Ito et al., 2000). It has been theorized that the shared ability of drugs of abuse and their associated cues to facilitate DA transmission is responsible for the reinforcing properties of the drug and drug-paired cues, such that the alterations induced in the DA system by drugs of abuse result in the pathological drug-seeking that ultimately culminates in addiction (Adinoff, 2004; Volkow et al., 2004b; Dalley and Everitt, 2009). To explain how experience with drugs produces the neural changes that result in addiction, several theories have been proposed.

#### **1.4 Theories of Drug Addiction**

Many theories have been proposed to address how drug use transitions into addiction, resulting in compulsive drug seeking, in which an addict continues to use out of a feeling of necessity, despite the possibility of severe negative consequences and a reduced enjoyment derived from taking the drug. Some have focused on the dysregulation of fronto-striatal brain regions induced by chronic drug use that results in exaggerated responses to drugs and drug-associated cues and impaired executive control (Jentsch and Taylor, 1999; Volkow and Fowler, 2000), with this reduced inhibitory control being responsible for compulsive drug taking. Others have theorized that chronic drug use decreases the hedonic response to the drug while recruiting stress-related circuitry, altering the normal reward setpoint and producing the “hedonic-allostatic” state (Koob and Le Moal, 2001), meaning that drug use continues in an

addicted fashion in order to quell anxiety and obtain a feeling of normalcy. There have been two other prominent theories proposed; the incentive sensitization theory (Robinson and Berridge, 1993) and the habit learning theory (Everitt et al., 2001). We have decided to focus on the incentive sensitization theory and the habit learning theory for a number of reasons. The incentive sensitization theory has garnered significant theoretical and empirical support and is considered to be amongst the more prominent theories of addiction. In addition, the habit learning theory has significant support in animal models, and may account for some aspects of addiction that are not as easily explained by the incentive sensitization theory. Finally, our lab has extensive experience with sophisticated behavioral techniques that we can employ in order to study these two theories and dissect their behavioral components and contributions to addiction.

### *Incentive Sensitization theory*

The central thesis of the incentive sensitization theory of addiction is that repeated exposure to addictive drugs can induce persistent alterations in neural systems (i.e. mesotelencephalic dopamine circuits) that regulate the attribution of incentive salience to stimuli and motivate behavior (Robinson and Berridge, 1993; Berridge and Robinson, 1998; Berridge, 2007). Thus, continued use of drugs of abuse produces neuroadaptations in this neural system, rendering it hypersensitive to drugs and drug-associated stimuli, leading to drug craving and relapse. Although a variety of experimental findings suggest that drug-associated cues can motivate drug seeking, as I argued above, most tests of cue-induced reinstatement do not provide a direct assay of the motivational properties of cues and their ability to provoke reward seeking. In order to study the invigorating effects of drug-paired cues on drug seeking behavior, the PIT paradigm can be used. Since drug administration should induce neuroadaptations that enhance the incentive salience of drug-related stimuli, one would predict that cues paired with drugs should potentiate drug-seeking actions and facilitate PIT. Although there have been



studies showing PIT with alcohol as the outcome (Glasner et al., 2005; Corbit and Janak, 2007), the PIT paradigm has not yet been applied to other drugs, so *I will explore how cocaine associated cues gain incentive salience and control reward-seeking behavior using the PIT paradigm.*

### *Habit learning theory*

As discussed previously, DA has been implicated in reward learning in general, and it is also involved in learning habitual behaviors in particular (Faure et al., 2005; Wickens et al., 2007), which has been suggested to contribute to compulsive drug seeking behavior (Wise, 2004; Belin and Everitt, 2008). The habit learning theory proposes that chronic drug exposure results in aberrant reward learning by pathologically subverting normal learning and memory systems. The alterations in these systems results in drug-related responses becoming ingrained and automatic, resulting in persistent drug-seeking behavior and leading to the establishment of compulsive drug-seeking habits (Tiffany, 1990; Robbins and Everitt, 1999; Everitt et al., 2001; Everitt and Robbins, 2005). As previously mentioned, habitual food seeking is usually achieved through extended instrumental training, well beyond what is required to achieve and maintain the predetermined response requirement. The habit learning theory suggests that psychostimulant pre-exposure can facilitate this process, leading to habitual behavior without extended training. Thus, repeated drug exposure produces a transition from goal-directed, A-O based actions to habitual, S-R based actions (Everitt and Robbins, 2005; Ostlund and Balleine, 2008; Pierce and Vanderschuren, 2010). Consequently, as drug consumption increases, the choice to seek out and use drugs should become increasingly dependent on environmental cues and, at the same time, relatively less sensitive to the undesirable consequences of this behavior. Studies have shown that rats will persist in cocaine seeking despite punishment (i.e. shock) (Vanderschuren and Everitt, 2004), or devaluation of orally administered cocaine that has been paired with flavored sucrose (Miles et al., 2003), or

after extinguishing the drug taking response (Zapata et al., 2010). Thus, drug seeking becomes a compulsive habit under the control of the S–R process, even in parallel with an apparent reduction in those subjective effects of the drug which initially established self-administration.

## **1.5 Methods and Attributes of Drug Administration**

### *The contingency of drug delivery*

Drug delivery can be either response-contingent (RC), where delivery of the drug is dependent on the action of the subject (self-administration), or non-contingent (NC), in which the drug is delivered irrespective of the actions of the subject. NC delivery can be achieved through experimenter administration, in which the drug is injected intraperitoneally (IP) or subcutaneously (SC), and also via a yoking procedure. With yoked drug delivery, the subject receives the drug intravenously (IV) based on the schedule of delivery produced by a self-administering subject (the ‘master’). Each time the master performs an action to receive the drug, the yoked subject will receive the drug at the same time but has no control over the delivery. This pharmacological control allows for the investigation of the effects of drug exposure independently from the volitional control or predictability that is associated with self-administration.

The majority of research on habit learning and incentive motivation has used experimenter delivered drugs, and this method of drug delivery has been shown to produce a range of significant effects. Experimenter-delivered drugs have been shown to produce increased motivation for drug reward (Vezina, 2004), incentive sensitization of cue wanting (Robinson and Berridge, 2000; Di Ciano, 2008), cognitive impairment (Schoenbaum and Shaham, 2008), and stronger S–R habits (Miles et al., 2003; Nelson and Killcross, 2006). However, there is growing evidence that self-administered cocaine produces greater glutamate and DA release than NC administration in brain regions involved in processing reward value and

motivation (Hemby et al., 1995; McFarland et al., 2003; Kimmel et al., 2005; Lecca et al., 2007). Furthermore, compared to NC delivery, RC delivery of cocaine through self-administration can produce an increase in dopamine D2 receptors and persistent long-term potentiation in the ventral tegmental area (VTA)(Stefański et al., 2007; Chen et al., 2008), which would likely upregulate the DA cells' response to eliciting events. While NC drug exposure might be sufficient to produce incentive motivation or induce habitual behavior, there is clearly a difference in the neuroadaptations associated with RC compared to NC cocaine, and this could have an important impact on the behavioral consequences of drug exposure. *The experiments conducted here will explore the behavioral effects of RC and NC cocaine exposure on motivated behavior.*

#### *Drug-exposure on natural reward-motivated behavior*

All drugs of abuse alter dopamine release and dopaminergic neural circuitry. Both the incentive sensitization theory and the habit learning theory propose that these alterations in the dopamine system, which is also involved in normal reward processing, result in the pathological drug-seeking that characterizes addiction. Interestingly, since the circuits sensitized by drugs of abuse are the same circuits that attribute incentive salience under normal circumstances, the incentive sensitization theory predicts that drug exposure could affect general motivation to obtain other drugs and natural rewards (Robinson and Berridge, 2008), which has received significant support (Taylor and Horger, 1999; Avena and Hoebel, 2003; Olausson et al., 2004). Some evidence for this cross-sensitization between drugs and food rewards has been shown using amphetamine pre-exposure for sucrose seeking (Wyvell and Berridge, 2001). This cross-sensitization would allow us to not only control the amount of cocaine delivered, but also investigate the effects of cocaine sensitization independently of the trained behavior with a food outcome, thereby studying how sensitization with cocaine alters motivation to obtain rewards.

Similarly for the habit learning theory, drug exposure is proposed to be subverting the normal, dopamine dependent reward learning processes to result in compulsive drug seeking. This alteration of reward learning induced by drug exposure can be generalized to other drugs of abuse and natural rewards, inducing habitual action selection for other rewards in situations that would otherwise induce goal-directed behavior. Consistent with this hypothesis, there has been evidence suggesting that alcohol (Ostlund et al., 2010) or amphetamine exposure (Nelson and Killcross, 2006; Nordquist et al., 2007) can make instrumental responding for food insensitive to devaluation. Once again, this ability of drugs to alter natural reward-motivated behavior will allow us to examine the effects of cocaine treatment on the development of habitual control. *The experiments I have done investigate the ability of cocaine exposure to either alter incentive motivation for food rewards or alter the behavioral control of food-motivated actions.*

## **1.6 Specific Aims**

One of the well-established characteristics of addiction is the ability of drug-paired cues to drive drug-seeking actions despite a desire to abstain, which may be due to the incentive motivation power of these cues. In the laboratory, this attribute of drug-paired cues has most often been studied using the cue-induced reinstatement task, but this paradigm allows for multiple explanations of how the cue might be motivating drug-seeking behavior. The PIT task is preferable to more clearly study the Pavlovian incentive properties of drug-associated cues on drug-seeking actions. Though PIT has been shown for food rewards and alcohol reinforcement, it has never been demonstrated with a psychostimulant as the reward. *To further elucidate the mechanisms by which drug-paired cues contribute to the addicted state, I will investigate the ability of cocaine-paired cues to affect cocaine-motivated actions using PIT.*

In order to explain the transition from drug use to addiction, several theories have been proposed that focus on different aspects of the addicted state. Of these theories, both the

incentive sensitization theory and the habit learning theory suggest that repeated exposure to drugs of abuse induces neuroadaptations within the dopaminergic circuitry, but they differ in their predictions of how these adaptations result in addictive behaviors. While prolonged drug exposure may affect the motivation to obtain the drug, drug exposure could also affect reward-motivated behavior in general via alterations to the dopaminergic system. *Here, I examine the ability of repeated cocaine exposure to alter food-motivated behavior while investigating both the incentive sensitization theory and the habit learning theory of addiction.*

The habit learning theory suggests that repeated exposure to drugs of abuse subverts the circuitry involved in normal reward processing, causing what would be goal-directed actions to become habitual in nature, later transitioning to compulsive drug-seeking. While there have been several studies demonstrating that drug exposure produces habitual behavior under conditions in which untreated animals are goal-directed, the learning mechanism by which this habitual behavior is produced has not been determined. Drug exposure could be increasing the speed of the transition from goal-directed to habitual control, or biasing drug-treated animals towards using a habitual strategy while learning, or impairing the ability of drug-treated animals to utilize a goal-directed strategy. *To further explore the habit learning theory, I examine the effects of cocaine exposure on goal-directed behavior.*

Finally, though numerous studies have investigated the impact of drug exposure on motivated behavior, the vast majority has used experimenter-delivered methods. While this mode of drug delivery clearly has significant effects, it has been shown that the method of drug delivery can make a difference in the effects of drug treatment. For example, recent studies have demonstrated that self-administered cocaine has a significantly greater impact on the dopamine system and neural excitability than passively infused cocaine, as noted above. However, there have yet to be any studies utilizing both self-administered and yoked cocaine that investigate the differential effect of mode of delivery on incentive motivation. *To determine whether volitional control or predictability of drug intake affects reward-motivated behavior, I*

*examine the effects of both self-administered and yoked cocaine exposure on incentive motivation.*

## **Chapter 2**

### **Pavlovian-to-Instrumental Transfer in Cocaine Seeking Rats**

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## **2.1 Introduction**

It has been well established that drug-associated cues can exert a potent source of control over drug seeking behavior, leading to relapse even after a prolonged period of abstinence (Heather and Greeley, 1990; Rohsenow et al., 1990). The cue-induced reinstatement paradigm is probably the most widely used method for animal studies targeting the excitatory influence of drug-paired cues on instrumental actions (Weiss et al., 2001; Shaham et al., 2003; Homberg et al., 2004; See, 2005). In such studies, response-contingent drug deliveries are typically accompanied by an extraneous cue. After significant training and extinction of the self-administration response in the absence of cue and drug delivery, the ability of the cue to reinstate the instrumental response is tested. This procedure typically generates a robust invigoration of drug seeking, and is commonly used to assess the impact of various treatments (e.g., brain lesion or inactivation, drug withdrawal period, pharmacological intervention) on cue-elicited drug seeking.

Despite the popularity of the cue-induced reinstatement paradigm, the behavioral processes that allow drug-paired cues to exert their influence over drug-seeking behavior have not been well characterized. It is generally assumed that this phenomenon depends on some form of interaction between Pavlovian and instrumental learning systems (Everitt et al., 2001; Berridge and Robinson, 2003; Everitt and Robbins, 2005; Weiss, 2005), with the cue acquiring its incentive motivational properties by virtue of its Pavlovian (stimulus-outcome) relationship with drug delivery. This account is supported by a large body of work showing that cues paired



with natural rewards, like food, can facilitate instrumental reward-seeking behavior (Rescorla, 1994a; Dickinson et al., 2000; Crombag et al., 2008a). However, the standard cue-induced reinstatement of drug seeking effect described above does not necessarily depend on a Pavlovian incentive motivational process. In such studies, the cue is paired with drug delivery in a response-contingent manner (e.g., response  $\rightarrow$  (cue + drug)) (Meil and See, 1997; Fuchs et al., 2004; Gál and Gyertyán, 2006; Lee et al., 2006; Cooper et al., 2007; Zavala et al., 2008; Kufahl et al., 2009) or is used as a discriminative stimulus to explicitly signal that a particular response is active (e.g., cue: response  $\rightarrow$  drug) (Bradberry et al., 2000; Weiss et al., 2001; Yun and Fields, 2003). Both procedures confound Pavlovian and instrumental contingencies. This is a major problem because the presence of the cue during instrumental training makes it possible for that stimulus to become directly associated with the drug-seeking action, providing alternative routes for response selection and elicitation. Furthermore, in many cases, cues are presented in a response-contingent manner at test, which does not distinguish their presumed ability to provoke and/or invigorate drug seeking actions from their ability to reinforce those actions through new learning (i.e., conditioned reinforcement)(Wyvell and Berridge, 2001).

The Pavlovian-to-Instrumental transfer (PIT) paradigm was developed specifically to target the incentive motivational effects of reward-paired cues on instrumental performance. In this case, the subject is given stimulus-reward and action-reward pairings in separate phases of the experiment in order to prevent associations from developing between the cue and instrumental action. The cue is then *noncontingently* presented during testing with the action available. Any increase in the performance of this action in the presence of the reward-paired cue must therefore be the result of the conditioned response-invigorating properties of that cue and not the product of a cue-response association or a conditioned reinforcement process. Despite its advantages over the conventional cue-induced reinstatement procedure, use of PIT has been largely restricted to studies of natural reward seeking actions, and the few studies that have used the PIT procedure to assay cue-elicited drug seeking have used orally administered

alcohol as the rewarding outcome (Glasner et al., 2005; Corbit and Janak, 2007; Milton et al., 2011). The reluctance to use this approach may stem from experienced or perceived difficulties in generating the PIT effect using conventional drug self-administration procedures (Kruzich et al., 2001). Indeed, Everitt and Robbins noted in their review (Everitt and Robbins, 2005) that:

...neither approach to a CS predictive of a drug, nor enhancement of drug seeking by the unexpected presentation of a drug-associated CS has been clearly demonstrated in laboratory studies of drug seeking or relapse, although both are readily seen in animals responding for natural rewards. It may be that the experimental conditions for demonstrating these phenomena in a drug seeking setting have not yet been optimized, but it may also be that the behavioral influence of CSs associated with drugs and natural reinforcers differ fundamentally in this regard (p.1482).

The current study aims to establish an effective PIT procedure for studying the effects of cocaine-paired cues on instrumental intravenous cocaine seeking actions in rats.

## **2.2 Methods**

### *Subjects*

Male Long Evans rats, weighing on average 337g before surgery, were housed singly in a climate-controlled vivarium and were tested during the light phase of the light/dark cycle (lights on from 7am to 7pm). Food and tap water were provided *ad libitum* in the home cage throughout behavioral training and testing. All procedures were approved by the UCLA Institutional Animal Care and Use Committee, and were performed in accordance with National Research Council's *Guide for the Care and Use of Laboratory Animals*. Five subjects were excluded from the experiment due to loss of catheter patency ( $N = 15$ ).

### *Apparatus and Training*

Rats were trained in eight identical Med Associates (East Fairfield, VT) operant chambers

housed within sound- and light-resistant shells. The chambers contained two retractable levers that could be positioned on left and right side of one end wall. A 3-W, 24-V houselight was mounted on the top center of the opposite end wall provided illumination. The chambers were also equipped with a tone generator and a clicker. Microcomputers equipped with the MED-PC program (Med Associates) controlled the equipment and recorded lever presses.

### *Drugs*

Cocaine hydrochloride (NIDA Drug Supply Program), dissolved in sterile saline (0.9% NaCl) and filtered-sterilized, was administered at 0.2mg/infusion over 4.35s using a Med Associates 100 pump during both instrumental and Pavlovian training.

### *Catheter surgery*

Rats were deeply anesthetized with isoflurane (4-5% induction, 1.5-2.5% maintenance), and a silicon catheter (O.D. 0.63mm x I.D. 0.30mm x wall 0.17mm, CamCaths, Cambridgeshire, England) was placed into the right or left jugular vein. The catheter was advanced approximately 35 mm caudally to the right atrium. The proximal end was attached to a coiled length of wider bore tubing that exited through a mount inserted under the skin between the scapulae. Rats were given 5 days to recover from surgery and catheters were maintained with twice daily heparin injections (0.1 ml of 10 units/ml) for the duration of the experiment. The antibiotic, sulfamexazole (TMS), was placed in the drinking water (0.05%) for the duration of the experiment. Catheter patency was evaluated twice daily, before and after each self-administration session, by checking for backflow of blood in the flushing syringe. Any catheter of questionable patency was tested by evaluating the sedative effectiveness of 0.2ml of 1% propofol. Any subject not sedated was excluded. Cocaine was self-administered through polyethylene tubing threaded through a spring tether that was connected to a liquid swivel attached to a balance arm, allowing the animals free range of motion.

### *Pavlovian training*

In the first conditioning session, which lasted approximately 2 hours, rats received 12 non-reinforced presentations of one of the two auditory stimuli (CS-; either a 3 kHz, 75dB tone or a 2Hz, 75dB click, 2-min duration) using a variable inter trial interval of 5 minutes (range: 3-7 minutes). In four daily subsequent 2-hour Pavlovian conditioning sessions, rats received 12 reinforced presentations of the alternate auditory stimulus (CS+), the onset of which signaled the delivery of a single infusion of cocaine (0.5 mg/kg/injection).

### *Instrumental training*

Pavlovian training was followed by 10-12 days of instrumental training, during which the rats were allowed to self-administer cocaine by performing a two-action, seeking-taking chain (Balleine, 1995; Olmstead et al., 2000; Corbit and Balleine, 2003; Vanderschuren and Everitt, 2004). This behavioral paradigm requires the animal to press an initial, distal, lever to gain access to a second, proximal, lever, an action on which delivers the reward, and is designed to isolate the processes that control drug 'seeking' actions from those controlling drug 'taking' actions. For the current study, we used a modified version of the seeking-taking chain procedure employed by Corbit and Balleine (2003) to examine the effects of reward-paired cues on *food* seeking and taking. Rats initially received continuous reinforcement training with only one lever present (either the left or right lever), the 'taking' action. Sessions lasted until 20 outcomes had been earned, or until 2 hours had elapsed. Having reached criterion on the taking lever (earning 20 outcomes for two consecutive days) rats were trained on the full seeking-taking chain. The alternate lever, which served as the cocaine 'seeking' action, was inserted into the chamber at the beginning of each of these training sessions. A single press on this lever resulted in the insertion of the taking lever. Performing the taking response resulted in delivery of a cocaine infusion and immediate retraction of both the seeking and taking levers, followed by

a 20-sec time out period. The seeking lever was then reinserted into the chamber, signaling that the seeking-taking contingency was once again active. As during taking lever training, these sessions were terminated after 20 outcomes had been earned or 2 hours had elapsed. Both components of the chain (the seeking-taking contingency and the taking-cocaine delivery contingency) were continuously reinforced for the first 2 sessions. The reinforcement schedule for each component was then shifted to random ratio (RR)-2 for 2 sessions, during which each response was reinforced with a probability of 0.5. The schedule was then shifted to RR-4 ( $p = 0.25$ ) for both levers for the remainder of training until stable lever pressing was obtained (20 outcomes in 2 hours over 2 consecutive days). This ratio schedule further distinguishes between the seeking and taking components of the chain by weakening the temporal contiguity between the seeking action and the outcome delivery. We used the same reinforcement schedule on both levers to encourage the development of similar robust and persistent levels of responding on the seeking and taking levers, which should facilitate detection of the PIT effect (Corbit and Balleine, 2003). Importantly, no cues were used to signal cocaine infusions during instrumental training sessions.

