THE SPEED OF INTRAVENOUS COCAINE DELIVERY ALTERS ITS EFFECT ON THE BRAIN AND DRUG-TAKING BEHAVIOR: IMPLICATIONS FOR ADDICTION LIABILITY

By

Ken Taro Wakabayashi

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Neuroscience)
in The University of Michigan
2010

Doctoral Committee:

      Professor Terry E. Robinson, Chair
      Professor Jill B. Becker
      Professor Kent C. Berridge
      Professor Martin F. Sarter

DEDICATION

Dedicated with love and gratitude to my mother and father, Yumiko and Akio.

ACKNOWLEDGEMENTS

It is with profound gratitude that I thank my advisor and mentor, Dr. Terry Robinson. Training, as with all things in life, is a path and journey. Thank you for letting me get lost among the trees a few times along the way, and providing the wisdom, experience, guidance, and above all the patience to let me find my way back each time. You have taught me so much about how to be a scientist, words seem hardly adequate. Thank you.

I would also like to thank the members of my doctoral committee for their service. I would like to thank Dr. Jill Becker, who always challenged me to think about my project in novel ways and to always keep an open mind. I would like to thank Dr. Kent Berridge for introducing me to the theoretical depth and historical richness of my field, and who always took time to explain things with amazing clarity when I was feeling overwhelmed by the scale of the whole endeavor. I would like to thank Dr. Martin Sarter, whose admonishment to me during my second year of graduate school to hold myself up to a higher standard of scientific understanding has continued to inspire me to push back the boundaries of my ignorance just a little more each day.

My time in graduate school has been shaped by the present and past members of the Robinson Lab, who as colleagues and friends have helped me, taught me, supported me, and encouraged me over the years. In particular, I would like to thank Mark Weiss and Kristin Pickup, who assisted in the experiments presented in this dissertation. Additionally, I would like to thank Dr. Anna-Noël Samaha and Dr. Hans Crombag for their advice and encouragement over the years. Both of your work has served as an inspiration for me during my time in graduate school.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

THE SPEED OF INTRAVENOUS COCAINE DELIVERY ALTERS ITS EFFECT ON THE BRAIN AND DRUG-TAKING BEHAVIOR: IMPLICATIONS FOR ADDICTION LIABILITY

by

Ken Taro Wakabayashi

Chair: Terry E. Robinson

Cocaine addiction in humans is a severe health and social problem. Therefore, understanding how casual use transforms into addiction is critical. One of the many factors that can facilitate addiction is how rapidly drugs like cocaine enter the brain.

Faster rates of cocaine delivery have a greater neurobiological impact on brain reward systems, producing sensitization. This may facilitate the transition to addiction by changing systems in the brain underlying reward. Yet most studies in the rat have shown few effects of rate of delivery on drug-taking behavior. Recently, paradigms have been developed where rats given extended access to take cocaine develop addiction-like behaviors. If the rate of drug delivery influences its addictive liability, it can be predicted that faster rates of cocaine delivery will be associated with a greater neurobiological impact and addiction-like behavior.

The studies in this dissertation tested this prediction. In the first study, fast rates (5 sec) of infusion induced more Fos expression, a marker for neurobiological impact, than slower rates (25 – 100 sec). This effect was equally evident in the patch and matrix subcompartments of the striatum, a brain reward sub-system implicated in addiction,

suggesting that fast rates of infusion had a widespread impact on this structure. In the second study, faster rates (5 – 45 sec) of cocaine infusion facilitated an escalation in overall drug intake in contrast to slower rates (90 sec) when rats were given extended, but not limited, access to cocaine. In the third study, fast (5 sec) rates of cocaine infusion during extended access self- administration was associated with a persistence to reinstate drug-seeking behavior in response to a drug induced priming injection 45 days after their last self-administration session. This behavior in the 5 sec group was also associated with more persistent neuroadaptations in the brain.

Thus, faster rates of cocaine infusion have a greater and more persistent impact on brain reward systems, and facilitate the development of behaviors that resemble addiction. This may be one reason why routes of administration that result in the rapid entry of cocaine into the brain, may preferentially promote the transition to addiction.

CHAPTER 1

INTRODUCTION

Overview

Given that addiction is such a widespread and persistence public health problem, it is important to understand the mechanisms responsible for the transition from casual drug use to addiction. However, this is still poorly understood. Many factors such as those related to the individual and the environment contribute to addiction liability, but here I will focus on factors intrinsic to the drug, drug formulation and/or route of administration. In particular, the rate at which one drug, cocaine, reaches the brain will be discussed in detail as the rate at which cocaine is delivered influences both its behavioral and neurobiological effects. This may be why certain forms of cocaine that result in faster absorption into the brain are more addicting than forms resulting in slower uptake. Although there is some epidemiological and clinical evidence supporting this hypothesis in humans, very little basic research has been conducted on exactly how the rate of cocaine delivery influences the behavioral and neurobiological actions of cocaine. Recently, animal models of drug-taking that can produce addiction-like symptoms have been developed. Using one of these models, experiments in this dissertation will attempt to clarify how the rate of cocaine delivery influences behavioral and neurobiological processes that may contribute to addiction.

The goal of this Introduction is to provide sufficient background for understanding the scope and intent of these experiments. Thus, the Introduction will begin with a review of addiction, cocaine and its properties as a drug of abuse, including its acute and chronic actions in the brain and its impact on behavior. I will examine the current evidence that the rate of cocaine delivery influences its neurobiological and behavioral effects. I will then discuss the use and implications of changes in neuronal

gene expression as a method to measure the impact of cocaine on the brain. Finally I will introduce self-administration procedures in rats that produce symptoms of addiction similar to those in humans, the use of which may further expand our understanding of how rate of infusion influences cocaine's addictive potential.

What is addiction?

Drug addiction in humans is often defined as a pattern of compulsive drug-taking behavior (Robinson and Berridge, 1993; American Psychiatric, 2000). Although the addiction liability of a compound has often been determined in animals by testing its reinforcing effects – that is, whether or not the compound is reliably self-administered (Johanson and Balster, 1978; Johanson, 1984), taking drugs alone is not addiction. Human addiction refers to a complex array of symptoms in addition to compulsive use (Gawin and Kleber, 1988; Gawin and Ellinwood, 1989; Robinson and Berridge, 1993; American Psychiatric, 2000; Deroche-Gamonet et al., 2004; Robinson, 2004). Some of these hallmark symptoms of addiction include a marked increase in drug intake (Ahmed and Koob, 1998), the development a much greater 'wanting' or craving of drug (Hyman, 2001; Preston et al., 2009) than non-addict subjects (Walsh et al., 2009), so much so that the motivation for drug becomes pathological (Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Belin et al., 2008; Walsh et al., 2009) even when drug is not available (Deroche-Gamonet et al., 2004; Robinson, 2004). Other symptoms of addiction include continued drug seeking or taking in the face of adverse consequences (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Pelloux et al., 2007; Belin et al., 2008), and a persistent vulnerability towards relapse even after periods of prolonged abstinence (O'Brien, 1997; Deroche-Gamonet et al., 2004; Knackstedt and Kalivas, 2007; Belin et al., 2008; Walsh et al., 2009). Thus only when repeated drug self-administration gives rise to a constellation of these and other symptoms is a subject considered "addicted" (Deroche-Gamonet et al., 2004; Robinson, 2004; Vanderschuren and Everitt, 2004; Belin et al., 2008).

Factors that can influence addiction liability of cocaine

Not everyone who initiates drug use transitions to addiction (Robinson and Berridge, 1993). In fact, many factors contribute towards the addiction liability of drugs such as cocaine in humans. A few of the factors mediating addiction liability are stress (Bell, 1971; Briand and Blendy, 2009), genetic differences (Enoch and Goldman, 1999), gender (Becker, 2009), age (Hser et al., 2008), and environmental influences (Robins et al., 1975). In addition to these factors, pharmacokinetic factors intrinsic to the drug itself, the drug formulation, and/or route of administration also contribute towards addiction liability. Thus, drugs or drug formulations that reach the brain rapidly are thought to have the greatest potential to produce addiction. This will be the main focus of this dissertation.

A brief synopsis of the impact of formulation and route of administration on the history of cocaine addiction

One drug that differs in its addictive liability depending on its formulation and route of administration into the body is the naturally occurring alkaloid cocaine. In its native form, cocaine is extracted from the coca plant *Erythroxylon coca* (Gay et al., 1975), a plant that has been cultivated in South America from at least the 6th century (Carroll, 1977; Petersen, 1977). In this form, cocaine is self-administered by humans by chewing the coca leaf along with an alkali and binder (Gay et al., 1975). In this form, the amount of cocaine absorbed is very low (Gay et al., 1975), and the rate of absorption through the oral mucous membrane is very slow (Fischman, 1984). When taken this way, there is little evidence for toxic effects or coca leaf addiction (Carroll, 1977; Jones, 1984). Although the euphorigenic properties of the coca plant have been documented as early as 1596 by the Spanish (Petersen, 1977), the use of cocaine became more widespread after it was chemically isolated and purified in the 1860's by Albert Niemann and others (Niemann, 1860; Petersen, 1977). At the time, the availability of more purified and concentrated forms of cocaine that could be administered more rapidly into the body

3

such as by insufflation led to increasing reports of problems, including addiction and fatal overdose (Gay et al., 1975; Petersen, 1977). However, despite subsequent regulation (Gay et al., 1975; Gawin and Ellinwood, 1989), debate over cocaine's addictive liability persisted into the early 1980's (Gawin and Kleber, 1988). Part of this skepticism was due to the unique clinical symptoms of cocaine abstinence, which in contrast to opiates and alcohol, lacked physiological withdrawal symptoms (Gawin and Kleber, 1988). In addition, only some people developed severe symptoms of addiction (Petersen, 1977). Although many factors influence the transition to addiction, one possible explanation for this trend may have been that in the late 1970s and early 1980s the majority of cocaine in the United States was taken intranasally ("snorting"), a moderate rate of administration resulting in relatively slow delivery of drug to the brain (Hatsukami and Fischman, 1996).