#### *Pavlovian-to-instrumental transfer testing*

After the self-administration criterion was reached, the rats underwent the first of two PIT tests to assess the impact of the cocaine-predictive cues on their performance of the seeking-taking chain. PIT studies using food reward have shown that the expression of this effect is particularly sensitive to the conditions present at test (Holmes et al., 2010). The response-outcome contingency at test appears to be a particularly important factor; the transfer effect tends to be considerably more robust when subjects are tested in extinction (i.e., in the absence of response-contingent reward). Therefore, in the first test, separate groups of subjects were tested under extinction or rewarded conditions to determine if this factor influences the expression of PIT on a cocaine seeking-taking chain of actions.

To lower response rates and thereby facilitate detection of the excitatory effects of the CS+, both groups began the test with 5 minutes of extinction. This was directly followed by 4 non-contingent trials in which two auditory cues (CS+ and CS-) were strictly alternated (tone, click), with each CS period being followed by an ITI. For the rewarded group ( $n = 8$ ), the seeking component of the chain was in place throughout the test, such that responding on the seeking lever resulted in insertion of the taking lever according to a RR-4 schedule. Responding on the taking lever resulted in retraction of that lever according to a RR-4 schedule, but did not result in cocaine delivery during the extinction period of the test. Following the extinction period, both components of the chain were fully intact, allowing rats to earn cocaine on a RR-4 schedule. This phase of the test was nearly identical to instrumental training sessions except for presentations of CS+ and CS-. To encourage low pre-CS response rates, scheduled cue deliveries were delayed until rats withheld responding on either lever for a period of at least 60 seconds, without any relationship to the cocaine delivery itself. This ensured an ITI of at least one minute. For the extinction group ( $n = 7$ ), we were particularly interested in comparing the influence of the cues across the seeking and taking levers. Therefore, in this condition, rats were given access to both levers for the duration of the transfer test in the absence of either the seeking → taking or taking → cocaine contingency. Cue presentations were separated by an ITI of 2 min. All lever presses were recorded during this session but no reinforcement was delivered. All rats (from both groups) were subsequently given three daily Pavlovian CS+ extinction sessions, which consisted of 12 CS+ presentations in the absence of outcome delivery, with an average ITI of three minutes (range 1-5 min), followed by an instrumental retraining session. They were then administered a second test performed under extinction conditions with both levers available throughout. Cue presentations began 10 minutes after the start of the session, following the procedure of the extinction group in the first test, but with a fixed 6-minute ITI to minimize the carryover of post-cue responding into the next pre-cue period.

### *Data analysis and statistics*

To assess the influence of the cues on lever press performance, we subtracted the number of lever presses occurring in the minute before the cue onset (i.e., Pre-CS baseline) from the number of lever presses performed during each of the next three minutes, which included the two-minute CS delivery and a one-minute post-CS period. We included the first minute of post-CS responding in this analysis because previous studies have shown that the excitatory impact of reward-paired cues on lever pressing can persist beyond the initial CS delivery period (Lovibond, 1983). For Test 1, statistical analysis of these data was conducted using a mixed ANOVA with CS (CS+ vs. CS-), action (Seeking vs. Taking) and period (CS minute 1, CS minute 2, post-CS minute 1) serving as within-subjects factors and group (rewarded or extinction test conditions) serving as a between-subjects factor. Test 2 was analyzed with the same mixed ANOVA, except with no between-subjects factor.

### **2.3 Results**

Although there was no direct measure of learning for Pavlovian conditioning, all rats displayed characteristic behavioral effects of cocaine administration (e.g., increased locomotor behavior, rearing, stereotypy) after each training session. They were then trained to self-administer cocaine by performing a seeking-taking chain of actions. Over the course of training, rats showed a significant increase in both seeking and taking lever presses, with significantly more lever pressing on the seeking lever (see Figure 2-1). A repeated-measures ANOVA detected a significant main effect of day ( $F(1,14) = 35.284, p < 0.001$ ), action ( $F(1,14) = 31.438, p < 0.001$ ) and a significant interaction of day and action ( $F(1,14) = 5.601, p < 0.05$ ). The difference in rate across actions is likely due to persistent responding on the seeking lever, which remained in the chamber during periods when the second (taking) component of the chain was active.

After Pavlovian and instrumental training, we tested the tendency for cocaine-paired cues to motivate lever-pressing behavior in the absence or presence of response-contingent cocaine deliveries. Pre-CS baselines are reported in Table 2-1. Surprisingly, a mixed ANOVA conducted on the pre-CS baselines revealed a significant main effect of CS ( $F(1,13) = 6.19, p < 0.05$ ), and a significant action by group interaction ( $F(1,13) = 5.89, p < 0.05$ ), with no other significant main effects or interactions (largest F value:  $F(1,13) = 4.26, p > 0.05$ ). These results indicate higher baselines for the CS- than the CS+, and more responding in the baseline period on the taking lever for the extinction group. Since these cues were delivered in strict alternation, the relatively elevated baseline response rate going into CS- trials may reflect carry over of the excitatory influence of recent CS+ deliveries.

The results of the first PIT test are presented in Figure 2-2. Although the findings from this initial round of testing were not particularly clear, some features of these data are worth noting. First, it appears that rats tested in extinction show a slight increase in lever pressing during cue presentations, an effect that was at least numerically greater during CS+ trials and was most prominent once the cues were terminated. Second, for rats that were rewarded with cocaine at test, there was little indication that the CS+ was capable of invigorating cocaine seeking or taking behavior. An ANOVA performed on these data found no effect of CS ( $F(1,13) = 1.958, p > 0.05$ ) or action ( $F(1,13) = 1.396, p > 0.05$ ), nor was there a significant interaction between these variables ( $F(1,13) = 0.177, p > 0.05$ ), or between these variables and test group ( $F = 0.03, p > 0.05$ ). However, though not specific to action or CS, there was a significant effect of period ( $F(2,26) = 4.907, p < 0.05$ ) and a period by group interaction ( $F(2,26) = 4.365, p < 0.05$ ), indicating that the groups differed in the rate at which they lever pressed during and immediately after the cue presentations. To explore this effect further we conducted separate CS x Action x Period ANOVAs for each test group. Both groups demonstrated a significant main effect of period ( $F(2,12) = 4.23, p < 0.05$  – extinction group,  $F(2,14) = 3.90, p < 0.05$  –



rewarded group), with no other significant main effect or interactions (largest  $F$  value:  $F(2,14)=2.741, p > 0.05$ ).

In light of these results, we conducted a second test using procedures likely to facilitate the expression of PIT. First, given the clear lack of effect in the rewarded group, all rats were tested under extinction conditions in Test 2. Second, we increased the initial extinction period from 5 to 10 minutes to further suppress pre-CS response rates and avoid a potential behavioral “ceiling” or upper limit on responding. Third, we increased the interval between trials to 6 minutes to minimize carryover effects. Fourth, we gave rats 3 sessions of extinction of the CS+ before Test 2. Though counterintuitive, recent evidence suggests that such extinction of the CS+ can enhance the PIT effect, presumably by weakening competing conditioned responses (Holmes et al., 2010).

Pre-CS baselines for Test 2 are reported in Table 2-2. There was a significant main effect of action ( $F(1,13) = 7.05, p < 0.05$ ), but no other significant effects (largest  $F$  value:  $F(1,13) = 2.17, p > 0.05$ ), reflecting more pre-CS responding on the taking lever than the seeking lever. Figure 2-3 presents the results of the Test 2. As is clear from these data, rats displayed a stimulus-specific increase in lever pressing to the CS+, a pattern indicative of PIT. A repeated-measures ANOVA showed a significant main effect of CS ( $F(1,13) = 6.752, p < 0.05$ ), indicating that the enhancement in lever pressing was greater for the CS+ than for the CS-. There was no effect of action ( $F(1,13) = 0.324, p > 0.05$ ) or period ( $F(1,13) = 0.208, p > 0.05$ ), nor were there any significant interactions between any of these factors (largest  $F$  value:  $F(1,13) = 2.25, p > 0.05$ ). Although these analyses failed to identify action-specificity in the PIT effect, inspection of the data in Figure 2-3 suggests that the CS+ produced a more pronounced and longer-lasting enhancement in performance of the taking action than of the seeking action, consistent with previous food PIT studies (Corbit and Balleine, 2003). Indeed, when we confined our analysis to one or the other action, we found a significant effect of CS for the taking lever ( $F(1,13) = 5.795, p < 0.05$ ), but found no such effect for the seeking lever ( $F(1,13) = 1.68, p > 0.05$ ).

In view of the trend towards a difference in the degree of transfer on the seeking and taking levers, an additional analysis of transfer test performance was conducted to determine if the rats performed the chain task during the test as trained, i.e. shifting from the seeking lever to the taking lever rather than moving in the opposite direction. To quantify these shifts in performance we followed Balleine and colleagues' example (Corbit and Balleine, 2003) in assessing the probability that the taking lever action was performed in each of the 10 seconds that followed performance of the seeking action, and generated a similar probability distribution for seeking presses that occurred in the 10 seconds that followed each taking action. These data, which are shown in Figure 2-4, clearly demonstrate that the rats were more likely to shift from seeking-to-taking, which was the order of actions reinforced by cocaine delivery, than from taking-to-seeking. This shift in performance appeared to be most frequent during approximately the first five seconds in the post-seeking period. A repeated measures ANOVA using action order (Seek-Take vs. Take-Seek) and time bin (1-10) as factors confirmed this analysis, revealing a significant main effect of action order ( $F(1,14) = 22.121, p < 0.001$ ), a significant main effect of bin ( $F(9,126) = 8.176, p < 0.001$ ), and a significant interaction between action order and bin ( $F(9,126) = 8.029, p < 0.001$ ).

## **2.4 Discussion**

To our knowledge, this study represents the first demonstration that environmental cues paired with intravenous cocaine administration acquire the ability to provoke and invigorate cocaine self-administration in rats specifically through a Pavlovian-to-instrumental transfer process. As such, the data support the hypothesis that drug-paired cues can invigorate drug-related activities by inducing a state of incentive motivation or "craving".

The vast majority of studies examining the influence of drug-paired cues on drug-seeking behavior have used the cue-induced reinstatement paradigm. (Weiss et al., 2001; Shaham et al., 2003; Homberg et al., 2004). However, as noted in the Introduction, this

approach confounds a number of distinct action selection strategies that may contribute to drug relapse. For instance, the cue may enter into a direct association with the self-administration response, allowing a habit to control reinstatement performance (Balleine and Ostlund, 2007; Ostlund and Balleine, 2008). By exposing subjects to the cue-drug and action-drug relationships in separate training phases, the PIT procedure makes it possible to isolate the influence of Pavlovian learning on drug self-administration, a behavioral process that has been assigned a fundamental role in mediating incentive motivation (Dickinson et al., 2000; Berridge and Robinson, 2003) and compulsive drug-seeking behavior (Robbins and Everitt, 2002). Furthermore, since the PIT effect is elicited by unexpected (response-independent) presentations of a reward-paired cue, it cannot be explained by that stimulus's ability to increase behavior through conditioned reinforcement, unlike certain versions of the reinstatement procedure (Kruzich et al., 2001).

Our findings also shed light on some of the factors controlling the expression of drug-motivated PIT. For instance, in Test 1 rats that were given response-contingent cocaine reward at test failed to show any evidence of response invigoration during presentations of the cocaine-paired cue, indicating not only that rewarded conditions are not necessary to produce PIT, but that receiving the drug at test may in fact disrupt the expression of this effect, consistent with similar studies using food self-administration tasks (Azrin and Hake, 1969; Lovibond, 1981, 1983; Rescorla, 1994a; Dickinson et al., 2000). It is possible that the lack of cue-elicited responding in the rewarded group was due to the rats' tendency to control their drug intake. For instance, rats tend to self-administer cocaine to maintain a preferred level of drug in their bloodstream (Tsibulsky and Norman, 1999, 2001; Suto and Wise, 2011). Thus, it is possible that a short-term satiety for cocaine was responsible for attenuating the PIT effect. Indeed, studies using food-motivated PIT have established that this effect can be abolished by satiating rats on food prior to the test session (Balleine, 1994; Corbit et al., 2007).

As with food-motivated PIT studies, we found that testing rats in extinction facilitated the expression of cue-evoked behavior. For instance, in Test 1, the rats tested in extinction showed a significant increase in responding during and immediately after CS+ presentations. Surprisingly, however, these rats also showed a somewhat similar increase in responding to the CS-, a stimulus that was never paired with cocaine. This pattern of results could reflect a nonspecific effect of these stimulus presentations (e.g., disinhibition or arousal) (Brimer, 1970), or it could have resulted from a cue discrimination impairment, perhaps brought about by the repeated administration of cocaine. However, we chose to test an alternative hypothesis: that expression of PIT to the CS+ was at least partially being masked by that cue's tendency to evoke incompatible conditioned responses, including locomotor activity (Ma et al., 2010). For food motivated tasks, it is known that response competition between conditioned orienting and approach behaviors can interfere with the expression of PIT (Overmier et al., 1979; Baxter and Zamble, 1980; Lovibond, 1983; Delamater and Oakeshott, 2007), and that extinguishing the CS+ will eliminate the competing response and allow the full excitatory impact of that cue to emerge (Holmes et al., 2010) without eliminating the transfer effect (Delamater, 1996). Therefore, we extinguished the CS+ over three sessions before giving the rats a second PIT test. Consistent with the response competition account, we found that rats selectively increased their rate of lever pressing to the CS+ during this round of testing, relative to baseline periods and CS- trials.

Although the CS extinction procedure is likely to have played a role in facilitating the expression of PIT, procedural differences between the two tests may have also contributed to our ability to detect a significant, CS+ specific PIT effect in Test 2. First, the initial extinction phase of the test was increased from 5 to 10 minutes to further suppress baseline response rates during Test 2 in an attempt to avoid a potential "ceiling effect" that may have countered cue-induced increases in responding during Test 1. It should be noted, however, that a significant (albeit nonspecific) elevation in responding was detected in Test 1, indicating that an

absolute upper limit on lever pressing did not prevent rats from increasing their rate of responding following the cue deliveries. We also lengthened the ITI to minimize carryover of cue-evoked behavior into the baseline period of subsequent trials and further suppress baseline responding.

Another important factor controlling the expression of PIT appears to be the position of the target action in the chain of events leading up to reward delivery. The current study used a seeking-taking chain designed to distinguish between those actions required to seek out or pursue cocaine and those involved in cocaine taking or consumption. Previous studies using a food-rewarded seeking-taking task have established that food-paired cues have a greater influence over the performance of the taking response (Balleine, 1995; Corbit and Balleine, 2003). Based on such findings, it has been argued that distinct motivational processes control these two types of actions; whereas reward seeking is guided by value estimates for specific behavioral (instrumental) goals, reward taking is dependent on Pavlovian incentive motivation generated by environmental cues (Balleine, 1995; Corbit and Balleine, 2003). We also found some evidence of taking-specific PIT in the second test session, suggesting symmetry between drug- and food-motivated PIT, and indicating that a fundamental feature of reward-paired cues is their ability to motivate actions that are associated with imminent reward delivery or consumption. However, this should not be taken as evidence that reward-paired cues have no effect on reward-*seeking* behavior. Just as in the previous reports that PIT is specific to food taking behavior, the current study found a stronger PIT effect on the taking lever in a test in which both levers were continuously available. During training, this situation predicted that the taking lever was active, making the reward-seeking action obsolete. So it is possible that the cocaine-paired cue would have had a stronger impact on cocaine seeking if that action were tested in isolation. However, it should also be noted that although the taking lever was, in this sense, more predictive of reward delivery, our rats distributed their actions across the two levers at test just as if the full chain contingency was in effect, performing the taking action shortly after

they performed the seeking action, but not the other way around. Therefore their behavior at test was not confined to a simple strategy of focusing their performance on the taking lever.

This study confirms that PIT can be generated in rodents performing a cocaine self-administration task. Studies of food-motivated PIT have established that dopamine signaling plays a particularly important role in mediating the response-invigorating effects of reward-paired cues (Dickinson et al., 2000; Lex and Hauber, 2008; Wassum et al., 2011; Ostlund and Maidment, 2012). Furthermore, studies using the PIT paradigm have shown that repeated psychostimulant sensitization could potentiate the cue-evoked food seeking behavior (Wyvell and Berridge, 2001; Saddoris et al., 2011), providing support for the incentive sensitization theory of addiction (Robinson and Berridge, 1993, 2000; Robinson and Berridge, 2001; Robinson and Berridge, 2008). It will be of interest to see if dopamine plays a similar role in cocaine-motivated PIT and whether this phenomenon can be modulated by treatments that sensitize the dopamine system, like repeated drug pre-exposure. Future studies should also examine whether other drugs of abuse, such as opioids or nicotine, can support PIT. Establishing these effects will make it possible to advance our understanding of the behavioral and neural processes underlying cue-motivated drug-seeking behavior.

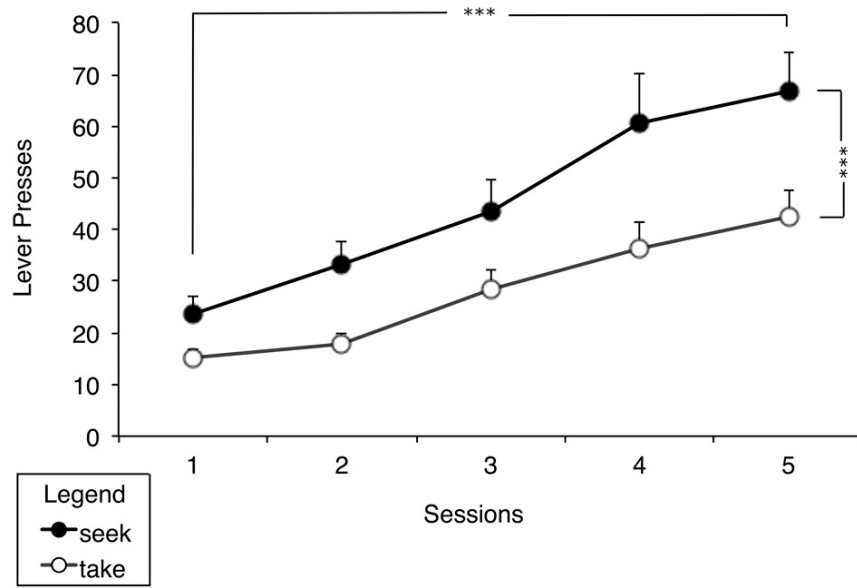
Group	Lever	CS+	CS-
Extinction Group	Seeking lever	1.43±0.69	3.14±0.83
	Taking lever	3.71± 2.17	8.57±2.99
Rewarded Group	Seeking lever	1.75±1.08	2.63±0.89
	Taking lever	0.625±0.38	3.13±1.37

**Table 2-1. Test 1 Pre-CS baselines and extinction responding.** CS = conditioned stimulus. Values are mean ± SEM.

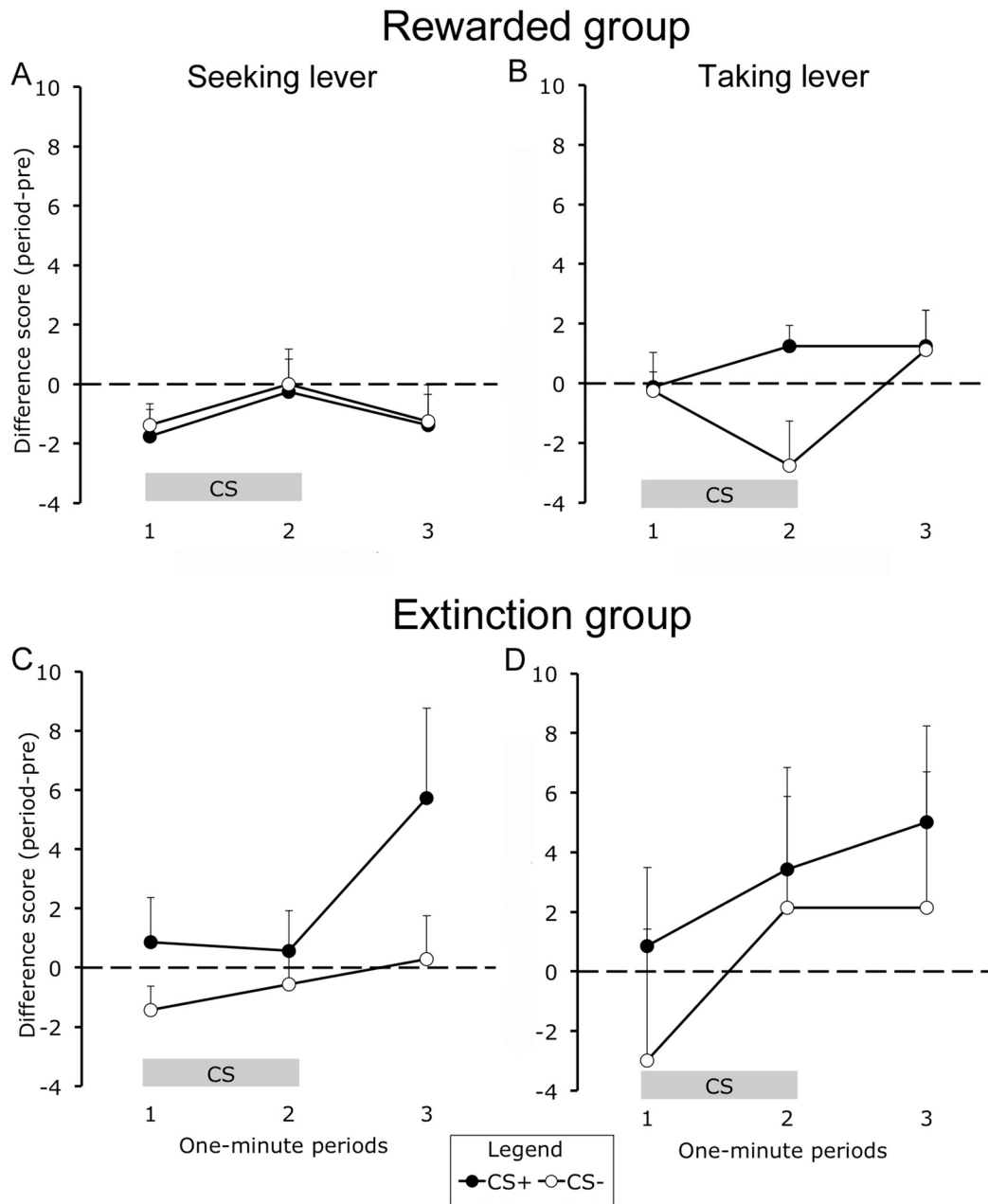
Lever	CS+	CS-
Seeking lever	0.43±0.29	0.29±0.13
Taking lever	1.14±0.43	1.86±0.53

**Table 2-2. Test 2 Pre-CS baselines.** CS = conditioned stimulus. Values are mean ± SEM.

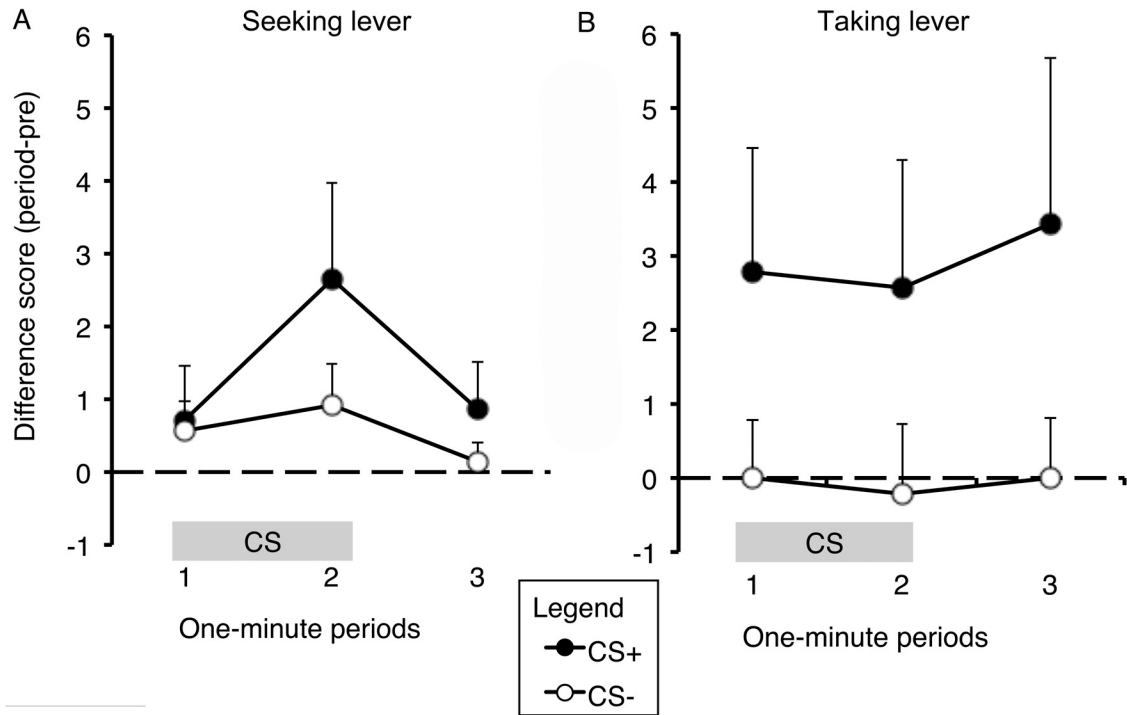




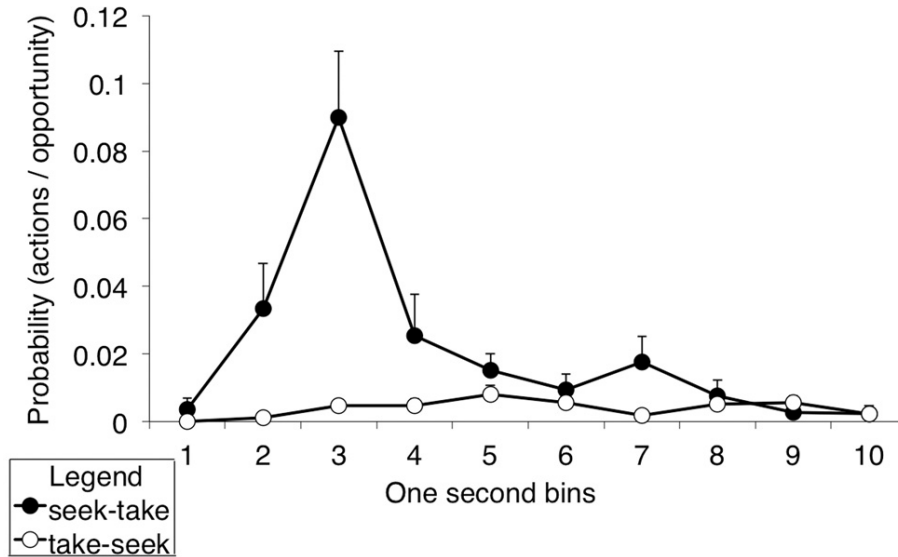
**Figure 2-1. Acquisition of the seeking-taking chain, shown as average lever presses over the session for the last five sessions of training. Means + SEM. \*\*\*  $p < 0.001$ .**



**Figure 2-2. Results of the first Pavlovian-to-instrumental transfer test.** Difference scores for each minute of the CS and the first minute of the post-CS period, displayed separately for action (seeking or taking) and group (extinction or rewarded). A-B, results for the rewarded group for the seeking lever (A) and taking lever (B). C-D, results for the extinction group for the seeking lever (C) and taking lever (D). Means + SEM.



**Figure 2-3. Results of the second Pavlovian-to-instrumental transfer test.** Difference scores for each minute of the CS and the first minute of the post-CS period, separately plotted for seeking (A) and taking (B) levers. Means + SEM.



**Figure 2-4. Probability of transitioning from the seeking to the taking lever vs. transitioning from the taking to the seeking lever.** Probabilities are calculated by dividing the total number of transitions in each 1-second bin by the total number of 1<sup>st</sup> action lever presses in the session. A transition was operationally defined as the first response on the 2<sup>nd</sup> action lever within 10 seconds after a response on the 1<sup>st</sup> action lever. Means + SEM.

### **Chapter 3**

## **Repeated Cocaine Exposure Facilitates the Expression of Incentive Motivation and Induces Habit Formation**

### 3.1 Introduction

Many theories have been proposed concerning the mechanism by which recreational drug use transitions to addiction, a state characterized by the compulsive pursuit of drugs despite the severe negative consequences of this behavior. The incentive sensitization theory argues that extended drug use sensitizes the neural circuitry involved in assigning incentive salience to drug-paired cues, allowing these cues to exert greater control over drug-seeking behavior (Robinson and Berridge, 1993, 2008). Virtually all addictive drugs stimulate, and typically sensitize, the dopamine system, a central mediator of incentive motivation (Ikemoto and Panksepp, 1999; Dickinson et al., 2000; Berridge and Robinson, 2003; Lex and Hauber, 2008; Ostlund et al., 2011; Wassum et al., 2011; Ostlund and Maidment, 2012). It is proposed that drug-induced adaptations in the dopamine system render it hypersensitive to drugs and their associated cues, allowing these stimuli to elicit intense drug craving and trigger drug seeking.

In contrast, the habit theory of addiction posits that chronic drug use causes a transition in the systems controlling drug-seeking behavior (Tiffany, 1990; Berke and Hyman, 2000; Everitt and Robbins, 2005; Hyman et al., 2006; Everitt et al., 2008). This account is based on research showing that rodents performing an instrumental action (e.g., pressing a lever) for food reward rely on two competing strategies: a goal-directed (or action-outcome) strategy that involves considering the consequences of potential actions, and a habitual (or stimulus-response) strategy that involves reacting – without deliberation – to prevailing stimuli that have acquired the ability to trigger certain actions (Balleine and Dickinson, 1998). Importantly, the control of actions shifts over the course of training, with the goal-directed control dominating early in training and habitual control taking over as the action becomes firmly established. The dopamine system is known to play a critical role in habit formation (Bayer and Glimcher, 2005; Faure et al., 2005; Wickens et al., 2007; Wang et al., 2011), and there is evidence that dopamine release reports errors in reward prediction (Bayer and Glimcher, 2005; Wassum et

al., 2012), which are assumed to serve as teaching signals for habit learning according to model-free theories of reinforcement learning (Barto, 1992; Montague et al., 1996; Daw et al., 2005). Drug-induced sensitization of this dopamine-mediated reinforcement process may therefore facilitate the habitualization of drug seeking.

Thus, the incentive sensitization and habit theories of addiction share a common mechanism: heightened responsiveness in the dopamine system. While attempting to explain the compulsive nature of *drug-seeking* behavior, there is considerable evidence that repeated exposure to drugs can have long-lasting effects on the control of actions motivated by natural rewards like food (Wyvell and Berridge, 2001; Nelson and Killcross, 2006; Nordquist et al., 2007; Ranaldi et al., 2009; Ostlund et al., 2010; Shiflett, 2012). For instance, previous studies have shown that rats given repeated exposure to the psychostimulant amphetamine show a heightened sensitivity to the incentive motivational effects of food-paired cues (Wyvell and Berridge, 2001) and exhibit accelerated habit learning (Nelson and Killcross, 2006; Nordquist et al., 2007). However, the impact of cocaine, another widely abused psychostimulant, on these phenomena has not been as well characterized. While both cocaine and amphetamine are known to cause persistent changes in behavior and neurotransmission, they have distinct modes of action on dopamine signaling and appear to engage and induce adaptations in nonoverlapping components of circuitry underlying learning and motivation (Pierce and Kalivas, 1997; White and Kalivas, 1998), raising questions about cocaine's ability to dysregulate these behavioral processes. In this study, we investigated whether rats repeatedly exposed to cocaine exhibit either enhanced incentive motivation for food reward or a bias towards habitual control.

### **3.2 Methods**

#### *Subjects and apparatus*

Male Long Evans rats (mean weight:  $330 \pm 10.13$ g) were used as subjects. Rats were group housed in a climate-controlled vivarium and were tested during the light phase of the light/dark

cycle (lights on from 7am to 7pm). Rats had *ad libitum* access to tap water throughout the study and were food deprived (10-14g of chow per day) to maintain them at ~85% their free-feeding body weight. All procedures were approved by the UCLA Institutional Animal Care and Use Committee, and were performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Rats were trained in 8 identical Med Associates (East Fairfield, VT) operant chambers housed within sound- and light-resistant shells. The chambers contained two retractable levers that could be inserted to the left and right side of a recessed food cup on one end wall. A 3-W, 24-V houselight mounted on the top center of the opposite end wall provided illumination. The chambers were also equipped with a tone generator and a clicker. .

### *Drugs*

Cocaine hydrochloride, provided by the National Institute on Drug Abuse Drug Supply Program, was dissolved in sterile saline (0.9% NaCl) and filter-sterilized prior to injection.

### *Instrumental Training*

Rats were first given two magazine training sessions in which they received 20 grain-based food pellets (45mg, Bioserv, Frenchtown, NJ) on a fixed time 1-min schedule. This was followed by 14 d of instrumental training, consisting of 30-min sessions with constant access to an active and inactive lever. Pressing on the active lever (left or right; counterbalanced with Pavlovian training and cocaine exposure conditions) resulted in the delivery of grain pellets, while pressing on the inactive lever was without consequence. The schedule of reinforcement used for the active lever progressed through consecutive days of continuous reinforcement, random interval (RI) 5s, RI-15s, RI-30s, followed by 10 d of RI-45s. Two subjects failed to discriminate between the two levers (< 90% total presses on the active lever during the last day) and were excluded from the rest of the study.



### *Pavlovian Training*

Rats were then given 14 daily 30-min sessions of Pavlovian training. During the first 11 sessions, the presentation of one of two auditory stimuli (CS+; either a 3 kHz, 75dB tone or a 2Hz, 75dB click, 30-s duration) was followed by delivery of 3 grain pellets at the offset of the cue; 10 CS+ presentations were delivered on a variable time 2-min schedule. The last 3 sessions were the same as the first 11 sessions, but with the addition of two non-reinforced presentations of the alternative auditory stimulus (CS-) during the middle and end of the sessions. Magazine entries were recorded to monitor acquisition of conditioned approach behavior.

### *Cocaine sensitization*

Rats were divided into two groups: a cocaine exposure group (n=11) and a saline exposure group (n=13) receiving 6 once-daily intraperitoneal (IP) injections of 15mg/kg cocaine HCl or saline (1 ml/kg), respectively, before being placed in the behavioral chambers for 45 min and subsequently returned to their home cages. Subjects were then abstinent for 10 d during which they remained in their homecages. The sensitization protocol was based on an earlier study finding sensitization of cue-evoked reward seeking in rats given repeated amphetamine injections (Wyvell and Berridge, 2001). For cocaine, similar dosage, duration, and abstinence parameters have been shown to support other forms of behavioral sensitization (Mayfield et al., 1992; Kalivas and Duffy, 1993; Sorg et al., 1993).

### *Pavlovian-to-instrumental transfer (PIT) testing*

Subjects were retrained for 3 d on the instrumental response on a RI-45s schedule. On the following day, rats received a 30-min extinction session in which both levers were available but produced no rewards. During the PIT test, both levers were extended into the chamber and

were retracted at the end of the test. All lever presses were recorded during this session but no rewards were delivered. The two auditory cues (CS+ and CS-) were non-contingently presented 4 times each in alternation (tone, click) to assess their ability to influence lever press performance. The number of presses performed on each lever during the min before the cue onset was used as the Pre-CS, or baseline, response rate. The magazine entry detector in one of the operant chambers was not functioning properly, and the data for two vehicle subjects was lost.

### *Instrumental Retraining*

Following the PIT test, subjects were retrained on the previously inactive lever for a new outcome (sucrose or chocolate purified pellet, 45mg, Bioserv) over four daily sessions, each ending after 30 min or once 30 pellets had been earned, whichever came first. Lever pressing was reinforced on a continuous schedule on day 1 and an RI-30s schedule thereafter. To ensure equal exposure to the other (control) pellet type, rats were allowed to consume 30 of these pellets (presented in a stainless steel cup) in an alternative context similar to their homecages either 30-min before or immediately after each instrumental training session, alternating over days.

### *Devaluation test*

We used a specific satiety outcome devaluation procedure to assess the rats' ability to adjust their lever pressing according to a change in outcome value. Subjects were given unlimited access (>30g) to either the pellet used to reinforce lever pressing (for the devalued test) or the other pellet (for the nondevalued test) in an alternative context for 1h before each test. Test sessions began with 5 min of extinction during which the lever was available but did not produce reward. This was immediately followed by a 25-min rewarded phase during which lever pressing was reinforced with the outcome previously delivered by that action on a RI-30s schedule. Thus,

we assessed the sensitivity of instrumental performance to reward devaluation in the absence (extinction test) and in the presence (rewarded test) of response-contingent feedback about the current value of the training outcome. After one day of retraining on the lever using an RI-30s schedule, the second devaluation test was administered with rats fed to satiety on the alternate (trained or control) outcome. One subject in the vehicle group responded for the devalued outcome to a statistically anomalous degree (Chauvenet's criterion  $< 0.5$ ) and was excluded from the analysis. Another subject from the vehicle group died between test 1 and test 2 and his data was also omitted from the devaluation tests.

#### *Data analysis and statistics*

Data from the PIT test were calculated as an elevation ratio ( $CS/(pre-CS + CS)$ ), which reflects the change in responding (either presses or magazine entries) during the cue relative to the total responses performed during the baseline and cue periods. An elevation ratio of 0.5 occurs if lever pressing during the cue and pre-cue baseline periods are equal, and more lever pressing during the cue presentation results in an elevation ratio  $> 0.5$ . Data from the extinction and rewarded portions of the devaluation tests were analyzed as a percentage of baseline response rates, which were taken from the final training session before each test. Data were analyzed with mixed ANOVAs using within- and between-subjects factors as appropriate. For reward devaluation testing, the analysis also included test order (which outcome was devalued first) as a covariate.

### **3.3 Results**

Rats acquired the instrumental response rapidly (see Figure 3-1), distinguishing between the active and inactive lever from the first training session. A lever  $\times$  day  $\times$  group ANOVA revealed a significant main effect of lever ( $F(1,22) = 261.26, p < 0.001$ ) and day ( $F(13,286) = 33.47, p < 0.001$ ) and a significant lever by day interaction ( $F(13, 286) = 31.11, p < 0.001$ ),

indicating that rats further learned to distinguish the active lever from the inactive lever over days. There were no significant interactions with group (largest F value:  $F(13,286) = 0.6, p > 0.05$ ), and no group effect ( $F(1,22) = 0.025, p > 0.05$ ). Figures 3-2A and 3-2C shows that rats displayed higher levels of magazine approach behavior during the CS+ compared to the pre-CS+ period during Pavlovian training. The period x day x group ANOVA found a significant main effect of period ( $F(1,19) = 16.31, p = 0.001$ ) and day ( $F(13, 247) = 5.67, p < 0.001$ ) and a significant period by day interaction ( $F(13,247) = 8.61, p < 0.001$ ). There were no interactions with group (largest F value:  $F(13,247) = 1.1, p > 0.05$ ), and no main effect of group ( $F(1,19) = 0.534, p > 0.05$ ). Both groups also learned to discriminate between the CS+ and CS-, as shown in Figure 3-2B and 3-2D. A period x CS x day x group ANOVA detected a significant main effect of CS ( $F(1,20) = 13.61, p = 0.001$ ) and period ( $F(1,20) = 17.5, p < 0.001$ ) and a significant CS by period interaction ( $F(1,20) = 14.19, p = 0.001$ ), representing greater approach behavior to the CS+ than the CS-, and greater approach during the CS+ than during the pre-CS+ period. There were no other significant interactions ( $F(2,40) = 2.16, p > 0.05$ ), and no main effect of group ( $F(1,20) = 1.5, p > 0.05$ ). The sensitization data is presented in Chapter 4.

After undergoing the cocaine administration and abstinence procedures, rats were administered a PIT test to assess the influence of the reward-paired cue on their instrumental performance. The conditioning and PIT testing parameters, modeled after a similar study (Wyvell and Berridge, 2001), were carefully selected to ensure that normal rats would show minimal levels of cue-motivated behavior. We reasoned that these suboptimal conditions would facilitate detection of an enhancement of the PIT effect in cocaine-treated rats. Indeed, as illustrated in Figure 3-3A, the cocaine-exposed group did show a significant increase in lever pressing during the CS+ but not during the CS-, while the vehicle group's lever pressing did not appear to be affected by either cue. A CS x group ANOVA revealed a significant main effect of CS ( $F(1,22) = 11.80, p = 0.002$ ) as well as a CS by group interaction ( $F(1,22) = 4.23, p = 0.05$ ), but no main effect of group ( $F(1,22) = 0.341, p > 0.05$ ). Separate analysis of the data from each

group found a significant effect of CS for the cocaine-exposed group ( $F(1,10) = 10.93, p < 0.01$ ) but not for the vehicle group ( $F(1,12) = 1.34, p > 0.05$ ), and direct comparison of CS+ responding between the two groups revealed that the elevation in pressing was significantly greater for the cocaine-treated rats (unpaired t-test:  $t(22) = 2.25, p < 0.05$ ). These effects cannot be attributed to differences in baseline responding, since there was no difference between groups (cocaine group -  $3.10 \pm 0.42$ , vehicle group -  $5.17 \pm 0.99$ ;  $t(22) = -1.80, p > 0.05$ ). Though vehicle treated rats did not show a significant PIT effect, they did show elevated magazine approach behavior during the CS+, relative to the pre-CS period, as did cocaine treated rats (Figure 3-3B). A CS x group ANOVA found a significant main effect of CS ( $F(1,20) = 69.1, p < 0.001$ ), but no CS by group interaction ( $F(1, 20) = 0.041, p > 0.05$ ) or main effect of group ( $F(1,20) = 0.127, p > 0.05$ ). This elevation was also not attributable to differences in baseline approach behavior (cocaine group -  $4.69 \pm 0.87$ , vehicle group -  $4.86 \pm 1.03$ ;  $t(20) = -0.122, p > 0.05$ ). Thus, both groups showed anticipatory conditioned responding to CS+, revealing that the lack of a PIT effect in the vehicle group was not due to a general impairment in Pavlovian conditioning.