However, during the same time period, a few users injected cocaine intravenously (i.v.) or converted the water soluble form through an involved and dangerous "gourmet" process into a smokable base form (Hatsukami and Fischman, 1996). Smoking free base or i.v. injection results in cocaine being delivered more rapidly to the brain (Jones, 1990), and people using these very fast routes of administration began reporting greater euphorigenic effects: smoking freebase cocaine was described at that time as the "ultimate high" or the "greatest thing since sex" (Siegel, 1984). Reports by the beginning of the 1980s began documenting greater toxicity and escalating patterns of use associated with intravenous and smoking free-base cocaine (Siegel, 1984), relative to that seen with "snorting" cocaine hydrochloride.

Cocaine use underwent a demographic shift in the United States and other parts of the world during the mid-1980s when reports of a more easily producible freebase form of cocaine, "crack", first appeared in the Bahamas in 1983 (Jekel et al., 1986). In addition to differences in ethnicity and socioeconomic status, crack cocaine evolved its own distinct distribution and use patterns from cocaine hydrochloride. Although crack cocaine was sold at the retail level in a number of ways, studies interviewing individual users reported that the "crack house" was the method most often used to procure crack

cocaine (Mieczkowski, 1990). Although these crack houses all provided a milieu where crack cocaine could be bought and consumed, Gerter (1994) proposed a spectrum of crack house types, ranging from well kept semiprivate "party houses" catering to working class clients intent on the pleasure-seeking and social aspects of drug, to so called "bandominiums" (i.e. abandoned condominium) where poverty stricken addicts whose desire for drug was so strong that they could tolerate drug-taking in an extremely dangerous environment surrounded by aversive and practically unlivable conditions (Geter, 1994). Additionally, many crack houses provided a permanent and fixed locale where addicts could binge on crack cocaine. For example, Mieczowski (1990) reported that "customers" of these crack houses would "meet, smoke, exhaust their funds, conspire, leave, commit a property crime, return with merchandise, exchange it for crack, and the begin the whole process over again."

Initially, the rapid increase in crack cocaine use, especially by ethnic minorities, was largely attributed to its perceived lower market price and greater availability (Jekel et al., 1986; Hatsukami and Fischman, 1996). However, others such as Caulkins (1997) found no differences in price per unit between cocaine hydrochloride and crack cocaine, suggesting that other factors were responsible for its increasingly widespread use (Karch, 1999). Specifically, the ability of "crack" cocaine to be delivered to the brain more rapidly was proposed to be a major contributing factor to its increased abuse liability (Jones, 1990; Hatsukami and Fischman, 1996; Karch, 1999).

Although much of the data on increased addictive liability of crack cocaine in humans is anecdotal, several studies have supported this hypothesis. In one survey of cocaine addicts undergoing inpatient treatment for cocaine addiction, Gorelick (1992) found that cocaine smokers progressed more quickly from initial use to other stages of cocaine use than users whose first use was through insufflation. For example, the time between initial and regular use of the drug was significantly shorter in patients who initiated their use by smoking. In addition, smokers comparatively experienced their first cocaine-related intrapersonal conflict sooner than those who began by snorting cocaine. Epidemiological studies surveying a larger number of subjects have found that those who

5

initiate their cocaine use through smoking crack cocaine are more likely to develop clinical symptoms of addiction than users who initiate their use the intranasal route (Chen and Anthony, 2004). A more recent study reports that subjects who initiate their cocaine use either by crack or i.v. injection have a higher relative risk of developing symptoms of dependence within two years of initiating cocaine use (O'Brien and Anthony, 2005). In a related vein, others have found that many cocaine users transition from an initially slower intranasal route of administration to a more rapid route of infusion such as smoking or intravenous injection as their addiction progresses (Gorelick, 1992; Dunn and Laranjeira, 1999).

Today, the use of cocaine hydrochloride and crack cocaine is acknowledged to be a persistent contemporary public health problem. For example, in 2008 the prevalence of cocaine use (including crack) in the previous month amongst people 12 years or older in the United States was projected to be 1.9 million people (National Survey on Drug Use and Health). Clearly, there is considerable interest in understanding factors that influence cocaine's addictive liability, such as how rapidly it reaches the brain.

The acute behavioral and physiological effects of cocaine

Euphoric and reinforcing effects

In humans, cocaine has potent euphorigenic effects that are well documented historically (e.g. Freud 1884) and clinically depending on the rate and dose of drug (Resnick et al., 1977; Fischman, 1984; Volkow et al., 2000; Abreu et al., 2001; Nelson et al., 2006). Cocaine is highly reinforcing in animals based on a number of different tests. Importantly, animals can be easily trained to self-administer cocaine over a number of different operant rules that govern the relationship between the required behavior and the delivery of cocaine (Deneau and Seevers, 1964; Pickens and Thompson, 1968; Wilson et al., 1971; Johanson, 1984). For example, animals will administer cocaine over Fixed Ratio (FR) schedules, where a certain number of responses are required before cocaine is delivered (e.g. Wilson et al., 1971 ; Downs & Woods 1974), Fixed Interval (FI) schedules where reward is available at certain time intervals (e.g. Balster and Schuster 1973,

Goldberg and Kelleher 1976), and progressive ratio schedules (PR), were each successive drug delivery requires an increasingly larger number of behavioral responses (Griffiths et al., 1975). Cocaine is also reinforcing in a conditioned place preference paradigm (e.g. Mucha et al., 1982; Morency and Beninger 1986). In this paradigm, animals are administered drug in test chambers with distinct spatial and environmental cues. In later tests, the environment where the drug was experienced becomes preferred over one that was not.

Peripheral effects

Acute cocaine administration has many physiological effects in the periphery that are dose dependent. In rodents and in primates, cocaine produces pupil dilation as well as, increases in heart rate and respiration, blood pressure, and body temperature. At higher doses cocaine is also a convulsant, and may induce respiratory depression and cardiovascular collapse. A combination of these effects are responsible for lethal overdose (Woods, 1977).

Psychomotor activation

Given acutely, cocaine induces a range of behaviors termed "psychomotor activation," a term of German etymology meaning "movement induced by psychic or mental action" (Wise and Bozarth, 1987). In animals, acute cocaine results in an increase in locomotion at low doses, and repetitive and stereotyped behaviors at higher doses such as grooming a portion of the body (Woods, 1977); this property is shared by phenylethylamine stimulants like amphetamine, and many other drugs of abuse (Wise and Bozarth, 1987).

The rate of drug delivery can influence cocaine's subjective effects

Why is crack cocaine potentially more addictive than cocaine hydrochloride? Although it is the same drug, crack cocaine is delivered to the brain faster when smoked than when cocaine hydrochloride is insufflated (Jones, 1990; Hatsukami and Fischman, 1996). In human laboratory studies, faster rates of drug delivery produce greater feelings

of euphoria with a variety of drugs, including orally administered pentobarbital (de Wit et al., 1992), i.v. morphine (Marsch et al., 2001), and cocaine (Resnick et al., 1977; Fischman, 1984; Volkow et al., 2000; Abreu et al., 2001; Nelson et al., 2006). Indeed, these results support the widely held hypothesis that faster rates are more addicting – they increase the rewarding properties of the drug (Gorelick, 1998). Some animal studies have supported this hypothesis. In non-human primates, increasing the rate of drug infusion increases the reinforcing effects of cocaine over several different schedules of reinforcement, including during fixed interval (Balster and Schuster, 1973), fixed ratio (Kato et al., 1987; Panlilio et al., 1998), and progressive ratio schedules of reinforcement (Woolverton and Wang, 2004). However, in some of these studies, the rate of drug delivery varied to such an extent that the total amount of drug to reach the brain also varied, confounding these two factors. This topic will be discussed in more detail below.

Faster rates of drug infusion may induce changes in processes in the brain sensitizing incentive motivation

In addition to producing greater euphorigenic effects, Samaha and Robinson (2005) have suggested that faster rates of drug delivery can also preferentially engage and alter the brain, so that systems that mediate the attribution of incentive motivational salience become sensitized – leading to an increased "wanting" of drug or drug related cues.

When given repeated and intermittent injections of cocaine, animals exhibit a progressive increase in psychomotor activation to the same dose of drug (Magos, 1969; Wallach and Gershon, 1971; Segal and Mandell, 1974; Post and Rose, 1976; Eikelboom and Stewart, 1982; Robinson, 1984; Robinson and Becker, 1986). With repeated injections of a relatively low dose, there is a progressive increase in drug-induced locomotion with each successive treatment (Robinson & Berridge 1993). At more moderate doses, similar treatment will begin to elicit an increase in stereotyped behaviors, even when the initial response to the drug is limited to an increase in locomotion (Robinson & Berridge 1993). Similar changes in behavior have also been

observed as a consequence of self-administration (Phillips and Di Ciano, 1996; De Vries et al., 1998; Zapata et al., 2003; Ferrario et al., 2005).

This sensitization to the psychomotor activating effects of cocaine (psychomotor sensitization) is thought to be a behavioral symptom of persistent and long term neuroadaptations in brain systems that are also thought to be important in mediating incentive motivational processes (Robinson and Berridge, 1993, 2000). In support of this idea, rats previously sensitized to cocaine acquire cocaine self-administration at lower doses than control animals (Horger et al., 1990), exhibit greater response for conditioned rewards (Taylor and Horger, 1999), and show greater motivation for cocaine (Lorrain et al., 2000) under a progressive ratio schedule of reinforcement. Rats previously sensitized to amphetamine, cocaine or morphine also show enhanced conditioned place preferences to the same drug, relative to control animals (Lett, 1989; Shippenberg and Heidbreder, 1995). Effects of one drug can generalize in some cases to other drugs. For example, animals previously sensitized to amphetamine will show cross-sensitization in locomotion when subsequently tested with cocaine (Schenk et al., 1991; Hooks et al., 1992; Bonate et al., 1997), suggesting a common neural substrate (Robinson and Berridge, 1993).

If sensitization of incentive motivational processes (i.e. incentive sensitization) underlies addiction, and faster rates of drug delivery are more addicting, then faster rates should be more effective in producing psychomotor sensitization. This hypothesis was tested by Samaha and colleagues in a series of studies by examining the effects of rate of drug delivery on psychomotor sensitization.

Faster rates of infusion are associated with psychomotor sensitization

In an initial study examining the influence of rate of drug delivery on sensitization, Samaha and colleagues (2002) found that faster rates of cocaine delivery are more effective in inducing psychomotor sensitization, as assessed by locomotor activity in neurologically intact rats or by rotational behavior in rats with unilateral lesions of the nigrostriatal dopamine system (Ungerstedt and Arbuthnott, 1970).