Rats were retrained on the previously inactive lever for a novel outcome. There was no difference between the two groups in their acquisition of lever pressing, as similar levels of total lever presses were apparent on the last day (cocaine group (n=11):  $307.55 \pm 44.24$ , vehicle group (n=11):  $394 \pm 44.58$ ). A day x group ANOVA revealed a significant main effect of day ( $F(4,80) = 73, p < 0.001$ ), but no day by group interaction ( $F(4,80) = 2.04, p > 0.05$ ) or main effect of group ( $F(1,20) = 0.002, p > 0.05$ ). To assess habit formation, we then conducted outcome devaluation tests to determine the degree to which their performance of this new response was dependent on the current value of the training outcome. As shown in Figure 3-4A, the vehicle group exhibited goal-directed control, decreasing their lever pressing during the extinction (no feedback) session when satiated on the trained outcome (devalued test) relative to non-trained outcome prior to test (nondevalued test). The cocaine group, on the other hand, showed no

such selective devaluation, a profile indicative of habitual performance. A devaluation x group ANOVA of these data detected a marginal devaluation by group interaction ( $F(1,20) = 3.66, p = 0.07$ ) with no main effects of devaluation ( $F(1,20) = 2.42, p > 0.05$ ) or group ( $F(1,20) = 0.180, p > 0.05$ ). Separate repeated measures ANOVAs revealed a significant effect of devaluation for the vehicle group ( $F(1,10) = 14.73, p = 0.003$ ), but not in the cocaine group ( $F(1,10) = 0.40, p > 0.05$ ). The extinction test phase was immediately followed by a rewarded phase, during which rats were given response-contingent feedback about the current value of the reward. Our initial inspection of the data from this test indicated that, for most rats, sensitivity to devaluation (i.e., a suppression in responding in the devalued test relative to the nondevalued test) was most apparent in the first 10 min of the rewarded phase, presumably because rats were becoming satiated on the training outcome during the nondevalued test session. Therefore, we chose to focus our analysis on the first 10 min of this 25-min test (Figure 3-4B). A devaluation x group ANOVA found a significant main effect of devaluation ( $F(1,20) = 4.60, p < 0.05$ ) but found neither a devaluation by group interaction ( $F(1,20) = 0.96, p > 0.05$ ) nor a main effect of group ( $F(1,20) = 1.51, p > 0.05$ ). While this lack of an interaction or main effect of group indicates that the two groups did not significantly differ in their sensitivity to response-contingent feedback, inspection of the data in Figure 4b suggests that this sensitivity was at least numerically more apparent in the performance of saline-treated rats. Furthermore, ANOVAs conducted separately for each group detected a significant effect of devaluation in the vehicle group ( $F(1,10) = 6.53, p < 0.05$ ), but no effect in the cocaine group ( $F(1,10) = 0.54, p > 0.05$ ).

### **3.4 Discussion**

In this study we sought to determine if repeated exposure to experimenter-delivered cocaine increases rats' tendency to seek out rewards when presented with a reward-paired cue and/or biases their tendency to acquire a habitual response selection strategy when pursuing a food reward. Our results support both hypotheses. To the best of our knowledge, these results

provide the first demonstrations that repeated experimenter-delivered cocaine can 1) facilitate the expression of PIT for food reward, a relatively pure measure of cue-evoked incentive motivation, and 2) bias rats towards using a habitual control strategy when pursuing food reward. Furthermore, by using a within-subjects design to examine these behavioral phenomena, the current results indicate that these alterations in motivation and action selection can result from the same cocaine exposure regimen, and are therefore not likely to be particularly parameter dependent. This tendency for repeated cocaine exposure to profoundly dysregulate otherwise healthy behaviors directed towards natural rewards may play a central role in the development of pathological drug-seeking.

Our findings are in line with previous reports that repeated peripheral administration of amphetamine facilitates expression of PIT (Wyvell and Berridge, 2001) and habit formation (Nelson and Killcross, 2006; Nordquist et al., 2007), which is not surprising since both psychostimulants are potent indirect agonists in the mesencephalic dopamine system thought to be responsible for mediating these phenomena. A more recent study (Saddoris et al., 2011) found that rats trained to self-administer intravenous cocaine exhibited an enhancement in PIT. Our results demonstrate that this enhancement is not dependent on the mode of drug delivery, which supports the validity of using experimenter-delivered cocaine administration to model the effects of cocaine taking for these purposes. However, given recent findings that passive and self-regulated cocaine intake are differentially effective in eliciting dopamine release (Wilson et al., 1994; Hemby et al., 1997; Stefanski et al., 1999; Chen et al., 2008) and support distinct adaptations in the circuitry controlling dopamine signaling (Stefanski et al., 1999; Chen et al., 2008), further comparison of the effects of these treatments seems warranted.

Using a PIT design much like that used here, it has recently been shown that rats trained to self-administer cocaine increase their pursuit of cocaine when presented with a cocaine-paired cue (Leblanc et al., 2012). Although this finding suggests that Pavlovian incentive motivational processes contribute to drug seeking/taking behavior, the role of sensitization in

this phenomenon is difficult to determine since all subjects were given repeated exposure to cocaine during training. The effects of repeated drug exposure on motivation are easier to evaluate when the target response is motivated by a natural reward. The current findings add to a growing literature showing that repeated drug administration can enhance appetitive behaviors generated by non-drug rewards (Harmer and Phillips, 1998; Mendrek et al., 1998; Fiorino and Phillips, 1999b, a; Taylor and Jentsch, 2001; Wyvell and Berridge, 2001; Nocjar and Panksepp, 2002; Clark and Bernstein, 2004; Olausson et al., 2006; Klein et al., 2007; Nordquist et al., 2007; Di Ciano, 2008; Mendez et al., 2009; Ranaldi et al., 2009). Such findings suggest that extended drug exposure produces nonspecific alterations in motivation, presumably via adaptations in the dopamine system. Indeed, while it is firmly established that repeated drug exposure can sensitize the dopamine response to future drug challenges, there is also growing evidence that such treatment generates broad cross-sensitization of dopamine release (Kantor et al., 1999) and increases dopamine responses to natural reward stimuli (De Luca et al., 2011). Linking these changes in dopamine signaling to alterations in incentive motivation will require further research.

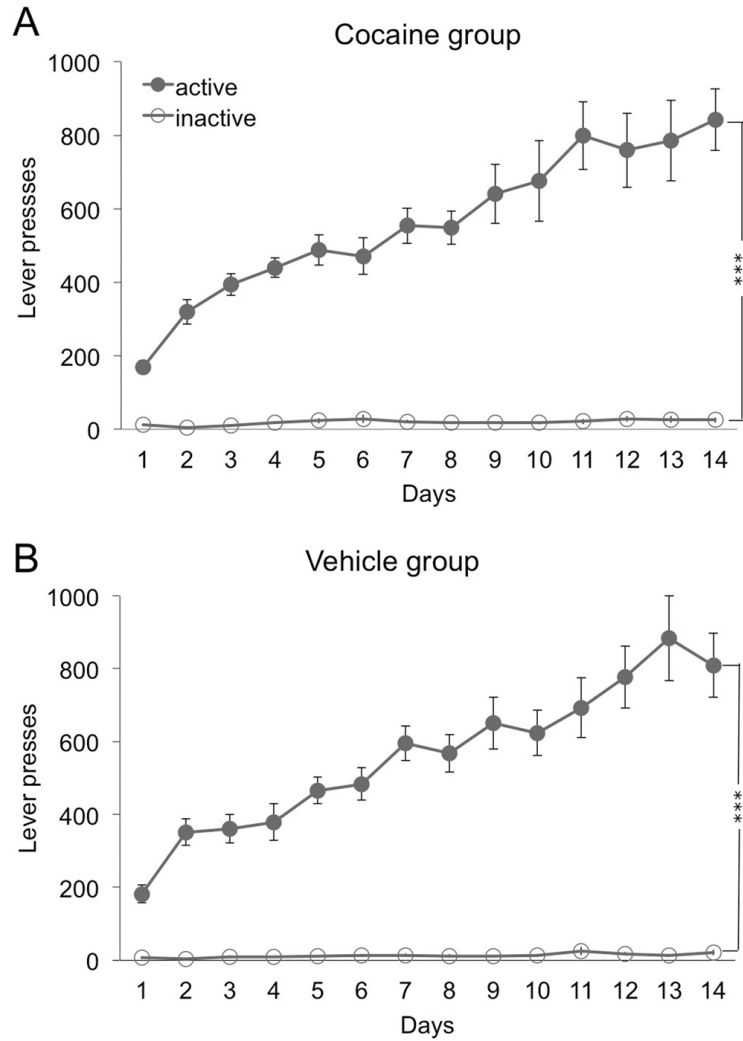
Our results also provide support for the habit theory of addiction, which posits that repeated exposure to drugs of abuse biases action selection towards the use of a habitual, rather than a goal-directed, response strategy. Evidence implicating the dopamine system in the acquisition of habits has led some to propose that drug-induced sensitization of this system facilitates acquisition of habitual control (Nelson and Killcross, 2006; Wickens et al., 2007). This enhancement in S-R learning should leave drug-seeking behavior less sensitive to its various negative consequences or to the desire to abstain. However, given the cross-sensitizing effects of cocaine and other drugs on dopamine signaling, it is predicted that repeated drug exposure should also facilitate habit formation during the pursuit of natural rewards. Our results suggest that cocaine can support such an effect. Similar findings have been obtained by pretreating rats with amphetamine (Nelson and Killcross, 2006; Nordquist et al., 2007), suggesting that this is a



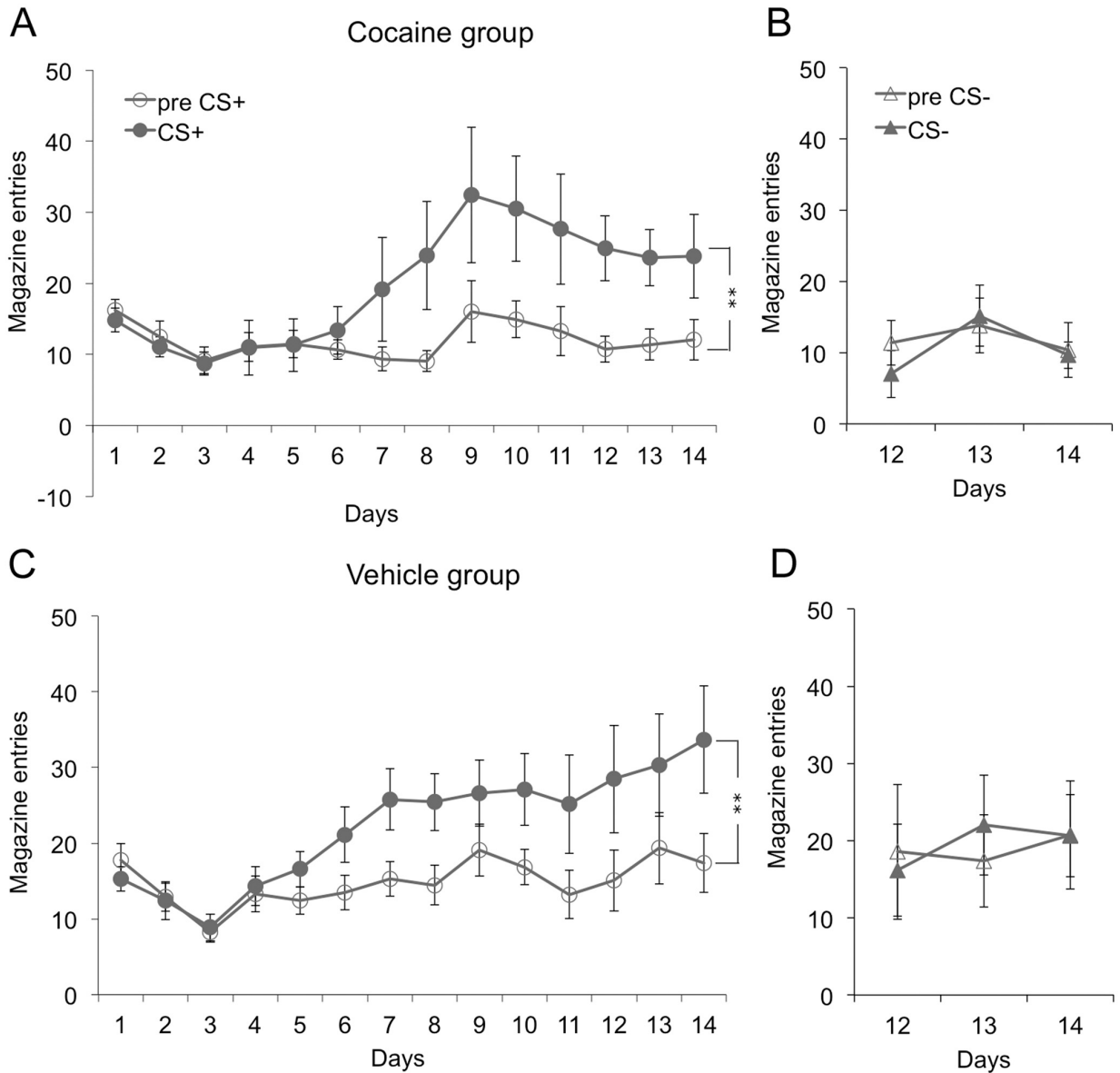
common outcome of repeated psychostimulant exposure. Such findings raise questions about the role of habit formation in drug addiction. Under normal conditions, habits will be suppressed if they produce undesirable outcomes, either through a weakening of stimulus-response associations or because the control of behavior is transferred to the goal-directed system under such conditions. Based on this reasoning, it has been argued (Ostlund and Balleine, 2008) that modeling drug-induced compulsive behavior in the laboratory requires a demonstration that the target behavior lacks sensitivity to both *anticipated* and *experienced* consequences (i.e., response-contingent feedback). This would demonstrate that long-term drug exposure both facilitates habit formation and disrupts the flexible transfer of control of behavior to the goal-directed system. In the current study, cocaine-treated rats appeared to have difficulty suppressing their instrumental performance even when given response-contingent negative feedback, although evidence of a group difference in this effect was not obtained. It is possible that an even clearer disruption would have been observed if the rats were given more frequent cocaine exposures or larger doses. However, the mode of cocaine delivery may have also played a role. Cocaine self-administration studies (Miles et al., 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Zapata et al., 2010) have found compelling evidence that such actions can become insensitive to negative feedback (electric footshock or devaluation procedures), although conflating the drug administration procedure with the target response makes it difficult to evaluate whether such effects are the result of action- and/or drug-specific processes (e.g., the over-valuation of cocaine as a behavioral goal) or whether they reflect broader changes in behavioral control or incentive motivation.

Determining how aberrations in habitual control and incentive motivation work together to generate compulsive drug seeking is an important goal for future research. One interesting possibility is that these processes make distinct, stage-dependent contributions to the development of addiction. They may also affect different components of drug-related behavior. For instance, it has been argued that while exaggerated habits may contribute to drug-taking or

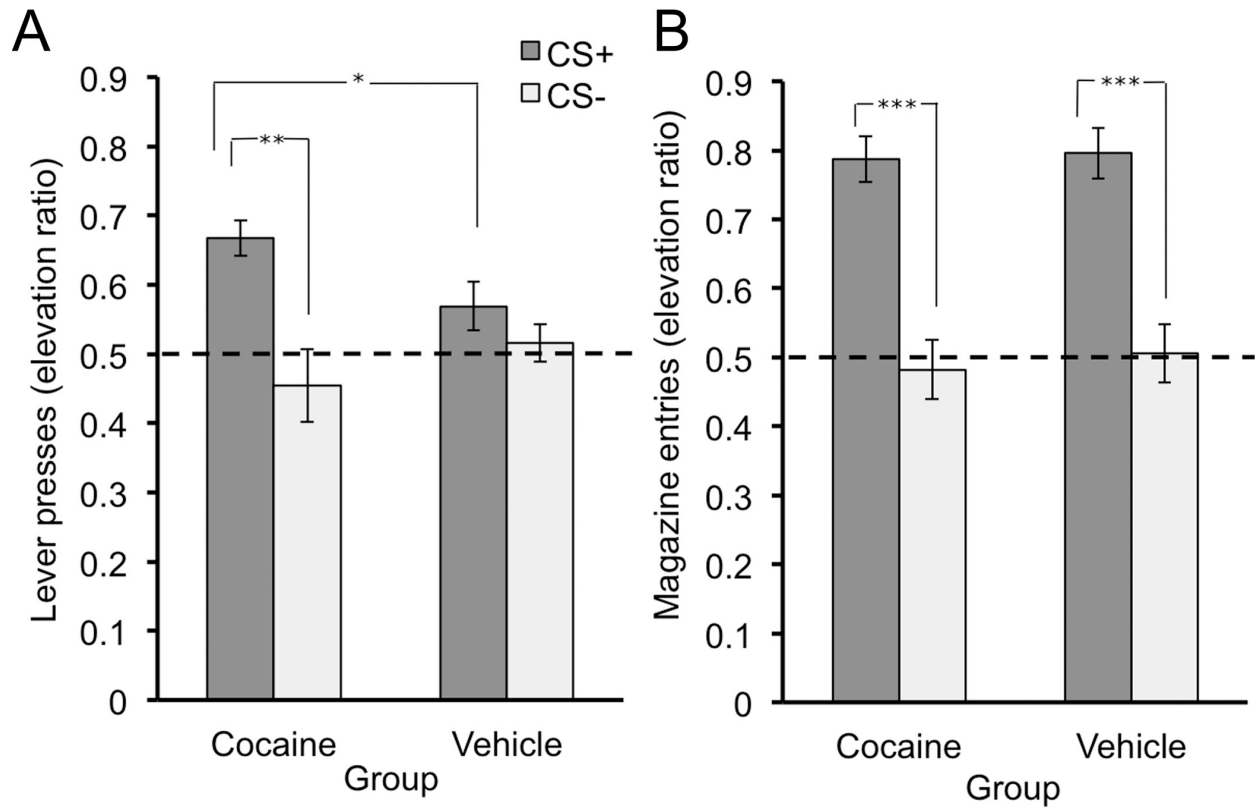
consumption, it is the sensitization of incentive motivation that maintains compulsive drug-seeking and disrupts attempts to abstain (Robinson and Berridge, 2008). Interestingly, basic behavioral research has shown that reward-paired cues tend to facilitate performance by engaging habits (Rescorla, 1994b; Holland, 2004), suggesting that these two processes work in tandem to control behavior. Our data show that, within a single set of rats, cocaine administration can sensitize both cue-evoked incentive motivation and habit formation, which is clearly compatible with this view. Finally, while this work suggests that drug-induced aberrations in motivation and behavioral control may contribute to addiction, the complex characteristics of this condition would seem to suggest that other cognitive and behavioral dysfunctions, such as alterations in prefrontal cortical areas and executive control, also play an important role.



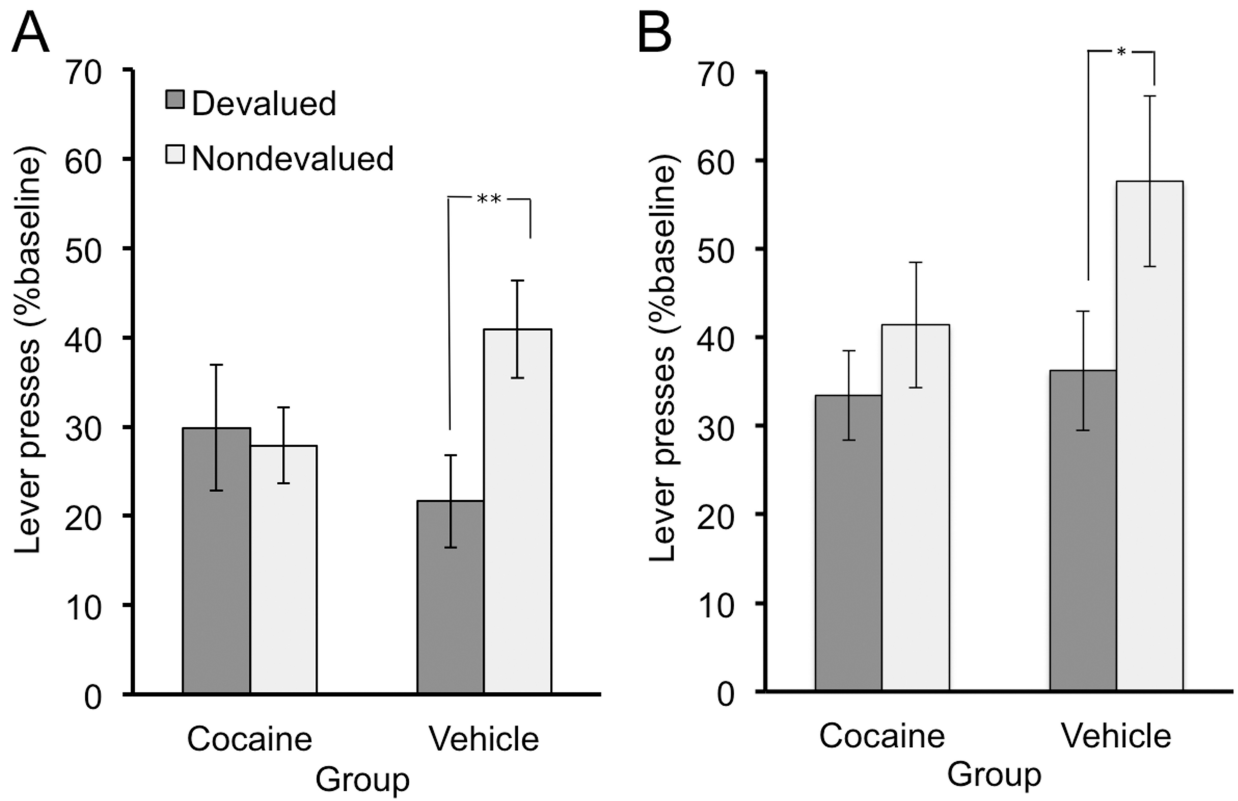
**Figure 3-1. Instrumental training on the active and inactive lever.** Responding is shown as average lever presses over days displayed separately for the cocaine group (1A) and the vehicle group (1B). Means +/- SEM. \*\*\* =  $p < 0.001$ .