Rotational behavior in lesioned rats is an especially sensitive indicator of sensitization (Robinson, 1984; Crombag et al., 1999). Samaha et al. (2002) reported that faster i.v. injections (3 – 16 sec) of 1.0 mg/kg of cocaine during 7 daily treatments produced greater sensitization of rotational behavior by the 7th and last day of treatment than slower rates (37 sec). After 5 days of withdrawal, only rats treated with faster rates (3 – 16 sec) expressed a sensitized response to a challenge infusion of either 5 sec or 34 sec. In neurologically intact rats, rats pretreated with a total of 5 rapid (5 sec) infusions of cocaine spaced several days apart showed a sensitized locomotor response to a challenge infusion of cocaine after 4 days of withdrawal across a range of treatment doses (0.5 – 2 mg/kg). In contrast, animals that had been treated with drug over 45 and 90 sec only showed a sensitized response to a challenge dose when their pretreatment doses were 2.0 mg/kg.

The results of this experiment showed by using two different behavioral measures that variation in the rate of cocaine delivery over a relatively narrow range (3 – 100 sec) had a large effect on its ability to induce psychomotor sensitization, and therefore, presumably to produce the changes in brain responsible for this form of drug-induced plasticity.

The effects of varying rate of infusion on behavior and neurobiology over 5 to 100 sec are not due to differences in the amount of drug reaching the brain

Variation in i.v. cocaine delivery between 5 –100 sec is similar to the variation in rates that addicts report when injecting drugs (Zernig et al., 2003), captures the variation between snorting and smoking (Jones, 1990), and influences the subjective effects of cocaine (Abreu et al., 2001). A very simple explanation for these effects of drug delivery on psychomotor sensitization is that different amounts of cocaine reach the brain, raising the possibility that the rate of cocaine delivery could be conflated with dose effects. However, this is not the case. First, the pharmacokinetics of cocaine are such that varying an i.v. injection between 5 and 100 sec has no effect on the peak amount of cocaine to

reach the brain; see Fig. 1.1 adapted from Samaha et al., (2002); also see Shou et al., (2006). With faster infusions, cocaine levels reach their peak levels more rapidly, as would be expected; but there is no difference in the peak or the total amount of cocaine in brain when rate of infusion is varied between 5 and 100 sec.

Within the brain, cocaine binds to monoamine transporters, preventing reuptake, and thereby increasing the synaptic concentration of dopamine, serotonin, and norepinephrine (Heikkila et al., 1975; Ritz et al., 1987; Giros and Caron, 1993). In particular, its effects on dopamine neurotransmission in the dorsal and ventral striatum are thought to be especially important in producing both its psychomotor activating and reinforcing effects (Roberts et al., 1977; Roberts et al., 1980; Ritz et al., 1987; Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Camp et al., 1994). Thus the influence of cocaine on dopamine "overflow" in the striatum is tightly coupled to its levels in brain (Shou et al., 2006), and therefore one can also ask how the rate of drug delivery influences dopamine levels measured with microdialysis. Microdialysis coupled with on-line capillary electrophoresis and laser induced florescence detection provides the ability to measure dopamine levels with reasonably good temporal resolution (one sample every 90 sec). Using this procedure, Ferrario et al. (2008) recently reported that cocaine given over either 5 or 100 sec proportionately increased dopamine to the same levels in the striatum. As expected from cocaine pharmacokinetics, after a 5 sec infusion, dopamine levels peaked faster than after a 100 sec infusion, but there was no difference in either peak dopamine levels or total dopamine overflow (as measured by area under the curve). (Fig 1.1– also see Shou et al., 2006)

Repeated cocaine and psychomotor sensitization are associated with neurobiological changes in the striatum

Although variation in the rate of drug delivery between 5 – 100 sec results in large differences in psychomotor sensitization, these differences are not due to differences in brain dopamine levels. This suggests that faster rates of drug delivery must influence other neurobiological actions of cocaine.

Animals with a history of chronic cocaine treatment and expressing psychomotor sensitization also have persistent and extensive neurobiological changes in the mesocorticolimbic circuit. For example, repeated non-contingent cocaine treatment is associated with potentiated release of dopamine in the dorsal and ventral striatum after a cocaine challenge (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990; Keller et al., 1992), an effect blocked by dopamine antagonists (Weiss et al., 1989; Kalivas and Stewart, 1991). Microinjections of amphetamine into the ventral tegmental area directly can lead to psychomotor sensitization (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1993; Perugini and Vezina, 1994; Cador et al., 1995; Vezina, 1996) and increased motivation for cocaine during subsequent self-administration under a progressive ratio schedule. An enhanced dopamine release upon a drug challenge is also seen (Vezina, 2004).

Although much of the research on this topic has focused on dopamine, it is important to emphasize that repeated administration of cocaine produces long-lasting changes in nearly every neurotransmitter system studied within these mesocorticolimbic circuits, including norepinephrine, serotonin, acetylcholine, amino acid transmitters, opioid peptides, and many others (Goodman, 2008). This may be because cocaine fundamentally alters the organization of these circuits, as indicated by changes in dendritic spine density and dendritic branching on MSNs in both the dorsal and ventral striatum, and pyramidal cells in the frontal cortex (Robinson and Kolb, 2004). This form of structural plasticity suggests that repeated exposure to cocaine alters patterns of synaptic connectivity in these brain regions.

In addition, repeated treatment (both non-contingent and self-administration) influences immediate early gene expression in mesocorticolimbic circuits. This will be detailed further in the following sections.

Immediate early gene expression as a marker for neurobiological impact

One frequently used measure of the neurobiological impact of drugs is their ability to alter the expression of immediate early genes (IEGs) such as c-*fos* (Sagar et al.,

1988; Harlan and Garcia, 1998). The Fos protein is a 55 or 62 kilodalton (kD) chromosomal transcription factor (Curran and Teich, 1982; Morgan and Curran, 1991) derived from the c-*fos* gene in the human and mouse genome (Curran et al., 1983), identified as the native homolog of the viral oncogene Finkel-Biskis-Jinkin (FBJ) Murine osteosarcoma virus (v-*fos*) (Curran et al., 1982).

Its suitability as a marker for biochemical activation arises from several common characteristics shared with other IEGs. Besides psychoactive drugs, neurons express c-*fos* rapidly after many different stimuli such as electrical stimulation (Sagar et al., 1988), nociceptive stimulation, heat stress and light stimulation (Morgan and Curran, 1991). c-*fos* induction is both transient and rapid (Hughes and Dragunow, 1995). For example, c-*fos* mRNA transcription occurs within 5 minutes and continues for 15-20 minutes, while Fos protein expression generally has a half-life of two hours (Morgan and Curran, 1991). c-*fos* expression is also independent of upstream protein synthesis (Sheng and Greenberg, 1990), indicating that the factors initiating the transcription of IEGs are pre-existing in the neuron, constitutively expressed, and activated by some method of post-translational modification (Hughes and Dragunow, 1995). In this way, c-*fos* expression has been hypothesized to couple acute stimuli received at the neuron's surface to longer term neuroadaptations by regulating gene expression (Sheng and Greenberg, 1990; Morgan and Curran, 1991). It should be noted that while Fos expression as a marker for brain activity and "activation" has many advantages, including high spatial resolution, its temporal resolution is low and should not be considered synonymous with increased neurophysiological activity (Kovacs, 2008).

Fos expression as a transcription factor initiating changes in the brain

Although IEG activation has been used primarily as a marker to map neurons biochemically activated after a stimulus (Sagar et al., 1988; Harlan and Garcia, 1998), the native role of Fos protein is as a transcription factor modulating a gene cascade linked to longer term neuroadaptations (Sheng and Greenberg, 1990; Morgan and Curran, 1991). Once synthesized, Fos protein forms heterodimers with Jun, another IEG protein, and

these heterodimers form activator protein complexes (AP-1) that bind to AP-1 binding sequences upstream of genes targeted for regulation (Morgan and Curran, 1991).

Chronic cocaine treatment may upregulate genes in D1-expressing MSNs that have AP-1 binding domains, including genes implicated in the dendritic reorganization of neurons (IGFBP6 and SDF1). This suggests that genes possibly targeted by Fos induction may also play a role in the enduring neurobiological changes associated with psychomotor sensitization (Zhang et al., 2005). Indeed, of particular relevance to the topic of this thesis, Zhang and colleagues (2006) recently found that the transgenic deletion of the c-*fos* gene in D1 receptor expressing striatal MSNs attenuated psychomotor sensitization and many of the associated neurobiological adaptations associated with it, including structural changes in MSN dendrites.

Thus, in addition to being a biochemical marker for neuronal activation, increased cocaine induced Fos expression in the striatum is functionally associated with the neurobiological adaptations of psychomotor sensitization.

Fos expression in the mesocorticolimbic system is upregulated by acute drug exposure

Acute exposure to many drugs of abuse increases c-*fos* gene expression in the intact brain. Neurons within the mesocorticolimbic system in particular express Fos in response to amphetamine (Graybiel et al., 1990; Young et al., 1991), cocaine (Graybiel et al., 1990; Young et al., 1991; Samaha et al., 2004), nicotine (Matta et al., 1993; Sharp et al., 1993; Kiba and Jayaraman, 1994; Samaha et al., 2005) and morphine (Chang et al., 1988; Liu et al., 1994; Garcia et al., 1995; Curran et al., 1996). Although each drug appears to induce Fos expression in unique patterns, suggesting that distinct cells within the circuit are biochemically activated (Harlan and Garcia, 1998), common changes to components of the mesocorticolimbic circuit by drugs of abuse, including cocaine, are believed to be a factor contributing towards the development of neural changes that may contribute to addiction (Robinson and Berridge, 1993; Hyman and Malenka, 2001).

Increased Fos expression in the striatum from acute cocaine is dependent on striatal dopamine

The induction of Fos expression by cocaine is largely dependent on dopamine, although other neurotransmitters can influence induction as well (Torres and Rivier, 1993; Couceyro et al., 1994; Szucs et al., 2005). Pretreatment with the dopamine D1 antagonist SCH-23390 results in a marked attenuation of Fos in the dorsal and ventral striatum (Graybiel et al., 1990; Young et al., 1991), and mutant mice with D1 receptor deletions have severely attenuated Fos expression after a cocaine challenge (Moratalla et al., 1996). Experiments suggest that D2 and D3 receptors may also play a role in modulating cocaine-induced Fos expression (Dilts et al., 1993; LaHoste et al., 1993; Ruskin and Marshall, 1994; Carta et al., 2000).