**Figure 3-2. Pavlovian training.** Training data is shown as magazine entries made in response to the CS and in the 30s period immediately before it (preCS), displayed separately for CS identity and group. CS+ trials for the cocaine group (2A) and the vehicle group (2C) are shown next to CS- trials for the cocaine group (2B) and vehicle group (2D). Means +/- SEM. \*\* =  $p < 0.01$ .



**Figure 3-3. PIT test results.** Results are calculated as an elevation ratio for both lever presses (3A) and magazine entries (3B) for the cocaine and vehicle groups. Means +/- SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .



**Figure 3-4. Habit learning test results.** Figures depict lever pressing during test as a percentage of baseline responding during training for the extinction (4A) and rewarded (4B) portions of the test, plotted separately for test condition: trained outcome devalued (devalued) vs. alternate outcome devalued (nondevalued). Means +/- SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

## **Chapter 4**

### **Repeated Cocaine Exposure Does Not Prevent Goal-Directed Learning But Alters How the Behavior is Performed**

## 4.1 Introduction

Amongst the many theories of addiction, the habit learning theory has gained significant support. This theory is based on the idea that drugs of abuse can pathologically subvert normal reward learning mechanisms, leading to compulsive drug seeking in response to drug paired stimuli despite a diminished rewarding effect of the drug or the possibility of negative consequences (Everitt and Robbins, 2005). Food-motivated instrumental behavior can become habitual with extensive training, becoming insensitive to devaluation of the outcome (Dickinson, 1985). When this occurs, control over behavior transitions from goal-directed, action-outcome (A-O) motivated behavior to a habitual, stimulus-response (S-R) mechanism of behavioral strategy. This learning process requires activation of the dopamine system when either food rewards (Faure et al., 2005) or drug rewards (Wickens et al., 2007; Belin and Everitt, 2008; Dalley and Everitt, 2009) are the outcome. The habit learning theory suggests that by coopting the dopaminergic reward-learning circuitry, drugs of abuse are able to induce habitual drug seeking that is characteristic of addiction and relapse.

Since it is the normal reward learning system that drugs of abuse alter, drug sensitization is able to affect reward-motivated behavior generally. Studies have shown that amphetamine treatment can produce habitual food seeking behavior with training conditions that normally produce goal-directed behavior (Nelson and Killcross, 2006; Nordquist et al., 2007). Though amphetamine treatment could bias action selection towards a habitual strategy, it did not interfere with previously learned, goal-directed behavior (Nelson and Killcross, 2006). Therefore, repeated exposure to amphetamine facilitates habit learning when treatment precedes the instrumental learning process, but does not interrupt goal-directed behavior that has been learned before drug treatment. Recently, we have found similar results on the facilitation of habit learning with repeated cocaine exposure (LeBlanc et al., 2012), however our results with reacquisition differed.



While Nelson and Killcross found that amphetamine treated animals showed an aversion to the devalued outcome during reacquisition, we found that during a rewarded test, cocaine treated animals still failed to show an effect of devaluation, although a trend in the correct direction was apparent. This result has three major possible interpretations. Either cocaine treatment 1) disrupts animals from learning a new instrumental behavior in a goal-directed fashion, 2) disrupts animals from switching from a habitual strategy to a goal-directed strategy when the devalued outcome is experienced, or 3) increases the speed at which action selection transitions from goal-directed to habitual control. Determining which of these options is occurring can greatly enhance our understanding of how drug treatment leads to compulsive drug seeking.

In this study, we will explore how cocaine treatment can affect animal's ability to learn instrumental tasks in a flexible, goal-directed way. Subjects are given repeated exposure to cocaine, then trained on a two-lever instrumental task that favors the use of a goal-directed strategy, even with extended training (Colwill, 1985; Colwill and Rescorla, 1988). We will investigate whether cocaine treated animals will learn this task in a goal-directed fashion, and if they will transition to a habitual strategy with extended training. These experiments will further our understanding of the effects of drug treatment on the flexible execution of reward-seeking behaviors, adding to our knowledge of how reward motivated behaviors are altered by drugs of abuse.

## **4.2 Methods**

### *Subjects and apparatus*

Male Long Evans rats (mean weight:  $267 \pm 2.65$ g) were used as subjects. Rats were group housed in a climate-controlled vivarium and were tested during the light phase of the light/dark cycle (lights on from 7am to 7pm). Rats had *ad libitum* access to tap water throughout the study and were food deprived (10-14g of chow per day) to maintain them at ~85% their free-feeding

body weight. All procedures were approved by the UCLA Institutional Animal Care and Use Committee, and were performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Rats were trained in 8 identical Med Associates (East Fairfield, VT) operant chambers housed within sound- and light-resistant shells. The chambers contained two retractable levers that could be inserted to the left and right side of a recessed food cup on one end wall. A 3-W, 24-V houselight mounted on the top center of the opposite end wall provided illumination. The chambers were also equipped with a tone generator and a clicker.

### *Drugs*

Cocaine hydrochloride, provided by the National Institute on Drug Abuse Drug Supply Program, was dissolved in sterile saline (0.9% NaCl) and filter-sterilized prior to injection.

### *Cocaine sensitization*

Rats were divided into two groups: a cocaine exposure group (n=12) and a saline exposure group (n=12) receiving 6 once-daily intraperitoneal (IP) injections of 15mg/kg cocaine HCl or saline (1 ml/kg), respectively, before being placed in the behavioral chambers for 45 min and subsequently returned to their home cages. During the 45 min period, both magazine entries and locomotor activity were monitored. Subjects were then abstinent for 10 d during which they remained in their homecages. Similar dosage, duration, and abstinence parameters have been shown to support other forms of behavioral sensitization (Mayfield et al., 1992; Kalivas and Duffy, 1993; Sorg et al., 1993).

### *Instrumental Training*

Rats were first given two magazine training sessions in which they received 15 each of two distinct food pellets (grain-based food pellets or chocolate purified pellets, 45mg, Bioserv,

Frenchtown, NJ) on a fixed time 1-min schedule. This was followed by instrumental training sessions, during which one lever was continuously available, and a press on the lever resulted in the delivery of a food pellet until 30 outcomes were delivered or 60 minutes had elapsed. Each lever was paired with one of the two pellet outcomes. On each day, rats received 2 training sessions, one on each lever, separated by 15 minutes. Lever pressing was reinforced on a continuous schedule on day 1 and an RI-30s schedule thereafter.

#### *Devaluation test*

We used a specific satiety outcome devaluation procedure to assess the rats' ability to adjust their lever pressing according to a change in outcome value. Subjects were given unlimited access (>30g) to either pellet (grain or chocolate) in an alternative context for 1h before each test. Test sessions consisted of 5 min of extinction during which both levers were available but did not produce reward. Rats were tested after the 4<sup>th</sup> day and the 12<sup>th</sup> day of training.

### **4.3 Results**

The cocaine group showed a significant locomotor sensitization effect during the cocaine treatment, with an increase in locomotor behavior over treatment days in the cocaine group without any change in the vehicle group, as displayed in Figure 4-1A. A mixed ANOVA with day (1 vs 6) as the within subjects factor and group (cocaine vs. vehicle) as the between subjects factor yields a significant main effect of day ( $F(1,22) = 8.28, p < 0.01$ ), a significant main effect of group ( $F(1,22) = 73.01, p < 0.001$ ), and a significant day by group interaction ( $F(1,22) = 10.54, p < 0.01$ ). This day by group interaction represented a significant increase from day 1 to day 6 for the cocaine group ( $F(1,11) = 11.33, p < 0.01$ ), with no difference from day 1 to day 6 for the vehicle group ( $F(1, 11) = 0.183, p > 0.05$ ). Interestingly, while general activity levels increased over days for the cocaine group, the rate of entry into the magazine did not (see Figure 4-1B). A mixed ANOVA with day (1-6) as the within subjects factor and group as the

between subjects factor showed a significant main effect of day ( $F(5,110) = 4.77, p = 0.001$ ), reflecting a decrease in entries over days for both groups, since there was no significant main effect of group ( $F(1,22) = 0.002, p > 0.05$ ) or day by group interaction ( $F(5,110) = 0.49, p > 0.05$ ).

This is in direct contrast to what we have found previously (LeBlanc, Maidment & Ostlund, 2012), when Pavlovian and instrumental training preceded cocaine treatment. In that study (Chapter 3) we found a significant increase in magazine entries over days for the cocaine group, but not for the vehicle group, as displayed in Figure 4-1C. A mixed ANOVA returned a main effect of day ( $F(5,100) = 2.48, p < 0.05$ ), a significant main effect of group ( $F(1,20) = 4.60, p < 0.05$ ), and a day by group interaction ( $F(5,100) = 2.58, p < 0.05$ ). A mixed ANOVA performed separately for each group produced a main effect of day for the cocaine group ( $F(5,50) = 2.35, p = 0.05$ ), reflecting an increase in magazine entries over days. This increase appears to taper off after day 4, perhaps due to extinction of the conditioned approach response, since no food rewards were delivered during this phase of the experiment. The mixed ANOVA for the vehicle group also produced a main effect of day for the vehicle group ( $F(5,50) = 7.46, p < 0.001$ ), reflecting a significant decrease in magazine entries over days.

After the abstinence period, both groups acquired the lever press response similarly, as shown in Figure 4-2. Rates of responding on the levers (4-2A) and rates of entry into the magazine (4-2B) were averaged across sessions for the two levers to produce one average number per day for lever presses and magazine entries. A mixed ANOVA on the lever responding with day (1-4) as the within subjects factor and group (cocaine vs. vehicle) as the between subjects factor found a significant main effect of day ( $F(3,66) = 77.14, p < 0.001$ ), but no main effect of group ( $F(1,22) = 1.60, p > 0.05$ ) or day by group interaction ( $F(3,66) = 0.92, p > 0.05$ ). The groups also learned to check for delivery of the outcome at approximately the same rate. A mixed ANOVA on magazine entries (specific parameters the same as for lever pressing) found a significant main effect of day ( $F(3,66) = 2.96, p < 0.05$ ), but no main effect of

group ( $F(1,22) = 0.041$ ,  $p > 0.05$ ) or day by group interaction ( $F(3,66) = 0.244$ ,  $p > 0.05$ ).

Since cocaine treated animals may be learning to perform the task differently, we conducted a mixed ANOVA on the ratio of lever pressing to magazine checking to explore the distribution of actions, since a higher lever press to magazine entry ratio could reflect the motivation to obtain the outcome. A mixed ANOVA on the ratio of lever pressing to magazine checking (Figure 4-2C) resulted in a significant main effect of day ( $F(3,66) = 35.00$ ,  $p < 0.001$ ), with no significant main effect of group ( $F(1,22) = 2.64$ ,  $p > 0.05$ ) or day by group interaction ( $F(3,66) = 0.924$ ,  $p > 0.05$ ).

After four days of training, both groups showed a significant devaluation effect, demonstrating that cocaine treatment does not prevent animals from being able to learn to perform an action in a goal-directed fashion. A mixed ANOVA on the percentage of baseline responding during test, with devaluation (devalued vs. nondevalued outcome) as the within subjects factor and group (cocaine vs. vehicle) as the between subjects factor revealed a significant main effect of devaluation ( $F(1,22) = 18.66$ ,  $p < 0.001$ ), but no main effect of group ( $F(1,22) = 0.150$ ,  $p > 0.05$ ) and no devaluation by group interaction ( $F(1,22) = 1.79$ ,  $p > 0.05$ ). After the devaluation test, subjects received another 8 days of training followed by a second devaluation test to determine whether performance of this goal-directed behavior can come under habitual control.

Additional training revealed that the cocaine group was behaving differently than the vehicle group in the way they distributed their food-motivated actions, which can be seen in Figure 4-4. Both groups showed an increase in lever pressing behavior over days (Figure 4-4A), but the cocaine group showed an overall higher rate of pressing than the vehicle group. A mixed ANOVA with day (5-12) and group (cocaine vs. vehicle) as factors found a significant main effect of day ( $F(7,154) = 46.33$ ,  $p < 0.001$ ), and a main effect of group ( $F(1,22) = 4.52$ ,  $p < 0.05$ ), but no day by group interaction ( $F(7,154) = 0.58$ ,  $p > 0.05$ ). The groups also differed in their magazine approach behavior (Figure 4-4B), with the vehicle group showing an increase in

magazine approach behavior over days that reflected their increase in lever pressing, whereas the cocaine group did not. A mixed ANOVA returned a significant day by group interaction ( $F(7,154) = 2.73, p = 0.01$ ), but no main effect of day ( $F(7,154) = 0.73, p > 0.05$ ) or group ( $F(1,22) = 1.13, p > 0.05$ ). The vehicle group showed a main effect of day ( $F(7,77) = 4.10, p = 0.001$ ), with increasing magazine entries over days, whereas the cocaine group did not ( $F(7,77) = 0.49, p > 0.05$ ). Finally, the ratio of pressing the lever to entering the magazine might provide a measure of the motivation to obtain the food reward, since this measure reflects bursts of responding on the lever between each outcome retrieval response. This analysis revealed a significant difference between groups, with the cocaine group showing a much higher amount of lever pressing compared to magazine entries than the vehicle group (Figure 4-4C). A mixed ANOVA found a significant main effect of day ( $F(7,154) = 11.79, p < 0.001$ ), a significant main effect of group ( $F(1,22) = 6.41, p < 0.05$ ), and a significant day by group interaction ( $F(7,154) = 4.16, p < 0.001$ ). When analyzed separately, both groups showed a significant main effect of day (vehicle group:  $F(7,77) = 3.94, p = 0.001$ ; cocaine group:  $F(7,77) = 8.61, p < 0.001$ ).

The second devaluation test revealed that both groups continued to behave in a goal-directed fashion, with both groups showing a significant devaluation effect (see Figure 4-3B). A mixed ANOVA (see devaluation test 1 for details) found a significant main effect of devaluation ( $F(1,22) = 10.42, p < 0.01$ ), but no main effect of group ( $F(1,22) = 2.09, p > 0.05$ ) or devaluation by group interaction ( $F(1,22) = 1.10, p > 0.05$ ).

#### **4.4 Discussion**

Our previous study (LeBlanc et al., 2012) raised the question of whether cocaine treatment is preventing animals from learning using a goal-directed strategy, accelerating the transition from goal-directed to habitual control of behavior, or preventing a transition from habitual to goal-directed control when the outcome can be experienced. Here, we've shown that cocaine treated animals can learn to perform a task in a goal-directed fashion. While the

action selection strategy at test may not be compromised, the performance of actions during training is clearly altered, with cocaine treated rats responding at a high lever press to magazine entry ratio. These results may suggest that cocaine sensitized animals can learn to perform a task using an A-O strategy, and may exhibit a greater motivation to obtain the outcome during training, reflected in an increased rate of lever pressing over the vehicle group.

Our results demonstrate that sensitization with cocaine does not prevent animals from learning to perform a task in a goal-directed fashion. While this may seem contradictory to our findings that this same drug treatment regimen facilitates habit learning, similar results showing enhanced habit learning but also goal-directed behavior after drug treatment have been found with amphetamine (Nelson and Killcross, 2006; Nordquist et al., 2007). This differential effect can either be due to the schedule of reinforcement used in training, or due to the timing of drug exposure, with drug treatment preceding training resulting in habitual behavior, while drug treatment after training resulting in goal-directed behavior. Previous studies showing a significant effect of drug exposure on S-R reward learning have used tasks that facilitate the development of a S-R strategy, either employing a Pavlovian learning task (Schoenbaum and Setlow, 2005), or a single lever interval schedule instrumental task (Nelson and Killcross, 2006; Nordquist et al., 2007). In this study, we use a two lever, two outcome task that favors an A-O response strategy, even with extensive training (Colwill, 1985; Colwill and Rescorla, 1988), which is often defined as at least 360 outcomes delivered in a response contingent manner. Despite the goal-directed nature of this task, it is possible that repeated cocaine exposure could facilitate the development of a S-R strategy after a sufficient amount of training. Our results do not support this account, instead showing that even with extensive training, cocaine treated animals remain goal-directed and sensitive to outcome devaluation.

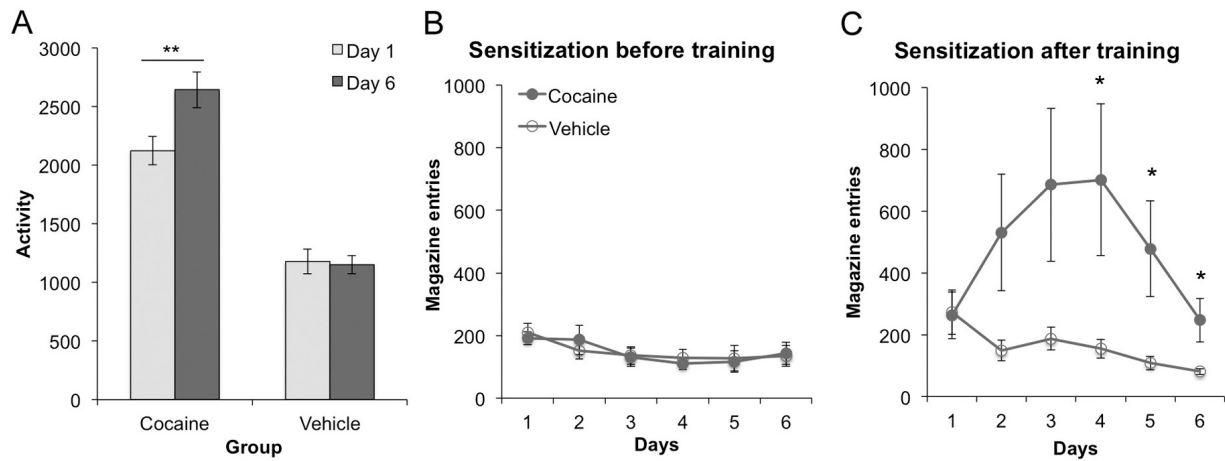
In our previous study, we found that the cocaine treated subjects showed an increase in magazine approach behavior during cocaine sensitization that was not apparent in the vehicle group. Importantly, this effect was not simply due to an increase in locomotor behavior,

because the cocaine-treated subjects in the current study exhibited an increase in locomotor behavior without any increase in magazine approach compared to vehicle treated rats. Since subjects received drug treatment before any training with the food outcome, the magazine was not yet associated with the delivery of food pellets, and thus did not elicit any Pavlovian approach behavior. However, in our previous study, drug treatment occurred after Pavlovian and instrumental conditioning, so the magazine would be associated with food delivery. These results suggest that cocaine treatment enhanced food-motivated approach behaviors, perhaps through an increase in incentive motivation. However, as noted above, such measures do not provide the kind of pure assessment of cue-invoked incentive motivation that PIT provides.