It should be noted that the majority of other drugs commonly abused by humans also increase dopamine in the striatum. In addition to cocaine, changes in dopamine have been observed with amphetamine (Di Chiara and Imperato, 1988), nicotine (Clarke and Pert, 1985), alcohol (Gessa et al., 1985; Mereu and Gessa, 1985), morphine (Matthews and German, 1984), and methylenedioxymethamphetamine (MDMA, but only the S(+)-enantiomer, see Fantegrossi, 2008). For this reason, dopamine is thought to play a critical role in reward and incentive motivational processes key in addiction, and is considered the major common mechanism by which drugs of abuse act in the brain and impact c-*fos* expression (Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Koob, 1992; Robinson and Berridge, 1993; Robbins and Everitt, 1996; Berridge and Robinson, 1998; Harlan and Garcia, 1998; Robinson and Berridge, 2000; Hyman and Malenka, 2001; Berridge and Robinson, 2003; Berridge et al., 2009).

Faster rates of infusion that can induce psychomotor sensitization have a greater acute impact on c-*fos* expression the brain

Samaha and colleagues (2004) demonstrated in rats that rapid rates of drug infusion (5 sec) capable of inducing psychomotor sensitization were associated with a

greater neurobiological impact on the brain. In one experiment, rats given a single acute infusion of cocaine over 5 sec – capable of inducing psychomotor sensitization in one day – showed greater c-*fos* and *arc* mRNA expression in specific regions of the brain. Areas of specifically high c-*fos* upregulation were parts of the mesocorticolimbic circuit, including portions of the medial prefrontal cortex (particularly the infralimbic cortex), the core and shell subdivisions of the nucleus accumbens in the ventral striatum, and the dorsomedial and dorsolateral subregions of the dorsal striatal division. Fos expression in the dorsal and ventral divisions also varied rostrocaudally. In the dorsal striatum, 5 sec infusions induced a pattern of expression where the caudal extent of the striatum had the highest expression. Conversely, the rostral extent of the nucleus accumbens had the greatest amount of c-*fos* expression.

Thus, faster rates of cocaine delivery were not only more effective in inducing sensitization, but also in initiating what is thought to be a key initial step for drug-induced neuroplasticity – the induction of IEGs. Furthermore, the pattern of c-*fos* expression suggested that specific functionally distinct nuclei projecting to the areas sampled (Willuhn et al., 2003) were being engaged by faster rates of cocaine.

The striatum is a heterogeneous structure which is part of a larger mesocorticolimbic circuit

Although Samaha and colleagues (Samaha et al., 2004) demonstrated that faster rates of an acute infusion results in greater Fos expression in the striatum, it is not a homogeneous structure. The striatum is a relatively large brain region embedded within mesocorticolimbic circuits, a reentrant loop of interconnected brain nuclei (Alheid and Heimer, 1988; Heimer and Van Hoesen, 2006) sharing general cellular and anatomical characteristics with other corticothalamic circuits (Zahm, 2006).

A major site of action of cocaine in this region includes synapses between dopamine neurons and the projection neurons in the striatum, the medium spiny neurons (MSNs). The dorsal striatum is innervated by dopamine cells located primarily in the substantia nigra and the ventral striatum (the nucleus accumbens) is innervated by

dopaminergic cells mainly from the ipsilateral ventral tegmental area (Ungerstedt, 1971; Fallon and Moore, 1978; Swanson, 1982; Tanaka et al., 1982; Alheid and Heimer, 1988). Although most drug related studies have focused on the nucleus accumbens, the dorsal striatum also plays a role in goal-directed behaviors as well (Yin et al., 2008).

In addition to dopaminergic input, MSNs in the striatum also receive excitatory glutamatergic input originating from pyramidal cells in layer 5 of the cortex (Glees, 1944; Webster, 1961, 1965; Reynolds and Zahm, 2005). MSNs receive glutamate input onto the heads of their spines, while receiving dopamine input on the necks of their spines (Totterdell and Smith, 1989; Sesack and Pickel, 1990; Meredith et al., 1999). This synaptic arrangement between glutamate and dopamine may allow an interaction modulating MSN activity (Smith and Bolam, 1990) and influencing output targets (Gerfen, 1992; Heimer and Van Hoesen, 2006).

The striatum is organized by subregions and subcompartments

MSNs in the striatum use γ-aminobutyric acid (GABA) as a neurotransmitter (Ribak et al., 1979; Kita and Kitai, 1988), and appear to be anatomically homogeneous (Kemp and Powell, 1971; Wilson and Groves, 1980). However, they are organized into complex and heterogeneous subregions that can be further classified into several distinct yet overlapping systems. This is of interest because acute cocaine injections have previously been shown to preferentially influence neurons in specific striatal subregions, suggesting that different functional areas of the striatum are activated in response to cocaine (Willuhn et al., 2003).

MSNs in the dorsal striatum and nucleus accumbens can be characterized along the rostrocaudal and mediolateral axis into subregions based on their glutamatergic innervation from functionally different areas of cortex (Webster, 1961; McGeorge and Faull, 1989; Berendse et al., 1992; Willuhn et al., 2003). For example, Willuhn and Steiner (2003) characterized 26 distinct striatal subregions based on a review of tract-tracing studies and functional mapping studies based on electrophysiology. They found that dorsal lateral portions of the striatum along the rostromedial axis receive glutamate afferents predominantly from the sensorimotor cortex, while dorsal medial portions

receive inputs primarily from the agranular cortex. In the ventral striatum, the core of the nucleus accumbens receives distinctly different input from the shell (Heimer et al., 1997; Willuhn et al., 2003). Generally, the core receives input primarily from prelimbic and agranular cortex, while the medial shell region receives its input primarily from the infralimbic and prelimbic cortex (Willuhn et al., 2003).

Acute cocaine injections have a greater neurobiological impact on gene expression in dorsal areas of the striatum, suggesting that functionally distinct cortical areas are being preferentially activated by cocaine (Willuhn et al., 2003). For example, lesions in the dorsal medial subregions of the striatum impact outcome devaluation, suggesting a role in goal directed learning (Yin et al., 2005). Dorsolateral subregions of the striatum are thought to play a role in mediating habitual responses to stimuli (Yin et al., 2004).

In the ventral striatum, acute cocaine has a greater neurobiological impact on gene expression on the shell of the accumbens than the core (Willuhn et al., 2003). Functionally, the shell of the nucleus accumbens may mediate the motivational impact of unconditioned stimuli like cocaine, while the core of the accumbens has been suggested to play a role in mediating the motivational impact of Pavlovian conditioned stimuli, or previously neutral stimuli that has been repeatedly paired with rewarding unconditioned stimuli (Cardinal, 2003).

In addition to subregions, the striatum (primarily the dorsal division) is also organized into biochemically distinct subcompartments. The striosome or "patch" compartment has a relatively high density of mu opioid receptors (Pert et al., 1976; Herkenham and Pert, 1980; Graybiel et al., 1981; Herkenham and Pert, 1981; Gerfen, 1992, 1992) whereas the surrounding matrix compartment is enriched with acetylcholinesterase (Graybiel and Ragsdale, 1978) and calbindin (Gerfen, 1985). Neurons in the patch are distinct from matrix neurons in many ways. During development, patch neurons arise earlier and have different patterns of migration during development than matrix neurons (van der Kooy and Fishell, 1987; Song and Harlan,

1994; Heimer et al., 1997). Patch neurons also have distinct neurophysiological characteristics (Miura et al., 2007).

Importantly, studies have suggested that glutamatergic cortical input into the patch and matrix compartments are partially segregated by both topography and by laminar origin within the cortex (Gerfen, 1984, 1989). Neurons in the patch receive input primarily from the prelimbic and infralimbic cortices (Gerfen, 1984) and the matrix receives afferents from the somatosensory and motor cortices (Donoghue and Herkenham, 1986). In addition, patch afferents originate from different layers of the cortex. Patches arise from neurons in Layer IV and deep Layer V of cortex, whereas matrix afferents originate from superficial layers of Layers III and V (Gerfen, 1989). The complexity of inputs suggests that striosomes represent distinct functional units in the dorsal striatum. Several studies support this hypothesis. For example, electrical stimulation of the striosome compartment is more reinforcing than stimulation in the matrix compartment (White and Hiroi, 1998), and animals with targeted striosomal ablations exhibit performance deficits on an accelerating rotarod, a complex motor task (Lawhorn et al., 2009). Importantly, repeated cocaine treatment shifts gene expression patterns so that genes important for long-term neuroadaptations express to a greater extent within the patch subcompartment. This will be described in the following section.

In summary, the striatum is a complex structure made up of subregions that are differentially influenced by cocaine (Willuhn et al., 2003). Increases in activity in specific areas and compartments of the striatum may be associated with the activation of functionally distinct areas of the brain, and will be taken into account in the following studies.

The effect of chronic cocaine treatment on Fos expression in the brain

Shifts in c-fos expression to the patch subcompartment relative to the matrix

One neuroadaptation associated with psychomotor sensitization to cocaine is a shift in c-*fos* expression to the patch subcompartment relative to the matrix (Graybiel et al., 1990; Moratalla et al., 1996; Tan et al., 2000), although in these studies the shift was

due to a relative decrease in the matrix rather than an increase in the patch. However, following extended withdrawal Jedynak and colleagues found that an amphetamine challenge increased Fos preferentially in the patch compartment in the dorsal striatum (Doctoral dissertation; unpublished, 2009). Since Fos expression patterns in the striatum may reflect the biochemical activation of distinct functional areas of the mesocorticolimbic circuit, this may reflect a change in the relative dominance of the patch vs. matrix subcompartments. One question examined in Chapter 2 of this dissertation was whether faster rates of an acute infusion induce greater Fos protein expression in the patch versus the matrix subcompartment.