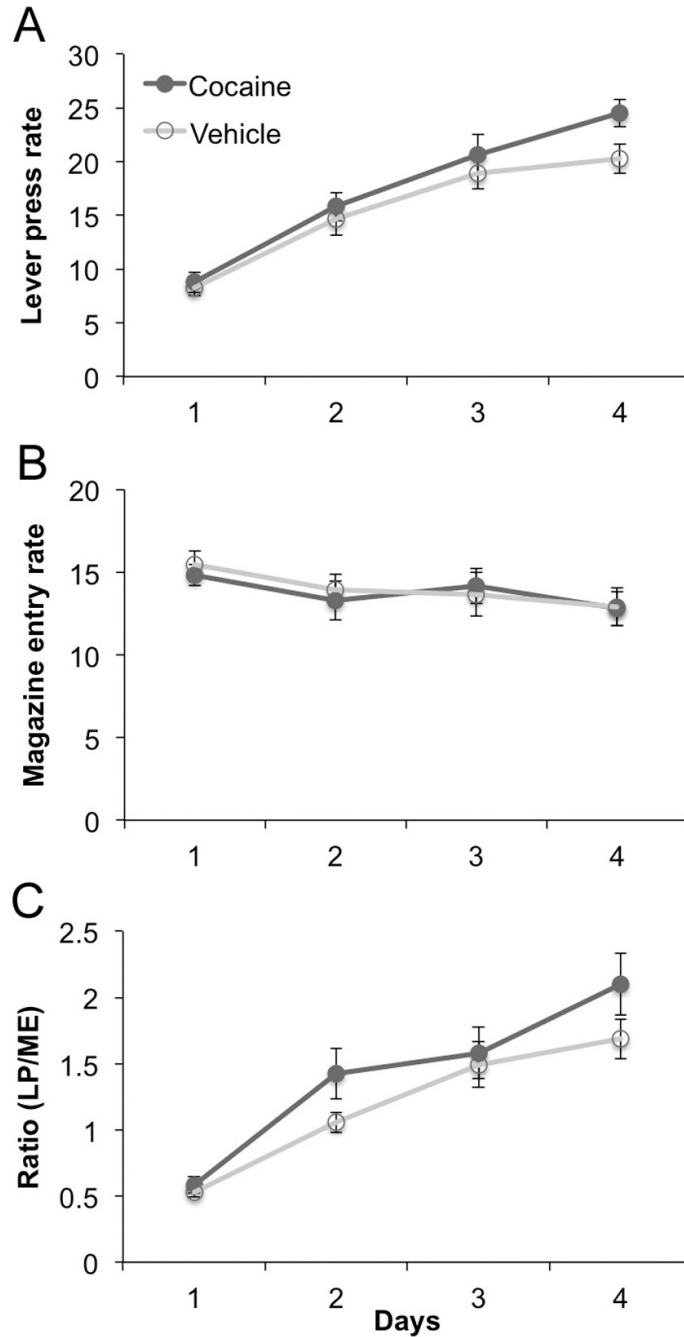
Our results also suggest that the enhancement of lever pressing exhibited by the cocaine treated group during training could reflect increased motivation to obtain the reward. Over the course of training, we found that cocaine treated rats continued to increase their rate of lever pressing without an increase in magazine checking behavior as seen in the vehicle treated animals. Since a short interval schedule favors frequent checking for delivery of the food pellet, the cocaine treated animals are expending more energy than necessary to obtain the reward. The idea that psychostimulant exposure promotes effortful behavior is supported in the literature, since treatment with psychostimulants have been shown to increase behavior on an action with a higher response requirement (Wardle et al., 2011) and increase break-points in a progressive ratio task for food rewards (Jones et al., 1995; Brown and Stephens, 2002). Our previous study on cocaine treatment on food-motivated behavior may also support this effort-based theory. In our previous study (LeBlanc et al., 2012), we have shown that while both cocaine and vehicle treated animals show an increase in magazine entries to a CS that predicts reward during a PIT test, only cocaine treatment increases lever-pressing actions during the CS+ presentation. This could be a reflection of increased effortful behavior to obtain a reward, since the training conditions predicted that the outcome should be delivered with no additional effort other than entering the magazine to retrieve it. While the cocaine group showed the low-



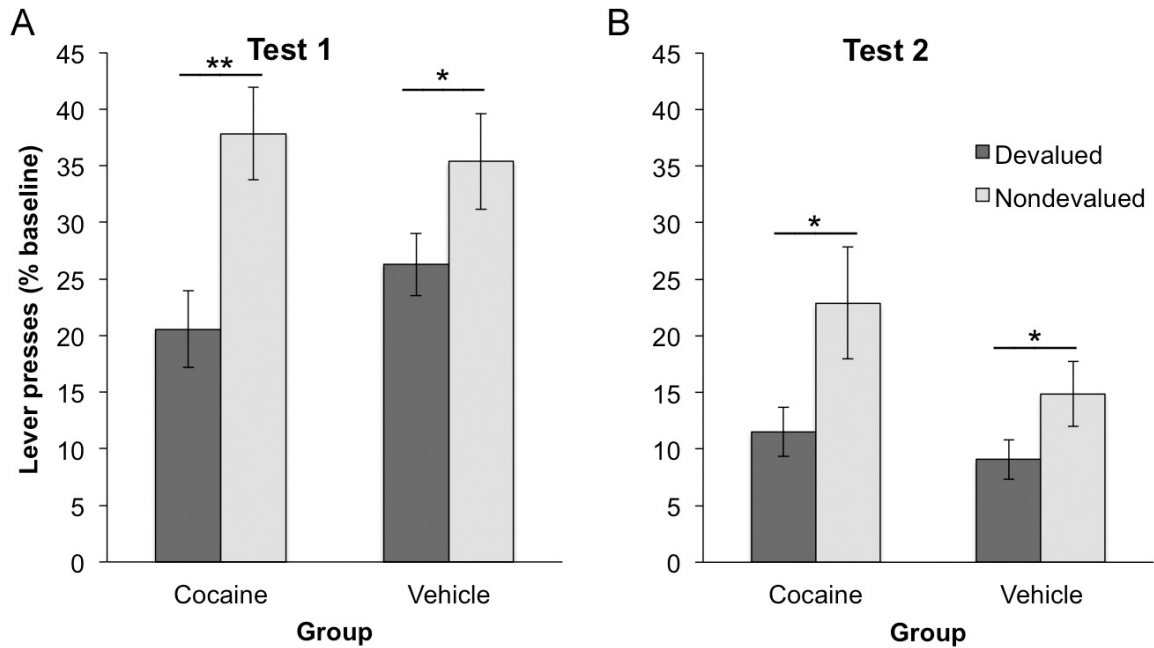
effort response (checking the magazine), they also engaged in the high-effort response (pressing the lever) that should be superfluous to obtaining the outcome when the CS+ is presented. It is possible then that our results reflect an increased willingness to exert effort to obtain the reward, which could be due to increased motivation to obtain it. Together, our findings further our understanding of how cocaine treatment may alter control over reward-motivated actions, which could have important implications for the loss of behavioral control that contributes to addiction.



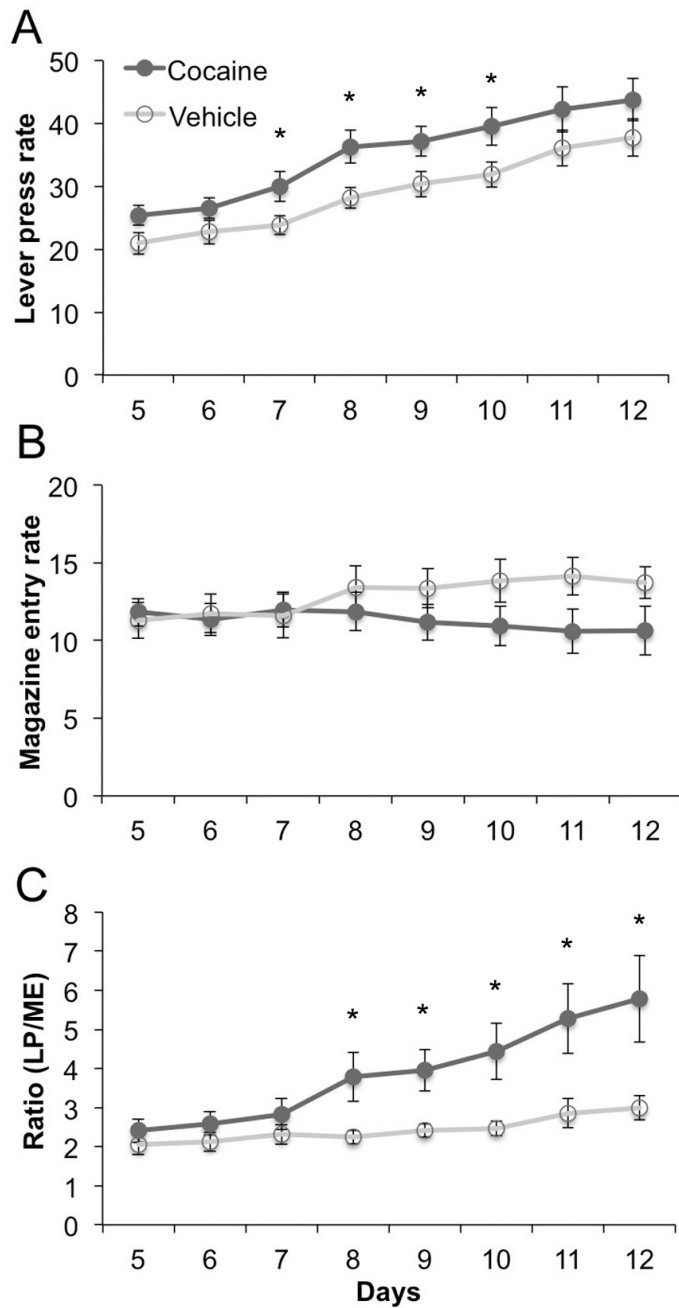
**Figure 4-1. Activity and magazine entries during cocaine sensitization.** Figure 4-1A: Total activity measurements over the 45min session on the first and last day of sensitization. Figure 4-1B: Average magazine entries over the 6 days of sensitization for the cocaine and vehicle groups from the current experiment (i.e. sensitization occurs before operant training). Figure 4-1C: Average magazine entries over the 6 days of sensitization for the cocaine and vehicle groups from our previous study (i.e. sensitization occurs after operant training). Values are means  $\pm$  SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .



**Figure 4-2. Instrumental training days 1-4.** Figure 4-2A: The rate of lever pressing over the first 4 days of training for both the cocaine and vehicle groups. Figure 4-2B: The rate of magazine entries over the first 4 days of training for both cocaine and vehicle groups. Figure 4-2C: The ratio of lever press rate (LP) to magazine entry rate (ME) over the first 4 days of training for both cocaine and vehicle groups. Values are means  $\pm$  SEM.



**Figure 4-3. Devaluation tests 1 and 2.** Figure 4-3A: Results from the first devaluation test, depicted as lever presses as a percentage of baseline lever responding for the devalued and nondevalued outcome for both the cocaine and vehicle group. Figure 4-3B: Results from the second devaluation test, depicted as lever presses as a percentage of baseline lever responding for the devalued and nondevalued outcome for both the cocaine and vehicle group. Values are means  $\pm$  SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .



**Figure 4-4. Instrumental training days 5-12.** Figure 4-2A: The rate of lever pressing over the last 8 days of training for both the cocaine and vehicle groups. Figure 4-2B: The rate of magazine entries over the last 8 days of training for both cocaine and vehicle groups. Figure 4-2C: The ratio of lever press rate (LP) to magazine entry rate (ME) over the last 8 days of training for both cocaine and vehicle groups. Values are means  $\pm$  SEM. \* =  $p < 0.05$ .

## **Chapter 5**

### **Prolonged Experience With Self-administered Cocaine, But Not Yoked Cocaine, Enhances Pavlovian-to-Instrumental Transfer For Food Rewards**

## 5.1 Introduction

Many theories have been proposed to account for the development of addiction and the phenomenon of relapse. One account that has received considerable attention is the incentive sensitization theory, which posits that extended drug use sensitizes the mesotelencephalic dopamine system and associated neural circuitry involved in assigning incentive salience to drug-paired cues, allowing these cues to exert greater control over drug-seeking behavior, resulting in compulsive drug use and provoking relapse during periods of abstinence or controlled drug intake (Robinson and Berridge, 1993). There is strong empirical support for the basic tenets of this view. Addictive drugs of virtually all classes can stimulate and, with repeated exposure, sensitize dopamine neurotransmission in the mesotelencephalic system (Wise, 1984; Rowell et al., 1987; Di Chiara and Imperato, 1988; Carboni et al., 1989), which has, itself, been repeatedly implicated in the attribution of incentive salience to reward-predictive stimuli (Ikemoto and Panksepp, 1999; Lex and Hauber, 2008; Wassum et al., 2011; Ostlund and Maidment, 2012).

Furthermore, although the incentive sensitization theory was intended to explain drug addiction, there is considerable evidence that repeated exposure to drugs can have a broad cross-sensitizing effect on incentive motivational processes (De Vries et al., 1998; Avena and Hoebel, 2003; Le Merrer and Stephens, 2006). For instance, rats that have been repeatedly administered psychostimulants, like amphetamine or cocaine, come to exhibit a more vigorous pattern of food seeking behavior (Wyvell and Berridge, 2001; Nelson and Killcross, 2006; Nordquist et al., 2007). Such treatment appears to specifically impact the motivational influence of reward-paired cues, which can be selectively assayed using the Pavlovian-to-instrumental transfer paradigm (Rescorla, 1994a). In such studies, rats are independently (i.e., in separate sessions) trained to press a lever for food reward and predict that reward in the presence of an auditory cue. At test, which is conducted under extinction to prevent new excitatory learning, rats tend to increase their lever pressing when presented with the reward-predictive cue, relative

to an unpaired control cue. This cue-evoked response invigoration is stronger for rats given repeated experimenter-delivered amphetamine (Wyvell and Berridge, 2001) or cocaine (LeBlanc et al., 2012) exposure. While experimenter-delivered drugs are clearly capable of eliciting neurochemical and behavioral alterations, they may not adequately model volitional drug-taking. This aspect of drug use is more directly modeled by allowing rats to self-administer a drug. Although we have recently used the PIT paradigm to demonstrate that Pavlovian cues predicting intravenous cocaine can invigorate cocaine-seeking behavior (Leblanc et al., 2012), it is not clear whether or to what degree incentive sensitization contributes to this effect. However, another recent study found that rats with a history of cocaine self-administration showed stronger food-motivated PIT than cocaine-naive rats (Saddoris et al., 2011), demonstrating that voluntary cocaine intake can replicate the incentive sensitizing effects of noncontingent, experimenter-delivered cocaine exposure. There has also been evidence to indicate that self-administration of cocaine produces both locomotor (Hooks et al., 1994; Phillips and Di Ciano, 1996) and neurochemical (Hooks et al., 1994) sensitization, supporting the use of self-administered cocaine in studying incentive sensitization. Interestingly, there is considerable evidence that the self-administered cocaine is more potent in stimulating dopamine release (Hemby et al., 1997; Kimmel et al., 2005; Chen et al., 2008) and can support stronger and more persistent neuroadaptations in the circuitry controlling dopamine signaling (Stefański et al., 2007) than passively administered intravenous cocaine. Thus the effects of cocaine exposure on the dopamine system do not appear to result simply from the direct pharmacological properties of that substance but are instead modulated by the mode of drug delivery.

The current study examined whether the sensitizing impact of intravenous cocaine on Pavlovian incentive motivation (assayed using PIT task) similarly depends on whether it is administered in an active (self-administration) or passive (yoked administration) manner. We found that rats with a history of active cocaine taking showed stronger cue-evoked incentive motivation than rats given non-contingent exposure to either cocaine or saline, with these latter



two groups exhibiting similar patterns of task performance. This finding demonstrates that the way in which cocaine is administered determines its long-term behavioral effects and has implications for addiction research and theory.

## **5.2 Materials and Methods**

### *Subjects*

Adult male Long Evans rats (mean weight:  $322 \pm 5.9$ g) were used in this experiment. Rats were housed in a climate-controlled vivarium and were tested during the light phase of the light/dark cycle (lights on from 7am to 7pm). Rats were kept on a food deprivation regimen (~12g home chow per day) throughout training and testing to maintain them at approximately 85% of their free feeding bodyweight, but were provided *ad libitum* access to tap water in the home cage. All procedures were approved by the Animal Research Committee of University of California, Los Angeles, and were performed in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

### *Apparatus and Training*

Rats were trained in eight identical Med Associates (East Fairfield, VT) operant chambers housed within sound- and light-resistant shells. The chambers contained two retractable levers on one of the two aluminum end walls that could be inserted into the chamber to the left and right side of a recessed food magazine, into which grain-based food pellets (45mg, Bioserv, Frenchtown, NJ) could be delivered. The side walls were composed of transparent acrylic and the floor consisted of parallel stainless steel bars. During self-administration training, the boxes were outfitted with a nosepoke hole (Med Associates) on the opposite wall from the levers (which were retracted) and magazine. A 3-W, 24-V houselight was mounted on the top center of the opposite end wall provided illumination. The chambers were also equipped with a tone generator (3 kHz, 75dB) and a clicker (2Hz, 75dB). Computers equipped with the MED-PC

program (Med Associates) controlled the equipment and recorded lever presses.

### *Drugs*

Cocaine hydrochloride, provided by the NIH, was dissolved in sterile saline (0.9% NaCl) and filtered for impurities.

### *Instrumental Training*

Before instrumental training began, subjects received two magazine training sessions in which they received 20 food pellets on a fixed time 1-minute schedule. Magazine training was followed by 14 days of instrumental training, consisting of 30-minute sessions with continuous access to an active and inactive lever. Pressing on the active lever (either left or right) resulted in the delivery of grain pellets, while pressing on the inactive lever was monitored but without consequence. The schedule of reinforcement used for the active lever was modified over days. On the first day rats were continuously reinforced, but were then shifted to a random interval (RI) schedule for the rest of training, with one day of RI-5s, one day of RI-15s, one day of RI-30s, followed by 10 days of RI-45s. Two subjects failed to discriminate between the two levers (< 90% total presses on the active lever during the last day) and were excluded from the rest of the study.

### *Pavlovian Training*

Rats were then given Pavlovian training, which consisted of 14 daily 30-minute sessions. During the first 11 sessions, the presentation of one of the two auditory stimuli (CS+; either the tone or clicker; 30-sec duration) was followed immediately by the delivery of 3 pellets. Each session consisted of 10 CS+ presentations, delivered on a variable time 2-min schedule. The last 3 sessions were otherwise identical to previous sessions, except for the addition of two non-reinforced presentations of the alternative auditory stimulus (CS-), which were made at the

middle and end of the sessions (i.e., after the 5<sup>th</sup> and 10<sup>th</sup> CS+ presentation). Magazine entries were recorded to monitor acquisition of conditioned approach behavior.

### *Catheter surgery*

Rats were deeply anesthetized with isoflurane (4-5% induction, 1.5-2.5% maintenance), and a silicon catheter (O.D. 0.63mm x I.D. 0.30mm x wall 0.17mm, CamCaths, Cambridgeshire, England) was placed into the right or left jugular vein. The catheter was advanced approximately 35 mm caudally to the right atrium. The proximal end was attached to a coiled length of wider bore tubing that exited through a mount inserted under the skin between the scapulae. Rats were given 5 days to recover from surgery and catheters were maintained with twice daily heparin injections (0.1 ml of 10 units/ml) for the duration of the experiment. The antibiotic, sulfamexazole (TMS), was placed in the drinking water (0.05%) for the duration of the experiment. Catheter patency was evaluated twice daily, before and after each self-administration session, by checking for backflow of blood in the flushing syringe. Any catheter of questionable patency was tested by evaluating the sedative effectiveness of 0.2ml of 1% propofol. Any subject not sedated was excluded. Cocaine was self-administered through polyethylene tubing threaded through a spring tether that was connected to a liquid swivel attached to a balance arm, allowing the animals free range of motion. One subject died during surgery (yoked saline group).

### *Cocaine exposure*

Subjects were divided into three groups: a master group ( $n = 8$ ) that self-administered cocaine, a yoked cocaine group ( $n = 8$ ), in which rats were noncontingently administered cocaine using intervals set by their counterparts in the master group, and a yoked saline group ( $n = 7$ ), in which rats received saline based on the intervals of their master counterparts. Thus, each master rat set the cocaine delivery times for one rat and the saline delivery times for another. To

facilitate discrimination between training phases, no house light illumination was provided during this phase of the experiment. The chambers were also distinguished by adding a punched stainless steel floor plate, side wall panels with black-and-white vertical lines (1" wide), and an odor cue (0.1ml of 10% almond extract, placed on a paper towel positioned below floor plate). An LED light positioned within the nose poke hole was used to signal cocaine availability for the master group; this light was never illuminated for rats in the yoked groups. For the master group, each nosepoke resulted in the delivery of 0.23mg of cocaine over 4.35s followed by a 20-s time out during which the nosepoke light was extinguished. All groups received 14 once-daily sessions. Sessions lasted for 2 h or until 30 outcomes had been earned by a master group rat. After cocaine or vehicle exposure, rats were given 10 d of abstinence during which they remained in their homecages. One set of rats (a master rat and his yoked cocaine and yoked saline counterparts) was eliminated from the study because the master rat failed to meet criterion for adequate self-administration (at least 50 cocaine infusions over the 14-d period).

#### *Pavlovian-to-instrumental transfer testing*

The cocaine administration cues were removed from the chamber for the remainder of training and testing. Rats were retrained for 3 d on the instrumental response on a RI-45s schedule. On the following day, rats received a 30-min extinction session in which both levers were available but produced no rewards, which was done to suppress lever press rates to facilitate detection of the PIT effect. A PIT test was conducted on the following day. All lever presses were recorded during this session but no rewards were delivered. The two auditory cues (CS+ and CS-) were noncontingently presented 4 times (2.5 min ITI) each in strict alternation (tone, click) to assess their ability to influence lever press performance. Because the excitatory effects of a reward-paired cue on lever pressing tend to persist after that cue has been terminated (Lovibond, 1983), we took the total number of presses performed on each lever during the 30-sec CS period and the 30-sec post-CS period. From these values, we subtracted the number of presses

occurring 30-sec before cue onset (Pre-CS) to generate difference scores (CS – Pre-CS), which reflect the cue-related changes in lever press performance.