Chronic treatment is associated with desensitization of c-fos expression

In addition to shifts in the pattern of c-fos expression, chronic cocaine treatment typically results in an enduring desensitization of c-*fos* expression to a cocaine challenge in both the nucleus accumbens and dorsal striatum (Hope et al., 1992; Steiner and Gerfen, 1993; Couceyro et al., 1994; Daunais and McGinty, 1994; Rosen et al., 1994; Moratalla et al., 1996; Willuhn et al., 2003), and this is also seen after self-administration (Daunais et al., 1995). It is important however to note that in some cases it has also been reported that prior cocaine experience can *enhance* Fos expression in the nucleus accumbens produced by a drug challenge, although this is only seen after a long period of drug abstinence (Crombag et al., 2002; Todtenkopf et al., 2002), and only if cocaine was administered in association with environmental novelty (Hope et al., 2006). However, this hypersensitivity is not always seen (e.g. Ostranader et al., 2003; Willuhn et al., 2003), and it is not clear exactly what conditions lead to a sensitization vs. desensitization of the Fos response, even following extended abstinence.

Faster rates of nicotine infusions during chronic treatment induce a persistent suppression of c-fos expression in the brain

Although there have been no studies examining how varying the rate of cocaine delivery during chronic treatment impacts c-*fos* expression, Samaha and colleagues (2005) did investigate the influence of rate of infusion on psychomotor sensitization to

nicotine and the effect of chronic treatment on IEG expression in the mesocorticolimbic circuit. While an extensive review of nicotine and its effects are beyond the scope of this introduction, two key similarities between cocaine and nicotine should be stressed beyond their shared addictive potential (Balfour, 1990). First, cocaine and nicotine administration share the ability to facilitate the development of psychomotor sensitization (Stolerman et al., 1973; Clarke and Kumar, 1983; Ksir et al., 1985; Miller et al., 2001). Second, acute exposure to nicotine has similar abilities to induce c-fos gene expression in the mesocorticolimbic circuit (Matta et al., 1993; Kiba and Jayaraman, 1994).

In one experiment, Samaha and colleagues (2005) showed that 50 µg/kg nicotine infused over 5 sec was able to induce greater psychomotor sensitization than nicotine delivered more slowly (25 − 100 sec), resembling the results of the cocaine experiment (2004). In a second experiment, nicotine was delivered over 5 sec after a chronic treatment regimen (5 serial infusions of 50 µg/µL nicotine equally spaced over 10 minutes over 6 consecutive days). Following 4 drug-free days, the ability of a subsequent nicotine challenge to induce c-*fos* was diminished in both the dorsal striatum and the shell division of the nucleus accumbens.

The results of this experiment showed that rapid rates of nicotine administration can induce psychomotor sensitization, and that faster rates of infusions during chronic treatment can have an enduring impact on its ability to induce c-*fos* expression in the mesocorticolimbic circuit.

Rate of infusion and cocaine self-administration: effects unclear

Given the results of the previous studies and the importance of rate of infusion in human addiction, one would expect a clear effect on rat cocaine self-administration. However, studies examining the influence of rate of infusion on self-administration in the rat have been largely negative. In the first study describing the influence of rates of cocaine infusion on cocaine self-administration, Pickens and colleagues (1969) found no differences in drug-taking behavior when cocaine was delivered over 25 to 75 sec. In our laboratory, Crombag and colleagues (2008) found that 100 sec infusions of cocaine in

comparison to 5, 25, or 50 sec infusions slightly decreased the reinforcing properties of cocaine in animals well trained to self-administer cocaine under a continuous schedule of reinforcement (FR1). However, when animals were retrained to self-administer under a fixed ratio 2 schedule of reinforcement (FR2), the rate of cocaine infusion did not influence cocaine reinforcement. In addition, no effects of rate of infusion were found on the acquisition of self-administration of either cocaine or amphetamine over a range of doses − rats receiving their infusions over 100 sec acquired self-administration equally well as the animals receiving the drug over 5 sec. Likewise, varying the rate of amphetamine or cocaine infusion had no impact on self-administration on a progressive ratio schedule of reinforcement − rats were equally motivated to take drug delivered over 100 sec or 5 seconds. Finally, the rate of amphetamine or cocaine infusion had no impact on drug-primed reinstatement of drug seeking behavior.

Given these results, a plausible hypothesis might be that rats cannot discriminate between these relatively short infusion rates. However, Schindler and colleagues (2009) demonstrated that rats can discriminate between different rates of cocaine infusion in this range (1.7 − 100 sec). In one experiment, when given the choice between a nosepoke delivering cocaine over 1.7 sec or 100 sec during self-administration, rats preferred faster rates of infusion. When the association between the nosepoke and rate of infusion was reversed (e.g. the nosepoke previously delivering cocaine over 1.7 sec now delivered the same dose over 100 sec), rats reversed their preference to some degree.

Another study has shown that rats with a fairly extensive history of self-administration under a progressive ratio schedule of reinforcement gradually increased their breakpoints across successive sessions if their drug is delivered over 5 sec, but not when delivered over 25 or 50 sec (Liu et al., 2005). In this study, animals trained at a faster rate of infusion maintained elevated breakpoints even when the rate of infusion was lowered in subsequent test sessions, presumably showing a persistent sensitized motivation and increased willingness to work for drug. Yet when these animals were retested after their progressive ratio experience, varying the rate of infusion of cocaine during an FR1 schedule did not influence drug-taking behavior, despite groups having

different rate of infusion histories. Consistent with the studies by Crombag et al. (2008), Liu and colleagues suggested that rate of infusion had no effect on the acute reinforcing effects of cocaine.

How can these negative results on the effect of rate of cocaine delivery on self-administration behavior in the rat be reconciled with the preponderance of evidence that in humans the rate that drugs reach the brain is an important factor in its addictive liability? Perhaps one reason might be that the ability of faster rates of cocaine to foster addiction is not directly related to its greater reinforcing effects.

As discussed in the beginning of this introduction, while addiction liability in humans has primarily been determined by testing its reinforcing effects, drug-taking is only one of the many symptoms associated with addiction. This perhaps is the reason why experiments testing the addictive liability of faster rates of drug infusions on rat self-administration have been unclear; testing its reinforcing effects may not adequately model its effects as a factor influencing addiction. After all, animals given access to cocaine under limited access have long been known to show well regulated intake (Wilson et al., 1971; Johanson, 1984). Recently, extended access self-administration procedures that are thought to better model aspects of human addiction have been developed.

Extended access self-administration

Extended access procedures differ from conventional self-administration sessions by either allowing many more sessions (Deroche-Gamonet et al., 2004; Belin et al., 2008), providing increased access per day by lengthening each session (Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004), or both (Ahmed and Koob, 1999)**.** When rats are given extended access to drugs, some of them begin to manifest many striking behavioral symptoms resembling human addiction (Gawin and Kleber, 1988; Gawin and Ellinwood, 1989). Compared to animals only given limited access to drug, rats given extended access begin taking increasingly greater amounts of drug over the entire session. Some researchers have also reported a concomitant escalation during the first

hour of the session (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002; Ben-Shahar et al., 2004; Ferrario et al., 2005; Knackstedt and Kalivas, 2007; Briand et al., 2008). This marked increase in drug intake is accompanied by other addiction-like symptoms. For example, rats become increasingly resistant to aversive stimuli related to drug, either when it is presented simultaneously with drug reward (Deroche-Gamonet 2004), or when it is explicitly paired with the act of seeking drug (Vanderschuren and Everitt, 2004; Pelloux et al., 2007). Some of these rats show greater motivation for drug when tested on a progressive ratio schedule of reinforcement (Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Belin et al., 2008) and they persist in seeking drug even when they know it is not available (Deroche-Gamonet et al., 2004; Ferrario et al., 2005). Rats with extended access to cocaine develop "burst-like" patterns of rapid drug-taking that may lead to faster and greater delivery of drug to the brain (Belin et al., 2009). Additionally, some rats given extended access to cocaine self-administration also show a greater propensity to reinstate cocaine seeking behavior after extinction (Deroche-Gamonet et al., 2004; Mantsch et al., 2004; Ferrario et al., 2005; Kippin et al., 2006; Knackstedt and Kalivas, 2007; Belin et al., 2008; Belin et al., 2009). As is the case in humans, rats with a history of extended access to cocaine self-administration often manifest many of these addiction-like symptoms together. In summary, extended access procedures result in the development of a number of addiction-like symptoms, and thus may better model changes in brain and behavior associated with addiction in humans (Robinson, 2004).

I hypothesize, therefore, that these procedures may reveal effects of rate of drug delivery that are not evident when using more conventional limited access self-administration sessions because they are not especially effective in producing symptoms of addiction. The overall goal of this dissertation will explore this hypothesis.

Hypotheses and Specific Aims

1. The aim of the first study in this dissertation was to confirm and quantify the influence of rate of cocaine delivery on Fos protein expression, extending the findings of previous studies which showed that faster rates of an acute infusion of cocaine can induce greater c-*fos* mRNA in the mesocorticolimbic system and induce greater psychomotor sensitization (Samaha et al., 2002; Samaha et al., 2004). To pursue this aim, I first determined if the rate of cocaine delivery affected Fos protein in a similar fashion to mRNA, as recent studies suggested Fos protein expression was required for cocaine to produce psychomotor sensitization and related changes in the organization of MSN dendrites. Second, I sought to determine if the effect of rate of cocaine delivery on the number of Fos positive cells was specific to the striosomal "patch" compartments.

2. The second study in this dissertation focused on determining the influence of rate of cocaine delivery on self-administration behavior when animals were provided extended access to the drug. To the extent that rate of drug delivery influences the transition to addiction, I hypothesized that an effect may be evident only under conditions that lead to the development of addiction-like symptoms (Ahmed and Koob, 1998; Robinson, 2004; Samaha and Robinson, 2005). Thus, I compared the influence of rate of i.v. cocaine delivery on self-administration behavior when animals were allowed access to cocaine for only 1 hr each day ("short access," ShA) or for 6 hrs each day ("long access," LgA).

3. The third study involved rats with a history of extended access to rapidly infused cocaine. Under limited access conditions, we previously saw no effect of rate of cocaine delivery on cocaine-induced reinstatement (Crombag et al., 2008). Thus, one aim tested whether the rats would persist in seeking drug during extinction and reinstate their drug seeking behavior when primed with cocaine to a greater degree than animals with a similar self-administration experience but with slower rates of drug delivery. The second aim of this study tested the hypothesis that rats with a history of rapid infusions during extended access would have enduring neurobiological adaptations, as assessed by Fos expression.