### 5.3 Results

All rats acquired the instrumental response, with no significant difference in lever pressing behavior between groups on the last day (Masters:  $31.04 \pm 4.82$ , Yoked cocaine:  $27.17 \pm 3.68$ , Yoked saline:  $26.07 \pm 3.95$ ). A one-way between-subjects ANOVA found no effect of group ( $F(2,17) = 0.391$ ,  $p > 0.05$ ). All subjects also learned to discriminate between the CS+ and CS-. As shown in Figure 5-1A, on the final day of training rats in all groups showed higher rates of magazine entry during the CS+ compared to the pre-CS+ period, while entry rates during the pre-CS- and CS- periods were similar to each other and to the pre-CS+ rate. A mixed group x period (pre-CS, CS) x CS (CS+, CS-) ANOVA found a significant main effect of period ( $F(1,15) = 34.1$ ,  $p < 0.001$ ) and, more importantly, a significant main effect of CS ( $F(1,15) = 11.36$ ,  $p < 0.01$ ) and a significant CS x period interaction ( $F(1,15) = 18.35$ ,  $p = 0.001$ ), indicating that the CS+ elicited a greater increase in magazine entry behavior than the CS-. There were no interactions with group (largest F value:  $F(2,15) = 0.18$ ,  $p > 0.05$ ), and no main effect of group ( $F(2,15) = 1.91$ ,  $p > 0.05$ ) demonstrating that all groups learned the Pavlovian association equally well. All but one rat in the master group learned to self-administer cocaine by nosepoking. Group average nosepoke rates (pokes per minute) on the final day of training demonstrate a high rate of self-administration in the master group with a small and variable amount of superstitious nosepokes made by the yoked cocaine group but no nosepokes made by the yoked saline group (Masters:  $18.6 \pm 3.06$ , Yoked cocaine:  $7.17 \pm 5.26$ , Yoked saline:  $0 \pm 0$ ). A one-way between-subjects ANOVA found a significant main effect of group ( $F(2,15) = 5.63$ ,  $p < 0.05$ ). Average daily cocaine intake increased steadily over days in the Master group, as displayed in Figure 5-1B. A repeated measures ANOVA revealed a highly significant main effect of day ( $F(13,52) = 4.01$ ,  $p < 0.001$ ).

The results of PIT testing are presented in Figure 5-2A, with pre-CS baselines reported in Table 5-1. Whereas masters increased their rate of pressing during the CS+, rats in the yoked cocaine and yoked saline groups were apparently unaffected by this cue. The CS- did not influence lever pressing in any of the groups. A mixed group x CS ANOVA found no overall effect of CS ( $F(1, 15) = 0.448, p > 0.05$ ) or group ( $F(2, 15) = 2.82, p = 0.09$ ), but did detect a significant CS by group interaction ( $F(2, 15) = 5.16, p < 0.05$ ). The CS effect was analyzed separately for each group (one-way repeated measures ANOVA). We found no effect in the yoked saline group ( $F(1, 5) = 0.378, p > 0.05$ ) or the yoked cocaine group ( $F(1, 5) = 3.6, p > 0.05$ ), but detected a significant main effect in the master group ( $F(1, 5) = 7.63, p < 0.05$ ). Further analysis (one-sample, 2-tailed t-tests using 0 as the null hypothesis) found that the CS+ was effective in eliciting lever pressing in the master group ( $t(5) = 3.78, p = 0.01$ ) but not in the yoked saline ( $t(5) = 1.28, p > 0.05$ ) or yoked cocaine ( $t(5) = 1.00, p > 0.05$ ) group. To elucidate which aspect of cocaine self-administration seemed to account for the enhanced PIT effect, we performed a correlational analysis. We found that the amount of cocaine received during a single session predicted the level of PIT effect shown in the master group (Figure 5-2B), with a significant correlation between the maximum number of cocaine infusions received on any day and the degree of elevation to the CS+ over the CS- (subtraction of the difference score for the CS+ from difference score for the CS-) ( $r(5) = 0.81, p < 0.05$ ). No such correlation is evident for the yoked cocaine group ( $r(6) = 0.58, p > 0.05$ ).

## 5.4 Discussion

We have demonstrated that self-administered cocaine, but not yoked cocaine or yoked saline, produces a PIT effect for food rewards. This differential result between self-administered and yoked cocaine coincide with previous results showing a greater effect of self-administered cocaine than yoked cocaine on extracellular dopamine levels (Hemby et al., 1997; Kimmel et al., 2005; Lecca et al., 2007) and on neuroadaptations and excitability in the dopamine system

(Stefanski et al., 1999; Chen et al., 2008; Larson et al., 2010). This increased ability of response-contingent cocaine to induce changes in dopaminergic circuitry could account for the observed increase in food-motivated behavior, since the dopaminergic system that is differentially affected by mode of cocaine delivery is the same system that is responsible for reward-processing in general (Robinson and Berridge, 2008). Thus, self-administered cocaine, via greater adaptations in the mesotelencephalic dopamine system, alters food-motivated behavior when yoked cocaine does not. Our results provide another demonstration of the differences between self-administered and yoked cocaine, and is one of the first demonstrations of a behavioral difference in these two treatments, highlighting the importance of volitional control or predictability of cocaine administration on motivated behavior.

Our results also add to the findings of Saddoris et al, showing that it is not purely cocaine exposure that accounts for the effect of self-administered cocaine on PIT. In addition, we have shown that this effect is not due to new experience with the Pavlovian associations, since there were no Pavlovian reminder sessions given after drug treatment. We have also expanded on previous finding by demonstrating that reinforcement at test is not necessary to obtain an enhancement of PIT, ensuring that any facilitation of PIT is not due to experiencing an association between the cue, lever response and outcome. Unlike Saddoris et al., we did not obtain a PIT effect in yoked saline animals due to the sub-optimal training procedures we employed to ensure that a facilitation of transfer could be detected.

The correlation between the maximum amount of cocaine received on any day of training and the elevation of responding to the CS+ over the CS- for the self-administration group suggests that larger daily intake supports a stronger PIT effect, and that this effect is dependent on either volitional control or predictability of cocaine delivery since no such correlation was found for the yoked cocaine group. The importance of the maximum daily amount of cocaine delivered could be related to studies showing an enhanced effect of “binge” delivery or extended access on behavioral and neural substrates of addiction (Di Ciano et al.,

1996; Ahmed and Koob, 1998; Banks and Negus, 2009). However, this correlation may only hint at a relationship between maximum cocaine delivery and PIT effect, since the effect is dependent on a single animal and would not survive robust correlational analysis. Further research should be done to evaluate what aspect of self-administration most greatly predicts the differences seen in incentive motivation.

Our findings here support our previous findings that IP cocaine sensitization facilitates a PIT effect for food rewards (LeBlanc et al., 2012), and demonstrate that both experimenter-delivered and self-administered cocaine can alter food-motivated behavior. The lack of an effect in the yoked cocaine group may suggest an important role for predictability in the effects of cocaine treatment on incentive motivation. With both experimenter-delivered and self-administered cocaine, the delivery of the drug is predicted by either injection cues (special handling, needle insertion) or volitional action (nosepoke). The cues associated with IP injections have been shown to have a significant impact on the effects of the drug (Cepeda-Benito and Tiffany, 1995), suggesting that the injection ritual may provide highly salient cues that allow the animal to predict the ensuing psychological and physiological effects of drug intake. Furthermore, the experience of yoked cocaine delivery may be perceived as aversive (Mutschler and Miczek, 1998; Twining et al., 2009). Yoked cocaine delivery has been shown to inducing heightened cardiovascular effects in humans (Donny et al., 2006) and increased mortality in rats (Dworkin et al., 1995) compared to self-administered cocaine. If yoked cocaine is inducing negative affect, it could prevent the cocaine treatment from having an excitatory impact on incentive motivation. In line with our findings, Chen et al (2008) showed that both experimenter-injected and self-administered cocaine induce LTP in the VTA while yoked cocaine does not.

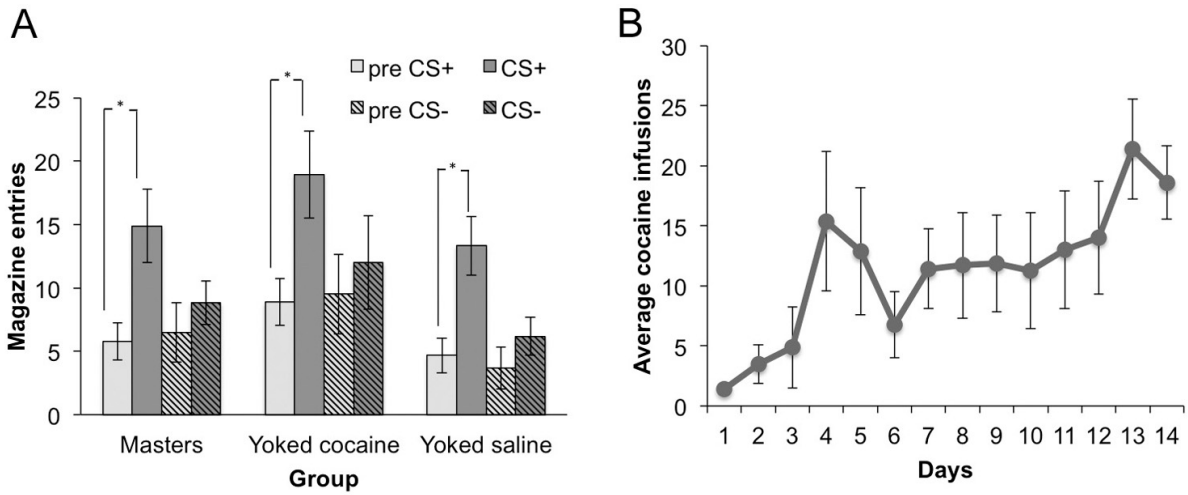
These results add to the growing literature showing that prolonged exposure to drugs of abuse such as psychostimulants and alcohol can result in cross-sensitization of the dopamine system and alter food motivated behavior (Wyvell and Berridge, 2001; Nelson and Killcross,



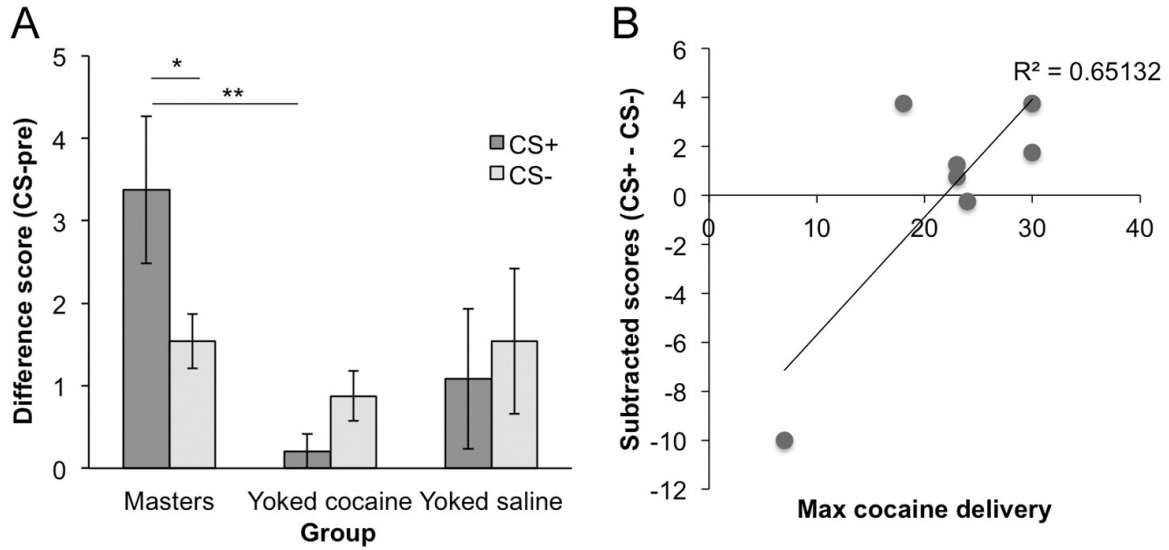
2006; Nordquist et al., 2007; Ostlund et al., 2010). In this study, we have shown additional support for the incentive sensitization theory of addiction, and indicated the importance of volitional control or predictability of cocaine intake on the ability of food-paired cues to motivate food-seeking behavior. These findings suggest that further research on the neural and psychological impact of the mode of drug delivery is needed. Although self-administered and experimenter delivered cocaine produce similar neuroadaptions and behavioral effects, much remains unexplored about how these treatments affect the brain and behavior or how these changes relate to the state of addiction. Our results elucidate a potential mechanism by which self-administered cocaine can alter motivated behavior in general, increasing the incentive salience of rewards and triggering reward seeking. This heightened reward seeking may translate into compulsive drug seeking, leading to addiction and relapse.

Group	Pre-CS+	Pre-CS-
Masters	1.42 ± 0.42	1.04 ± 0.48
Yoked cocaine	1.58 ± 0.52	1.67 ± 0.50
Yoked saline	2.79 ± 0.45	2.67 ± 0.45

**Table 5-1. Pre-CS baselines.** CS = conditioned stimulus. Values are mean ± SEM.



**Figure 5-1. Pavlovian training and cocaine self-administration.** 5-1A: Magazine entries during the CS+, CS-, and the 30s periods immediately preceding the CS+ (pre CS+) and CS- (pre CS-) on the final day of training. 5-1B: Average number of cocaine infusions earned by the master group over the 14 days of self-administration. Means  $\pm$  SEM. \* =  $p < 0.05$ .



**Figure 5-2. PIT test results and correlation with self-administration.** 5-2A: PIT test results, displayed as a difference score (baseline subtracted: CS – pre CS). 5-2B: Correlation between maximum number of cocaine deliveries earned on any one day during the self-administration period and the difference between difference scores for the CS+ and CS-. Means  $\pm$  SEM. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

**Chapter 6**  
**General Discussion**

Addiction to cocaine is a serious problem in the United States, costing millions of dollars to our health and law enforcement systems, in addition to severe consequences to the individual (loss of employment, incarceration, hospitalization) to which no dollar amount can be attached. Understanding how drug use transitions into abuse and addiction, and how stimuli associated with the drug elicit relapse could be the key to developing treatments or preventions for the development of addiction. Several theories have been proposed to explain how drug-associated stimuli influence and control drug-seeking behavior, resulting in compulsive drug seeking despite the threat of negative consequences, decreased pleasure derived from the drug, or a desire to abstain. In this set of experiments, I have explored two of these theories – the incentive sensitization theory and the habit learning theory.

## **6.1 Cocaine and Incentive Sensitization**

In chapters 2, 3, and 5, we have explored the capacity for cocaine to alter incentive motivation using the PIT procedure. We have tested the predictions of the incentive sensitization theory, which suggests that drug exposure induces alterations in the mesotelencephalic dopamine system that are responsible for the attribution of incentive salience to outcomes and their associated stimuli. According to the theory, these neuroadaptations sensitize this system, which increases the incentive motivational properties of drugs and drug-paired cues, leading to incessant motivation to obtain the drug, which results in addiction (Robinson and Berridge, 1993). In these studies, we have investigated the ability of cocaine treatment to alter incentive motivation for cocaine (Chapter 2), and for food rewards (Chapters 3 and 5), and explored the role of response contingency of cocaine delivery in generating these effects.

### *Cocaine-paired cues enhance incentive motivation for cocaine*

In chapter 2, we utilized the PIT paradigm and the seeking-taking chain to investigate the ability of cocaine paired cues to affect drug-seeking and drug-taking actions. This design allowed us to determine the mechanism by which drug-paired cues contribute to drug-motivated actions, and furthermore to determine which aspect of instrumental motivation – drug-seeking or drug-taking – is most affected by a drug-paired cue. This study revealed that cocaine paired cues exert their influence over instrumental actions to acquire the drug via a Pavlovian incentive motivational mechanism, which is further reflected by the cocaine-paired cue's prolonged enhancement of the taking action.

The cue-induced reinstatement paradigm is commonly used to assess the effects of a drug-paired cue on drug-motivated actions. This procedure is a reliable and effect way to generate strong cue-evoked reward seeking behavior after extinction. Despite its strengths, this procedure allows for a number of mechanisms by which these cues reinstate drug-seeking behavior, and is not particularly useful for targeting the Pavlovian incentive motivation process that allows reward-paired cues to provoke and invigorate reward seeking. Since the cue is present during instrumental training, either in a response-contingent manner (Fuchs et al., 2004; Lee et al., 2006; Cooper et al., 2007) or as a discriminative stimulus (Weiss et al., 2001; Yun and Fields, 2003), the Pavlovian and instrumental contingencies are confounded. Thus, the stimulus becomes directly associated with the drug-seeking action, and could therefore be reinforcing the drug-seeking action through new learning. This learning could take the form of conditioned reinforcement, in which the cue has acquired incentive value through its association with the drug-seeking action, or conditioned habits, in which the cue is able to trigger drug-seeking behavior due to the development of a stimulus-response relationship. In Chapter 2, we have demonstrated that the drug-paired cue can alter drug-motivated behavior due to a Pavlovian motivational process, without the possibility of conditioned reinforcement or

conditioned habits contributing to the cue's effect. Furthermore, using PIT, we have demonstrated the ability of the cue to provoke drug seeking rather than reinforce it.

The seeking-taking chain is a beneficial behavioral paradigm for dissecting motivational properties of instrumental actions, since it has been shown that performance of each of these actions is governed by different aspects of motivation (Balleine, 1995). Importantly, responding on the taking action seems to reflect Pavlovian incentive motivation, since food-paired cues enhanced performance of the taking action (Corbit and Balleine, 2003). Our results are similar, demonstrating that cocaine-paired cues have a prolonged and somewhat greater effect on the taking action than the seeking action, and thus a greater effect on Pavlovian incentive motivation. This pattern of action distribution, with greater and more persistent responding on the taking lever, could be attributed to the continuous access to both levers, since the taking lever is more closely associated with the outcome delivery. To determine whether subjects continued to perform the seeking-taking chain at test, we performed an analysis of the transitions between levers. As Corbit and Balleine found, subjects were significantly more likely to transition from the seeking lever to the taking lever, the order of actions learned during training. This study identified the conditions necessary to obtain PIT with intravenous cocaine as the outcome, and clarified the motivational mechanism by which cocaine-paired cues influence cocaine-motivated behavior. While we suggest that these results indicate that cocaine-paired cues are more likely to affect the consumption of the drug if it has already been acquired rather than to seek out the drug, this may seem counter to the human condition and should be considered with some caution.

#### *Experimenter-delivered cocaine increases incentive motivation for food rewards*

Chapter 2 demonstrated that cocaine exposure alters incentive motivation for the drug itself, but cocaine exposure could also have more global effects, altering incentive motivation for rewards in general. The premise behind the incentive sensitization theory suggests that this



sort of general reward processing could be affected by drug treatment, and recent studies have supported this effect. Amphetamine exposure has been found to enhance Pavlovian incentive motivation for food rewards (Nelson and Killcross, 2006; Nordquist et al., 2007; Shiflett, 2012), as has self-administered cocaine (Saddoris et al., 2011). In Chapter 3, we have shown that experimenter-delivered cocaine also increases Pavlovian incentive motivation for food rewards.

As reported in Chapter 4, the cocaine treated subjects in Chapter 3 showed an increase in magazine approach behavior during cocaine sensitization that was not apparent in the vehicle group. Importantly, this effect was not simply due to an increase in locomotor behavior, because the cocaine-treated subjects in Chapter 4 exhibited an increase in locomotor behavior without any increase in magazine approach compared to vehicle treated rats. Since subjects from Chapter 4 received drug treatment before any training with the food outcome, the magazine was not yet associated with the delivery of food pellets, and thus did not elicit any Pavlovian approach behavior. Any checking of the magazine was likely associated with locomotor or exploratory behavior. However, in Chapter 3, drug treatment occurred after Pavlovian and instrumental conditioning, and so the magazine would be associated with food delivery. These results suggest that cocaine treatment increases incentive motivation for food rewards by eliciting Pavlovian food-motivated behavior.

In addition to the effect of cocaine during the drug treatment phase, cocaine exposure also affected Pavlovian incentive motivation for food rewards after sensitization, with cocaine-treated animals demonstrating a PIT effect that is not evident in vehicle-treated subjects. Though only the cocaine treated animals showed an increase in lever pressing behavior during the CS+, both the cocaine and vehicle groups showed a similar increase in magazine approach behavior and locomotor behavior, demonstrating that both groups experienced an activational effect of the reward-paired cue that elicited the trained Pavlovian response. One possible explanation is that cocaine treatment, via sensitization of the dopamine system, may be resulting in more active, effortful reward seeking. Studies have shown that effort-based decision

making depend on dopamine (Depoortere et al., 1993; Walton et al., 2006; Salamone et al., 2007; Barbano et al., 2009), so enhancement of the dopamine system via sensitization may also enhance the effort exerted to acquire an outcome. In some cases, treatment with psychostimulants have been shown to increase performance on an action with a higher response requirement (Wardle et al., 2011) and increase break-points in a progressive ratio task for food rewards (Jones et al., 1995; Brown and Stephens, 2002). Therefore, cocaine exposure may be sensitizing the dopamine system in such a way that when the CS+ is presented, cocaine treated rats are more willing to exert the effort to press the lever to try to obtain the outcome than vehicle treated rats.