Fig. 1.1 Panel a: A pharmacokinetic model of predicted cocaine concentration (μM) in the brain of a 300 g rat after a 1.0 mg/kg injection. Successive curves show the time to peak and peak levels of cocaine with infusion rates over 5, 25, 50, and 100 sec (from left to right, respectively) used in these experiments. Note no difference in peak concentration of cocaine over this range of rates. Figure adapted from Samaha et al. (2002). Panel b: The mean concentration of extracellular dopamine in the striatum after an acute infusion of 2.0 mg/kg cocaine given either over 5 or 100 sec. Arrow indicates the time of the infusion. Note that similar to the predicted concentration of cocaine shown in Panel a, the peak concentration of dopamine released by cocaine over these rates of delivery does not differ – only the time to peak differs across these rates. The total amount of dopamine (as expressed by area under the curve) also does not differ across these rates (data not shown). Figure adapted from Ferrario et al. (2008).

27

a

b Extracellular Dopamine

■ 5 sec
○ 100 sec

% Baseline

Minutes

Brain Cocaine (μM)

Time (min)

28
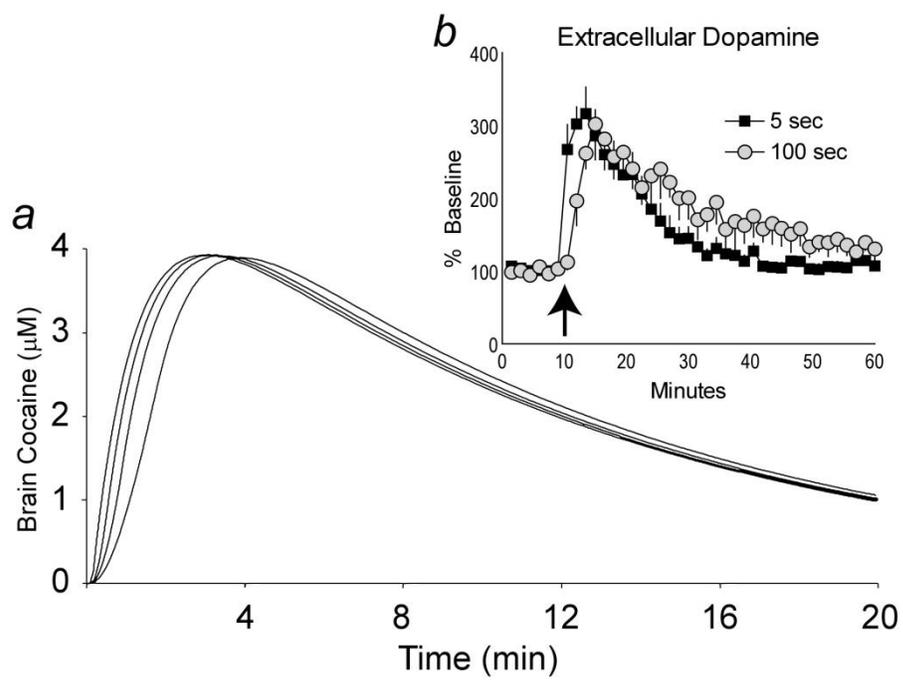
CHAPTER 2

THE INFLUENCE OF THE RATE OF INTRAVENOUS COCAINE DELIVERY ON FOS EXPRESSION IN SUBREGIONS AND SUBCOMPARTMENTS OF THE STRIATUM

Introduction

The time it takes drugs such as cocaine to reach the brain is thought to be an important factor in determining its abuse liability (Hatsukami and Fischman, 1996), but there is very little research on how rate of cocaine delivery influences its neurobiological actions (Samaha and Robinson, 2005). Previous work has shown that faster rates of drug administration, while delivering the same amount of drug to the brain and inducing similar peak levels of dopamine efflux in the brain (Pan et al., 1991; Samaha et al., 2002; Ferrario et al., 2008), nonetheless are more effective than slower rates in producing psychomotor sensitization and inducing immediate early gene expression (Samaha et al., 2002; Samaha et al., 2004; Samaha et al., 2005), as well as in altering brain temperature (Brown and Kiyatkin, 2005). The induction of psychomotor sensitization by faster rates of drug administration is considered a behavioral symptom of long lasting neurobiological alterations in brain circuits that may contribute to the transition to addiction (Samaha and Robinson, 2005).

The use of immediate early gene (IEG) expression is a well established method of mapping brain activation and the biochemical response of neurons to a number of stimuli, including drugs of abuse (Sagar et al., 1988; Hughes and Dragunow, 1995; Harlan and Garcia, 1998). Indeed, many researchers have shown that the mRNA for the prototypical IEG, c-*fos* (Curran et al., 1982; Curran and Teich, 1982), is upregulated in the mesocorticolimbic system, a circuit in the brain critical to addiction (Robinson and Berridge, 1993; Hyman and Malenka, 2001; Robinson and Berridge, 2003; Kalivas and Volkow, 2005) by the administration of drugs such as amphetamine, methamphetamine,

cocaine, morphine, and nicotine (Chang et al., 1988; Graybiel et al., 1990; Young et al., 1991; Persico et al., 1993; Konradi et al., 1994; Badiani et al., 1998; Uslaner et al., 2001; Uslaner et al., 2001; Crombag et al., 2002; Ferguson et al., 2003; Ostrander et al., 2003; Uslaner et al., 2003; Uslaner et al., 2003; Ferguson et al., 2004; Samaha et al., 2004; Samaha et al., 2005). Cocaine and nicotine are much more effective in inducing c-*fos* and *arc* mRNA in diverse areas of the neocortex and subregions of the striatum when given rapidly (5 sec) than when given even a little more slowly (25-100 sec) (Samaha et al., 2004; Samaha et al., 2005). This expression in subregions of the striatum is thought to reflect increased synaptic input from distinct and topographically arranged synaptic targets (Webster, 1961; McGeorge and Faull, 1989; Berendse et al., 1992; Willuhn et al., 2003).

However, the striatum is not a heterogeneous structure, and previous studies examining the effect of rate of drug administration on IEG expression have not taken this into consideration. In addition to differences in regional innervation, the striatum is also organized into biochemically distinct striosome ("patch") and matrix subcompartments (Pert et al., 1976; Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981). Neurons in these subcompartments differ in their development (van der Kooy and Fishell, 1987; Song and Harlan, 1994; Heimer et al., 1997), express different proteins (Graybiel and Ragsdale, 1978; Graybiel et al., 1981; Herkenham and Pert, 1981; Gerfen, 1985), maintain partially segregated afferent inputs and efferent projections (Gerfen, 1984, 1989), and have distinct neurophysiological characteristics (Miura et al., 2007). Behavioral studies have extended the idea that striosomes represent distinct functional units in the dorsal striatum. For example, electrical self stimulation of the striosome compartment is more reinforcing than stimulation in the matrix compartment (White and Hiroi, 1998), and animals with targeted striosomal ablations exhibit task-specific performance deficits (Lawhorn et al., 2009). Furthermore, it has been reported that animals behaviorally sensitized to cocaine as a result of repeated exposure have greater Fos protein expression in the patch subcompartment relative to the matrix, when compared to the pattern induced by an acute injection (Graybiel et al., 1990; Moratalla et

al., 1996; Canales and Graybiel, 2000; Canales et al., 2002). Thus, expression of c-*fos* mRNA and its protein product Fos preferentially in the striosome or matrix compartment is thought to reflect biochemical activation of functionally distinct populations of neurons (Donoghue and Herkenham, 1986; White, 1989; Gerfen, 1992; Eblen and Graybiel, 1995; Flaherty and Graybiel, 1995; White and Hiroi, 1998; Brown et al., 1999; Canales and Graybiel, 2000; Brown et al., 2002; Canales, 2005).

Given that the rapid delivery of cocaine is more effective in producing psychomotor sensitization, and also is more effective in inducing c-*fos* mRNA in various striatal subregions (Samaha et al., 2004), we hypothesized that variation in the rate of cocaine delivery may influence IEG expression in the patch compartment to a greater extent than the matrix. The purpose of this experiment was to test this hypothesis. In addition, in this experiment we quantified the influence of rate of cocaine infusion on the number of cells positive for Fos protein (in previous studies we quantified only c-*fos* mRNA). This may be an important variable because post-transcriptional regulation of c-*fos* mRNA may alter the amount of Fos protein (Rahmsdorf et al., 1987) in striatal MSNs containing the dopamine D1 receptor. This has been reported to be required for both cocaine-induced psychomotor sensitization and associated biochemical and structural changes in dendrites on MSNs in the dorsal and ventral striatum (Zhang et al., 2006; Xu, 2008).

Materials and Methods

Subjects and Housing

A total of 29 male Sprague Dawley rats (Harlan, IN) weighing between 225 – 250 g upon arrival were used in this experiment. Prior to the start of the experiment, rats were individually housed in a climate-controlled animal colony maintained on a 14/10 light/dark cycle (lights on at 0800), with food and water available *ad libitum*. Rats were given a minimum of 5 days to habituate to the animal colony. For the duration of the experiment, animals were housed individually in test chambers in a separate testing facility under the same housing conditions. To minimize the effects of extraneous noise

in the room, a room-wide white noise generator set to 70 dB was on continuously in the testing facility to mask other sounds. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Catheter Construction and Surgical Procedures

Chronically indwelling jugular catheters were placed in rats using a procedure similar to that described elsewhere (Weeks and Davis, 1964; Weeks, 1972; Crombag et al., 1996; Samaha et al., 2002). Briefly, catheters were constructed from SILASTIC® tubing (0.51 mm inner diameter, 0.94 mm outer diameter; Dow-Corning, Midland, MI), secured by polyolefin heat shrink tubing (Alpha Wire Company, Elizabeth, NJ) to a backport constructed from a 22 gauge, 11 mm cannula (Plastics One, Roanoke, VA) bent to a ninety degree angle. The cannula was supported by a 1 cm length of a trimmed 200 μL pipette tip, and secured to a 3 cm diameter circular 250 micron mesh (Small Parts, Miramar, FL) by dental cement (Bosworth, Skokie, IL). While not in use, catheters were capped with obdurators. All catheters had a dead volume of 24-26 μL.

Rats were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg, intraperitoneally, IP). The backport was secured between the animal's shoulder blades, while the silicon end of the catheter was inserted into the left or right external jugular vein. Catheters were then filled with 0.1 mL solution of 10 mg/mL gentamicin sulfate (Vedco, St. Joseph, MO) in 0.9% sterile bacteriostatic saline to minimize infections and catheter occlusions.

Animals were allowed to recover for a minimum 4 days. Starting the day after the surgery, catheters were flushed manually once per day with 0.2 mL of the gentamicin solution. Every 5 days animals received an i.v. infusion of 0.2 mL sodium thiopental solution (20 mg/mL, dissolved in sterile water, Hospira, Lake Forest, IL) to assess catheter patency. Animals that did not become ataxic within 5-10 seconds were removed from the study.

Testing Apparatus

Test chambers were the same as those described previously (Samaha et al., 2002). Briefly, test chambers consisted of red plastic buckets 25 cm in diameter and 36 cm in height, with corncob bedding on the floor. Each test chamber was equipped with a photocell rotometer which measured the number of quarter turns in each direction (McFarlane et al., 1992). Prior to a session, each rat was tethered to a 22 gauge liquid swivel (Instech Solomon, Plymouth Meeting, PA) fixed to a counter-balanced arm via a homemade spring, which allowed the animals to move freely in the test chamber. A length of Tygon microbore tubing serving as the infusion line was inserted through the spring and attached to the catheter. A second length of Tygon tubing attached the distal end of the swivel to a 1.0 mL syringe, which was mounted on a programmable Harvard Apparatus pump (Holliston, MA). The pump was calibrated to each individual swivel and line apparatus so that the pump delivered a volume of 10 µL over 5 (120 µL/min), 25 (24 µL/min), or 100 (6 µL/min) seconds.

Groups and Procedures

At the start of the experiment, rats were transported from the animal colony to the testing facility and individually placed into the test chambers, where they lived for the duration of the experiment. After one day of acclimatization, all rats underwent infusion habituation sessions for the next three days. Once per day, rats were attached to the infusion apparatus and infused with 10 µL of saline over 5, 25 or 100 seconds. Over the course of habituation sessions, all rats received each infusion rate once in a randomized order. Rats remained tethered to the infusion apparatus for 2 hours post-infusion, and then were disconnected.

On Day 4, the infusion apparatus was prepared for a 2.0 mg/kg cocaine or saline infusion in the same manner as previously reported (Samaha et al., 2004). Once animals were attached to the infusion apparatus, rats were left undisturbed for 40 minutes to establish baseline motor activity. Rats were then infused with 10 µL of 2.0 mg/kg cocaine over 5 (n=8), 25 (n=6), 100 seconds (n=8), or saline over 5 seconds (n=7), and locomotor activity (quarter turns) was recorded. To allow for sufficient time for transcardial

perfusion at the end of the experiment, infusions were staggered 40 minutes apart. After the infusion the animal remained tethered to the infusion apparatus for 2 hours.

Two hours after the injection, animals were disconnected and taken to an adjoining room and tested for catheter patency with sodium thiopental. Animals with patent catheters were immediately anesthetized with sodium pentobarbital (390 mg/kg, i.p., Vortech, Dearborn, MI), and perfused transcardially with 500 mL ice cold perfusion rinse (73 mM sucrose, 18 mM procaine hydrochloride, 139 mM sodium chloride in 0.1 M sodium phosphate buffer (SPB), pH 7.4), followed by 250 mL ice cold paraformaldehyde rinse (4% (W/V) paraformaldehyde dissolved in 0.1 M SPB buffer with 73 mM sucrose, pH 7.4). Brains were removed and placed in paraformaldehyde rinse overnight at 4 degrees Celsius, and then transferred to 30% (W/V) sucrose for 3 days. Brains were coronally sectioned to a 40 μm thickness with a freezing microtome. Series of sections were collected 120 μm apart. Sections processed for immunohistochemistry within 2 days were stored in 0.1 M SPB, otherwise sections were stored at -20 Celsius in liquid cryoprotectant.

Immunohistochemistry

Sections were processed for Fos and Mu opioid receptor protein immunofluorescence in a similar manner to Reynolds and Berridge (2008). Mu opioid immunoreactivity provides a robust marker for patch compartments (Harlan and Garcia, 1998). Sections were washed in 0.1 M SPB containing 0.2% (V/V) Triton-X with gentle agitation for 30 minutes, and then pre-blocked for 30 minutes in a 5% (V/V) Normal Donkey Serum (NDS) solution. Sections were then incubated overnight with a primary antibody solution containing 1:500 goat polyclonal antibody raised against the N-terminus of human Fos (sc-52-G, Santa Cruz, Santa Cruz, CA) and 1:1000 rabbit polyclonal antibody raised against the C-terminus of the rat Mu opioid receptor 1 (ab10275, Abcam, Cambridge, MA). After washing in 0.1 M SPB for 30 minutes, sections were pre-blocked in a solution containing 5% NDS and 5% (V/V) Image-iT FX signal enhancer (Invitrogen, Eugene, OR) for 30 minutes. Sections were subsequently incubated for 2 hours in a solution containing 1:250 donkey anti-goat AlexaFlor 488

34

antibody and 1:250 donkey anti-rabbit AlexaFlor 594 antibody (Invitrogen, Carlsbad, CA) and 5% signal enhancer. Next, sections were washed in SPB buffer and mounted, air-dried, and coverslipped with Prolong Gold anti-fade reagent (Invitrogen, Eugene, OR). Control tissue stained with only secondary antibodies revealed little or no unspecific binding.

Visualization

Sections were visualized at 100x total magnification with a Leica DMRx (Wetzlar, Germany) microscope with a Leica HBO-100 fluorescent light source coupled to a Sony DXC-970 MD (Tokyo, Japan) camera. AlexaFlor 488 was fluoresced using a Leica L5 bandpass filter cube, and AlexaFlor 594 was fluoresced using a N3 bandpass filter cube. The same section and region was imaged for Fos and Mu opioid receptor immunoreactivity. Separate Fos and Mu opioid images were captured as LZW lossless compression TIFF files to minimize image compression artifacts in dark-field areas of the image and were analyzed using the MCID Elite (Cambridge, UK) software package. Figures 2.2-2.4 provide sample images of both Fos and Mu opioid receptor staining in the dorsal and ventral striatum.

Sampling Areas

In all areas of the brain examined, data from the left and right hemisphere were summed together. All brain regions were identified by Paxinos and Watson (4th Edition, 1998). The dorsal striatum was sampled at +1.7 mm, +1.2 mm, and +0.0 mm from bregma. Fig. 2.2 illustrates the areas of the dorsal striatum sampled. At +1.7 mm from bregma, a sampling area of 0.508 mm^2 (725 µm x 700 µm) per hemisphere was used to account for the smaller area of the striatum. For the more caudal sections, a sampling area of 1.08 mm^2 (1200 µm x 900 µm) per hemisphere was used. The nucleus accumbens (NAc) shell and core were sampled at +2.0 mm from bregma. Fig. 2.3 illustrates areas of the NAc sampled. An elliptical sampling area of 300x900 µm^2 was used to sample the shell, and a smaller sampling area of 300x450 µm^2 was used for the core. To better follow the contours of the shell and the core, sampling ellipses were angled. The infralimbic (IL)

35

cortex was chosen as the cortical sampling area because this area showed the greatest influence of rate of cocaine infusion on c-*fos* mRNA expression (Samaha et al., 2004). The IL was sampled at 2.0 mm from bregma, with the lateral terminus of the forceps minor corpus callosum serving as the main morphological landmark for the location of the IL. Fig. 2.3 illustrates areas of the IL sampled. The IL was sampled using a circular sampling area 800 μm in diameter.

Quantification

For Fos nuclei quantification, Fos images were optimized for counting by applying the "Target Accentuate" filter available within MCID Elite with a matrix setting of 9x9, using a method previously reported by Vanderschuren and colleagues (2002). Briefly, the filter increases contrast between areas of differing intensity value, while decreasing the contrast between areas of similar intensity. A higher matrix value applies a more aggressive transformation to the target image. Using the "Autoscan Utility," a maximum of 5 Fos positive cells were visually identified in each image by the experimenter and sampled for hue and intensity values using a 4 micron diameter sampling circle. The average value provided the threshold for identifying and quantifying Fos positive nuclei in the image. In addition, a "Target selection criteria" optimized to reduce the incidence of image artifacts contributing to the final count was used. Targets thus identified by the "Autoscan Utility" were then overlaid on the original unaltered Fos image and visually inspected to ensure fidelity in quantification and to minimize the contribution of artifacts towards the final cell count. In cases where no Fos positive nuclei could be identified in the section, Fos-positive nuclei from the cortex immediately adjacent to the striatum were used to set the target threshold.

For patch identification, areas with high Mu opioid receptor expression were visually identified by the experimenter and manually selected using the "Autoscan B" utility. The number of cells within each patch, as well as the area of each patch in each sampling area, was recorded.

To calculate the patch versus matrix ratio, the Index of Striosome to Matrix Predominance (ISMP) (Canales and Graybiel, 2000) was determined by calculating the

ratio between the density of Fos-positive neurons in the patch and the density of Fos-positive neurons in the matrix.

All quantification was done by trained raters blind to the experimental condition of the animal.

Statistics

Fos counts and ISMP ratios were compared using analyses of variance and where appropriate post-hoc comparisons were made using Fishers LSD tests.

Results

Acute Locomotor Behavior

Fig. 2.1 shows the number of quarter turns in the first 12 minutes following a single i.v. injection of 2.0 mg/kg cocaine delivered over 5, 25, or 100 sec (control saline administered over 5 sec). This time period captures the duration of the behavioral effect. There was a main effect of treatment ($F(3,28)=5.031$, $p<0.007$), and post-hoc tests revealed that the effect arose because the drug treatments increased locomotor activity relative to saline (saline vs. 5 sec $p<0.007$, saline vs. 25 sec $p<0.002$, saline vs. 100 sec $p<0.038$). There were, however, no significant differences in the acute psychomotor activating effects of cocaine as a function of rate of infusion, consistent with our previous studies (Samaha et al., 2002; Samaha et al., 2004).

Dorsal Striatum (Total Fos Positive Cells)

Figs. 2.2-2.3 show sample images to illustrate the labeling and the location of the regions sampled. Fig. 2.5 shows the total number of Fos positive nuclei per mm^2 in four subregions of the dorsal striatum (dorsal medial, DM; dorsal lateral, DL; ventral lateral, VL; ventral medial, VM) across three rostrocaudal levels (+1.7 mm, 1.2 mm, +0.0 mm from bregma) in four groups of rats (those who received a single acute i.v. infusion of 2.0 mg/kg cocaine over 5, 25, or 100 sec, or saline over 5 sec).