This theory may also be supported by our results in Chapter 4 showing that cocaine treated rats exhibit a higher lever press to magazine entry ratio during training on an interval schedule of reinforcement. This task should favor a low lever press to magazine entry ratio, since animals should learn that delivery of the reward occurs after a period of time has elapsed (30s) and therefore check relatively frequently for the delivery of the reward. However, the cocaine treated rats persist in lever pressing, which may be a reflection of an increased willingness to exert effort to obtain the outcome, potentially due to a lack of behavioral control. Another possible explanation for the results of Chapter 3 is that cocaine exposure is enhancing the incentive motivational properties of the food-paired cue in such a way that the cue is then able to more powerfully induce reward-seeking behavior. This would potentially explain our findings on cocaine exposure with PIT and habit learning, perhaps providing a unified explanation for the effect of cocaine treatment on cue-motivated behavior. This theory will be discussed further in a later section.

#### *Self-administered cocaine increases incentive motivation for food rewards*

Finally, in Chapter 5, we explored the importance of contingency of drug delivery on cocaine's influence over motivation. While we have shown that experimenter delivered cocaine

can enhance PIT for food rewards, there is reason to believe that response-contingent, self-administered cocaine could have a greater effect. Since drugs of abuse are increasing the sensitivity to reward-paired cues by sensitizing the dopamine system, the neurotransmitter system critical for PIT, and since studies have found a greater effect of self-administered than experimenter-administered drugs on alterations to the dopamine system (Hemby et al., 1997; Stefanski et al., 1999; Stefański et al., 2007; Chen et al., 2008), this increased effect of self-administration over experimenter-delivery cocaine on the dopamine system could result in a stronger PIT effect. I found that self-administered cocaine produced a significant PIT effect, whereas yoked cocaine and yoked saline did not. While others have shown a similar potentiation of food PIT with self-administered cocaine (Saddoris et al., 2011), this study shows a difference in motivated behavior produced by self-administered and yoked cocaine, and could indicate a pivotal role for predictability or volitional control in cocaine's effects on incentive motivation.

Many of the studies that use a yoked cocaine control group do not include a cue that predicts cocaine delivery (Hemby et al., 1997; Kimmel et al., 2005; Donny et al., 2006; Larson et al., 2010), so it is not possible to determine whether the differences found between the self-administration group and the yoked group are due to control over drug-taking or due to predictability of the delivery of the drug. It is likely that both processes may be contributing to the differences that have been found between these groups. A study measuring neural temperature (believed to be a reflection of neural activity) in which the delivery of cocaine was cued for the yoked group found a similar pattern of temperature changes between the groups, but a greater degree of changes as well as an anticipatory temperature increase at the beginning of the session in the self-administration group (Kiyatkin and Brown, 2004), possibly indicating that control of drug-delivery is responsible for differences between self-administered and yoked cocaine. However, another study that also signaled the delivery of cocaine to the yoked group found no difference between the yoked cocaine or self-administered cocaine

groups on behavioral sensitization or the increase in dopamine release in the NAcc in response to cocaine (Zapata et al., 2003), suggesting that predictability may be the important factor. The importance of cues that predict the delivery of drug has also been demonstrated with alcohol (Weise-Kelly and Siegel, 2001). Since dopamine signaling may reflect the degree to which cues predict the delivery of reward (Schultz et al., 1997), the predictability of cocaine delivery could be responsible for the greater alterations in the dopamine system created by self-administration over yoked delivery (Hemby et al., 1997; Stefański et al., 2007; Chen et al., 2008). Furthermore, these changes in the dopamine system could be responsible for incentive sensitization, thereby producing the increased incentive motivation for food rewards in the self-administration group compared to the yoked cocaine group seen in Chapter 5.

If predictability is a critical factor, this could explain the how experimenter-delivered cocaine produced PIT whereas unsignaled yoked cocaine did not. Cues associated with the injection (altered handling, the sensation of the injection) are very salient, and can serve as a strong predictor of following pharmacological and psychological effects of the drug (Cepeda-Benito and Tiffany, 1995). Additionally, the experience produced by an intraperitoneal injection as opposed to an intravenous injection is significantly different in regards to the temporal dynamics and pharmacokinetics of its effects (Ma et al., 1999). It would not be surprising then that these methods of delivery also differ on their psychological experience and effects on neural circuitry (Chen et al., 2008), leading to a difference in their ability to alter incentive motivation for food rewards. In fact, a number of studies have shown that the yoked delivery of cocaine is aversive (Mutschler and Miczek, 1998; Twining et al., 2009), inducing heightened cardiovascular effects in humans (Donny et al., 2006) and increased mortality in rats (Dworkin et al., 1995) compared to self-administered cocaine. It could be these negative qualities of the yoked-cocaine experience that prevent this treatment from increasing the incentive motivation for food rewards.

## 6.2 Cocaine and Control of Action Selection

Repeated exposure to drugs of abuse can alter reward learning processes, such that goal-directed actions transition into S-R habits, resulting in compulsive drug-seeking despite decreased enjoyment derived from taking the drug or the threat of punishment (Everitt and Robbins, 2005). A number of studies have attempted to study this effect with a drug as the outcome motivating behavior; however, there are some issues with these procedures. Firstly, habitual behavior is often demonstrated through a devaluation of the outcome, and while studies have used footshock (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004), taking lever extinction (Zapata et al., 2010), and oral drug delivery paired with a distinctive flavor that is later devalued (Miles et al., 2003) as methods of 'devaluation', these procedures are not truly altering the value of the drug, but rather pairing it with punishment or signaling that the response to acquire the drug is ineffective. Secondly, it is difficult to evaluate the effects of drug sensitization on drug-seeking behavior since the outcome of behavioral training and sensitization are confounded. The drug administration and target response cannot be separated, making it difficult to determine whether the effects obtained are due to drug-specific processes (e.g., that drug's ability to reinforce the associations that mediate drug-seeking or that drug value as a goal of voluntary action selection -- i.e., instrumental incentive learning) or more general alterations in behavioral control or incentive motivation. Since drugs of abuse are affecting the same circuitry that is involved in normal learning for natural rewards, exposure to drugs could alter how food-motivated actions are learned. This effect can be tested indirectly using food-motivated behavior, since food outcome devaluation procedures have been well established (Adams and Dickinson, 1981; Dickinson et al., 1983). In Chapters 3 and 4, we explore how cocaine treatment can alter the learning strategy selection for food-motivated behavior.

### *Cocaine exposure promotes habitual control of food-motivated behavior*

In Chapter 3, we examined whether experimenter-delivered cocaine could facilitate habitual food-seeking behavior as demonstrated by an insensitivity to outcome devaluation. We found that cocaine sensitization biased rats towards a habitual action selection strategy, responding to the same degree whether the trained outcome or the alternative outcome was preferred while the vehicle group showed a significant devaluation effect. This lack of a devaluation effect in the cocaine group was found after a short amount of training that would normally result in goal-directed performance, so it cannot be attributed to overtraining. Both groups consumed approximately the same amount during the satiety periods, and the trained outcome was counterbalanced, so the effect cannot be attributed to a higher level of general satiety in the cocaine group or a preference for one or the other outcome. Our results with cocaine mirror the findings of similar studies with amphetamine (Nelson and Killcross, 2006; Nordquist et al., 2007), suggesting that psychostimulant sensitization in general is capable of encouraging habitual control of reward-motivated actions.

Interestingly, the insensitivity to devaluation present when no feedback was given remained even after the subjects were allowed to earn the outcome, a condition that normally produces a devaluation effect even in 'habitual' animals. Thus, not only were cocaine treated animals unable to use their knowledge of the value of the outcome to guide reward seeking behavior, but they were also unable to alter their behavior once they could experience the outcome in its devalued state. This is counter to what studies with amphetamine sensitization have found (Nelson and Killcross, 2006), providing a potential difference in the effects of cocaine and amphetamine on the control of food-motivated behavior. Additionally, our results support the theory that repeated drug exposure results in compulsive reward seeking despite decreased enjoyment derived from it (i.e. decreased reward value via satiety). However, it is important to note that our results in rewarded conditions were marginal, with a trend in the

cocaine group towards a devaluation effect, showing that this persistent behavior may not be entirely habitual in nature.

One explanation for these results is that cocaine sensitization is accelerating the rate at which goal-directed behavior transitions into habitual, S-R behavior (Nelson and Killcross, 2006). However, our results from the rewarded test could also be produced if cocaine sensitization is preventing animals from learning to perform behavior in a goal-directed fashion, or preventing subjects from transitioning from a S-R strategy back to an A-O strategy when faced with feedback. To determine which of these possibilities is responsible for our results, we investigated how cocaine sensitization affects a task that strongly favors goal-directed performance even with extensive training.

#### *Cocaine exposure does not prevent learning with a goal-directed strategy*

In Chapter 4, we show that sensitization with cocaine does not prevent animals from learning to perform a task that heavily favors goal-directed behavior using an A-O strategy. Previous studies showing a significant effect of drug exposure on S-R reward learning have used tasks that facilitate the development of a S-R strategy, either employing a Pavlovian learning task (Schoenbaum and Setlow, 2005), or a single lever interval schedule instrumental task (Nelson and Killcross, 2006; Nordquist et al., 2007). In this study, we use a two lever, two outcome task that favors an A-O response strategy, even with extensive training (Colwill, 1985; Colwill and Rescorla, 1988). We find that repeated cocaine treatment does not prevent animals from learning to perform the task in a goal-directed fashion, showing a significant devaluation effect after a short amount of training. This is similar to what has been found with amphetamine treatment on a ratio schedule task that also favors goal-directed behavior (Nordquist et al., 2007). Though this task favors goal-directed behavior even with extensive training, it is possible that repeated cocaine exposure could facilitate the development of a S-R strategy after a sufficient amount of training. Our results do not support this account, instead showing that even

with extensive training, cocaine treated animals remain goal-directed and sensitive to outcome devaluation.

### **6.3 Implications for Addiction**

When performing experiments using animal models of drug-seeking behavior, it is critical to consider their relevance to addiction in humans. Animal models allow us to explore the neural circuitry and motivational components of addiction in ways that are not possible in human studies, allowing for the investigation of specific attributes in a controlled manner. However, because of abstractions of the drug delivery procedure and the distinct possibility of species differences, it is important to confirm when possible that the results obtained from animal studies can be replicated in humans, and to consider the potential for variability when replication is not possible. In this section, the results of the preceding experiments will be discussed in their greater context. The mode and pattern of drug delivery, as well as the context in which the delivery occurs, are important considerations concerning their similarity to human drug-taking behavior. Furthermore, each of these experiments has made contributions to different theories of addiction, which, though often described as competing, are likely working together to produce the many traits of addictive behavior.

#### *Mode of drug delivery*

The results of Chapters 3 and 4 have been obtained using experimenter-delivered cocaine. While this mode of delivery may lack face validity, a large number of studies on the effects of drugs have been conducted by this method, and many of these findings have been validated in human studies (Stolerman and Jarvis, 1995; Laviola et al., 1999; O'Brien and Gardner, 2005), suggesting that this method of drug delivery produces similar changes to the brain and behavior in humans as it does in non-human animals. Self-administration has more face validity, and has been shown to induce greater changes to the dopamine system (Di Ciano



et al., 1996; Hemby et al., 1997; Lecca et al., 2007; Stefański et al., 2007; Chen et al., 2008) and have more significant impacts on motivated behavior (Chapter 5) than unsignaled, non-contingently delivered cocaine using the same route of administration (IV). Many of the self-administration studies with cocaine have used relatively short access (one or two-hour administration periods) (Stuber et al., 2005; Lecca et al., 2007; Chen et al., 2008; Saddoris et al., 2011), but humans often administer cocaine in binges (Gawin, 1991). Though short periods of cocaine self-administration clearly have significant effects, it has been suggested that using a binge method of delivery more closely models human drug use patterns. Several studies on cocaine self-administration have found a significant effect of binge delivery or extended access on cocaine seeking, reinstatement, and neural changes (Hemby et al., 1997; Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004; Kippin et al., 2006), suggesting that binge exposure might produce stronger effects than more limited access. Future studies should utilize the extended access or binge style of drug delivery to determine if it produces greater effects on incentive sensitization and produces faster or stronger S-R control of goal-directed actions.

### *The context of drug delivery*

Studies of drug addiction in humans have revealed that the context in which drugs are delivered can come to acquire conditioned incentive properties and consequently have significant influence over drug-seeking behavior (Wikler, 1973; O'Brien et al., 1992). Animal models have demonstrated a similar effect with conditioned place preference (CPP) and context-induced reinstatement. The CPP paradigm relies on the idea that a discriminable environment, when paired with an outcome that is reinforcing or rewarding, will come to elicit a preference for the reward-paired context as displayed by a greater amount of time spent there (Mucha et al., 1982), and it has been used extensively in the addiction literature (Tzschentke, 2007). Self-administration studies have also shown context to be a powerful mediator of drug-seeking behavior, since the drug-paired context is able to reinstate drug-seeking behavior after

the instrumental response has been extinguished (Post et al., 1981; Crombag et al., 2008b), modeling how drug-paired contextual cues can induce craving and relapse in human subjects. In Chapters 2, 3 and 4, the instrumental training and testing are occurring in the same environment as the drug-delivery. Since cocaine was the reinforcer in Chapter 2 this was unavoidable, but it is important to consider that our results could reflect an involvement of the cocaine-paired context in facilitating PIT. In Chapters 3 and 4, it is likely that context is playing a role in the effects of cocaine on food-motivated behaviors, though we are unable to draw this conclusion from our results alone. Studies with amphetamine have shown that receiving the drug treatment in the locomotor training and testing environment increases their behavior (Robinson et al., 1998), showing that both the induction and expression of behavioral sensitization are context-dependent.

The results of this study by Robinson et al. also suggest that experiencing amphetamine in a novel environment enhances the expression of behavioral sensitization. Interestingly, rats prefer to self-administer cocaine in novel (non-home cage) environments (Caprioli et al., 2007), and this preference has also been shown in human subjects (Caprioli et al., 2009). This findings could contribute to the explanation of results obtained in Chapter 5, since cocaine was self-administered in a novel context that was distinct from both the home cage and the chamber in which instrumental training and testing for food rewards occurs. Interestingly, even though a strong role of context in locomotor sensitization has been shown, the self-administration group still showed an enhancement of PIT despite being exposed to cocaine in an alternative context. Future studies should systematically examine the contributions of context to the effects of both experimenter-delivered and self-administered cocaine on incentive sensitization and habit learning. Importantly, these studies should distinguish between the effects of novelty and context, since previous studies have consistently compared a novel environment to a familiar home cage environment, confounding the role of novelty/familiarity with the role of context.

### *Evidence for incentive sensitization and habit in human addiction studies*

The incentive sensitization theory was based on clinical observations in human addicts, such as an increased desire for or craving for a drug despite decreased pleasure derived from consuming it (Fischman et al., 1985), which could be the result of increased value placed on the drug. An increased value of drug-paired cues could also lead to craving and relapse, as cue-induced drug craving has been well documented (Wise, 1988; Childress et al., 1993). While some aspects of the theory are supported in the human research literature, other aspects of the theory, such as persistent increases in dopamine neurotransmission and behavioral sensitization, have not been easily reproduced. Behavioral sensitization had been a purely animal phenomenon for a number of years, but recent studies have now shown evidence of sensitization in humans (Strakowski et al., 1996; Strakowski and Sax, 1998; Leyton, 2007). Not only can psychostimulant treatment produce behavioral sensitization in humans, but it also may produce dopamine sensitization in humans. Three separate injections of amphetamine induced an increase in psychomotor responses and dopamine signaling in the ventral striatum that remained significantly increased at 2 weeks and even 1 year after the drug treatment (Boileau et al., 2006). The ventral striatum, or nucleus accumbens, is a region that has been suggested to be critically involved in incentive sensitization. Increased dopamine transmission in the ventral striatum with chronic treatment is controversial, since the opposite was found in other neuroimaging studies (Volkow et al., 2004a) and there was a reduced tissue content of DA in post-mortem tissue from amphetamine users (Wilson et al., 1996).

Though support in the human literature for some features of the incentive sensitization theory may be controversial, the evidence in support of habit learning is limited and indirect, since drug devaluation is difficult to achieve. This theory is also based on clinical observations that drug use becomes compulsive, with subjects continuing to use the drug despite decreased enjoyment derived from it and the possibility of severe negative consequences, but suggests that these traits are due to the formation of a compulsive drug taking habit that makes the user

insensitive to the consequences of continued use. It has been found that chronic treatment with psychostimulants induces prolonged increases to dopaminergic neurotransmission in the dorsal striatum/putamen in response to drug-associated cues (Volkow et al., 2006; Wong et al., 2006), in an area known to be involved in S-R, habitual control of action selection (Yin et al., 2004). Additionally, the insensitivity to devaluation shown in rodents after extensive training has recently been shown to occur in humans as well, and to also depend on the dorsolateral striatum (Tricomi et al., 2012), confirming the validity of the animal model with food rewards at least. Finally, cue-induced drug craving has been correlated with dopamine in the dorsal, but not ventral, striatum in human cocaine addicts (Volkow et al., 2006), which raises the possibility that these cues are inducing S-R associations that are contributing to the motivation to take the drug. However, the dorsal striatum could also be involved in incentive motivation, and it is important to note that no direct test of the habit learning theory has been conducted with human addicts.

#### *Incentive sensitization, habit, and the power of reward-paired stimuli*

Our results have provided support for both the incentive sensitization theory and the habit theory of addiction, demonstrating that repeated exposure to cocaine can enhance the ability of cues associated with a reward (drug or food) to elicit reward-taking behavior (for both cocaine and food) and also subvert control of normal reward learning in favor of S-R habits. While our experiments have tested these theories separately, they may be contributing to different aspects of addiction, or they may each be responsible for different stages of the addiction process. For instance, it has been argued that while exaggerated habits may contribute to drug-taking or consumption, it is the sensitization of incentive motivation that maintains compulsive drug-seeking and disrupts attempts to abstain (Robinson and Berridge, 2008). Conversely, studies have shown that compulsive drug-seeking behavior can be induced independently of any increased motivation for the drug, by producing habitual control of

behavior (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Belin et al., 2009). Cocaine-induced increases in dopamine levels in the NAcc play a major role in the initial reinforcing effects of cocaine (Pierce and Kumaresan, 2006), supporting studies that have found an initial role of the ventral striatum in drug-seeking that later comes to rely on dorsal striatal regions (Porrino et al., 2004; Porrino et al., 2007), potentially through the activation of the corticostriatal loop circuitry (Haber et al., 2000). Based on these results, some have suggested that it is in fact increased incentive motivation for the drug that develops first, but it is the habitual, S-R action selection strategy that develops later that facilitates compulsive drug-seeking (Pierce and Vanderschuren, 2010).

Though our results, along with a significant body of animal literature, have shown that treatment with psychostimulants can enhance motivation for food rewards, human literature has found that addicts have a decreased appreciation for natural rewards such as money and sex (Garavan et al., 2000; Martin-Soelch et al., 2012). In addition, stimuli associated with food and drug rewards can differentially activate the same brain region (Baunez et al., 2005; Di Chiara and Bassareo, 2007). While this may seem contradictory, it could be due to the time frame of changes in the dopamine system during the process of addiction. It has been firmly established that acute use of psychostimulants causes increases in dopamine release in the NAcc (Ritz et al., 1987; Hurd and Ungerstedt, 1989), but this effect reverses with chronic use, with studies showing a decrease in dopamine release and D2 receptors in the NAcc (Volkow et al., 2004b). During the initial stages while dopamine in the NAcc is increased, motivation for rewards in general may be increased, which would support our results with food outcomes after the duration of cocaine treatment we used. Our results are also supported by studies showing a similar change in the dopamine system produced by drug addiction and obesity in humans (Wang et al., 2001) and a continued preference for saccharin under cocaine intoxication, sensitization or intake escalation in rodents (Lenoir et al., 2007). Since alterations to dopamine in the dorsal striatum seem to develop later in addiction after prolonged use, this could also

explain our results from Chapter 4. Since the cocaine treatment used was of relatively short duration, it might have been adequate to encourage habitual behavior under conditions that favor S-R control (Chapter 3), but not sufficient to induce habitual control of a strongly goal-directed task (Chapter 4).

Finally, while incentive sensitization and habit learning may be making stage-dependent contributions to addiction, they could also be having a common action through enhancing the power of drug-associated stimuli over drug-seeking actions. The incentive sensitization theory predicts that repeated exposure to the drug is going to increase the incentive motivation produced by drug-paired stimuli (Robinson and Berridge, 1993), which could result from an increase in the value of the cues. In addition to drug exposure increasing the salience of drug-paired stimuli, it may also be increasing the ability of these cues to exert control over behavior, facilitating S-R learning as the habit theory predicts (Everitt and Robbins, 2005), which has been suggested previously (Nordquist et al., 2007). This S-R process that contributes to compulsive drug seeking could either be a consequence of the increased value or salience of the stimuli, or a separate process. To distinguish between the contributions being made by these theories, behavioral tasks should be developed that can separate the motivational explanation from the habit learning explanation with distinct predictions. In addition to incentive sensitization and habit learning, other processes are likely to be significant factors, such as the decreased inhibitory control due to a decreased activation of fronto-striatal circuits in addicts (Jentsch and Taylor, 1999). In addition, the allostatic set-point of mood may be altered, in which the addict is in a negative mood state without the drug, and must use the drug in order to achieve a feeling of normalcy (Koob and Le Moal, 2001). Regardless, it is likely that both incentive sensitization and habit learning are contributing to the formation of addiction.

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