In the DM subregion, cocaine increased the number of Fos positive cells, relative to the saline control group, at all rates of infusion. An overall two-way ANOVA

analyzing the effect of group and rostrocaudal level on the number of Fos nuclei resulted in a main effect of group ($F_{(3,87)}=23.847$ $p<0.001$) and rostrocaudal level ($F_{(2,87)}=4.10$ $p<0.001$). The interaction trended towards significance ($F_{(6,87)}=2.073$ $p=0.065$). Similar to effects of cocaine on c-*fos* mRNA, cocaine increased the number of Fos positive cells in the caudal striatum more than in the rostral striatum. Pairwise comparisons showed that cocaine significantly increased the number of Fos positive cells in all groups (saline vs. 5 sec, $p<0.001$; vs. 25 sec, $p<0.001$; vs.100 sec, $p<0.016$). However, when delivered over 5 sec, cocaine increased the number of Fos positive cells to a significantly greater extent than when it was delivered over either 25 or 100 sec (p's<0.001). The 25 sec and 100 sec groups did not differ from one another ($p=0.137$).

In the DL subregion, a two-way ANOVA resulted in a main effect of group ($F_{(3,87)}=12.114$ $p<0.001$) and rostrocaudal level ($F_{(3.387)}$ $p<0.039$). The interaction was not significant ($F_{(6,87)}=0.510$). As in the DM striatum, cocaine induced more Fos positive cells caudally. In this region, only when cocaine was delivered over 5 sec did it significantly increase the number of Fos positive cells (saline vs. 5 sec, $p<0.001$; vs. 25 sec, $p=0.203$; vs. 100sec, $p=0.109$), and the 5 sec group differed significantly from both the 25 sec and 100 sec groups (p's<0.001)

In the VL, there was a main effect of group ($F_{(3,87)}=5.353$ $p<0.003$), but no effect of rostrocaudal level ($F_{(2,87)}=0.778$ $p=0.463$; interaction, $F_{(6,87)}=0.508$ $p=0.800$). Only when cocaine was delivered over 5 sec did it significantly increase the number of Fos positive cells ($p<0.001$).

In the VM, a two-way ANOVA analysis resulted in a main effect of group ($F_{(3,87)}=22.804$ $p<0.001$) and rostrocaudal level ($F_{(2,87)}=3.932$ $p<0.024$, but no interaction $F_{(6,87)}=1.005$ $p=0.427$). All groups given cocaine had significantly more Fos positive cells than the group given saline (saline vs. 5 sec $p<0.001$; vs. 25 sec $p<0.001$; vs. 100 sec $p<0.004$). However, when delivered over 5 sec, cocaine produced significantly more Fos positive cells than when it was delivered over either 25 sec or 100 sec (p's<0.001), and the latter two groups did not differ from one another ($p=0.373$). In

VM striatum, the number of Fos positive cells was lower at the level 1.2 mm from bregma than more rostrally or caudally.

Nucleus Accumbens and Infralimbic Cortex

Fig. 2.6 shows the number of Fos positive nuclei per mm^2 in the nucleus accumbens core (NAcc), shell (NAcs), and the infralimbic (IL) cortex sampled at +2.0 mm from bregma, as a function of treatment condition. In both the NAcs and NAcc, a one-way ANOVA resulted in significant effect of treatment (NAcs, F(3,29)=10.514, p<0.001; NAcs, F(3,29)=9.096 p<0.001). Post-hoc tests revealed that when compared to saline, all drug treatments produced an increase in the number of Fos positive cells (NAcs, saline vs. 5 sec p<0.001; vs. 25 sec, p<0.006; vs. 100 sec p<0.011; NAcc, saline vs. 5 sec, p<0.001; vs. 25 sec p<0.025; vs. 100 sec, p<0.030). However, when cocaine was delivered over 5 sec it induced a significantly greater number of Fos positive cells than when delivered over either 25 or 100 sec in both subregions of the accumbens (all p's<0.034), and the 25 and 100 sec groups did not differ from one another.

In the IL, there was a main effect of group (F(3,29)=3.116 p<0.042), and all rates of infusion increased the number of Fos positive cells, but there was no effect of rate of infusion.

The results presented above are consistent with previous studies regarding the effects of rate of cocaine infusion on the induction of c-*fos* mRNA in the dorsal and ventral striatum (Samaha et al., 2004). The results also extend these findings by showing that rate of cocaine infusion has a similar effect on the number of cells positive for Fos protein. However, the major purpose of this experiment was to determine if rate of infusion had a differential effect on the number of Fos positive cells in the patch (striosome) vs. matrix subcompartments in the dorsal striatum (Fig. 2.4), and those results are shown in Fig. 2.7.

Dorsal Striatum (Patch vs. Matrix)

Fig. 2.7 (Panel a) shows the number of Fos positive nuclei in the patch or matrix subcompartments of either the dorsal medial or dorsal lateral striatum. The Fos counts

were averaged across all bregma levels sampled (+1.7 mm, +1.2 mm, +0.0 mm), as this factor did not influence the results. There was a large effect of rate of infusion on the ability of cocaine to increase the number of Fos positive cells in both the patch and matrix compartments (Group effect: DM – $F_{(3,58)}=27.11$; DL – $F_{(23,58)}=10.29$, both p's<0.0001). However, the effect of rate of infusion was clearly the same in both the patch and the matrix compartments (effect of compartment non-significant: DM – $F_{(1,58)}=0.03$ p=0.8567; DL – $F_{(1,58)}=0.37$ p=0.5443; interaction DM – $F_{(3,58)}=0.19$ p=9.742; DL – $F_{(3.58)}=9.021$). Panel b shows the same data expressed as the Index of Striosomal Matrix Predominance (ISMP), and one-way ANOVAs comparing ISMP across groups resulted in no significant group differences (DM – $F_{(3,29)}=2.342$ p=0.094; DL – $F_{(3,29)}=0.274$, p=0.844).

Discussion

When cocaine is injected rapidly it is more effective in producing psychomotor sensitization and inducing c-*fos* mRNA in the dorsal and ventral striatum (Samaha et al., 2004). Here we quantified the influence of rate of cocaine delivery on the number of cells positive for Fos protein in the striatum, and also tested the hypothesis that the rapid delivery of cocaine would induce greater Fos immunoreactivity preferentially in the patch subcompartment. The results strongly support and extend our previous mRNA findings. Cocaine delivered i.v. over 5 sec increased the number of Fos positive cells in both the dorsal and ventral striatum to a much greater extent than when it was delivered over 25-100 sec. However, the effect of rate of cocaine delivery was equally evident in the patch and matrix subcompartments of the dorsal striatum. Therefore, even though there are many differences between the patch and matrix subcompartments (see Introduction) it appears that the influence of rate of cocaine delivery on IEG expression in the dorsal striatum is widespread, and not localized to either the patch or matrix subcompartments.

Cocaine is an indirect agonist of monoamine neurotransmitters, primarily binding to presynaptic proteins including those that transport dopamine, serotonin and norepinephrine (Heikkila et al., 1975; Ritz et al., 1987; Giros and Caron, 1993). The

blockade of the dopamine transporter by cocaine in particular increases synaptic concentrations of dopamine and other monoamines. The range of infusion rates used in this study (5 – 90 sec) produces the same peak concentration of cocaine in the brain (Pan et al., 1991; Samaha et al., 2002), produces similar peak concentrations of dopamine in the striatum (Ferrario et al., 2008), and are equally effective in producing acute psychomotor activation (present data; Samaha et al, 2004). Nevertheless, there was a large effect of rate of cocaine delivery on its ability to increase the number of Fos positive cells in the striatum. This suggests that small differences in how rapidly cocaine reaches the brain, and presumably small differences in the temporal dynamics of transporter blockade, can have large effects on downstream intracellular molecular cascades, including those responsible for IEG expression.

Fos expression was utilized in this study as a biochemical marker for activation of intracellular signaling cascades (Sagar et al., 1988; Sheng and Greenberg, 1990; Morgan and Curran, 1991; Harlan and Garcia, 1998), but its native role inside neurons is as a constituent in a transcription factor complex (Activation protein-1 (AP-1)) (Sheng and Greenberg, 1990; Hughes and Dragunow, 1995) capable of inducing the expression of downstream genes that contain AP-1 binding domains (Morgan and Curran, 1991). In the striatum, increased Fos expression produced by an acute injection of cocaine is dependent primarily on the D1 dopamine receptor (Graybiel et al., 1990; Young et al., 1991; Moratalla et al., 1996), and therefore expression of Fos in D1 dopamine receptor expressing striatal neurons is thought to initiate a gene expression cascade contributing towards a wide range of enduring neurobiological changes, including changes associated with psychomotor sensitization (Nestler, 2001). Consistent with this idea, transgenic mice with targeted deletions of Fos in D1 receptor expressing striatal neurons exhibit attenuated psychomotor sensitization compared to wild-type controls, and also fail to show associated structural modification in dendrites on medium spiny neurons (Zhang et al., 2006; Xu, 2008). Using gene microarrays, over 100 genes have been found to be upregulated by chronic cocaine treatment in wildtype mice compared to D1 receptor

deficient mice, including those implicated in enduring structural changes in neurons (Zhang et al., 2005).

It should be noted that although the acute effects of faster rates of cocaine infusion on Fos expression are thought to be mediated by D1 receptor expressing neurons, the selectivity for D1 mediated Fos expression to cocaine is heavily modulated by the environmental context in which the drug was delivered (Badiani and Robinson, 2004). Drug induced Fos expression in the mesocorticolimbic system and psychomotor sensitization is enhanced when given in a novel environment distinct from an animal's homecage (Badiani et al., 1995; Badiani et al., 1995; Badiani et al., 1998; Browman et al., 1998; Day et al., 2001; Uslaner et al., 2001). Notably, the pattern of Fos expression between "home" and "novel" drug experienced groups are distinct along the rostrocaudal axis of the caudate (Badiani et al., 1998; Uslaner et al., 2001; Ostrander et al., 2003), and activate a different subpopulation of MSNs expressing the D2 receptor and enkephalin (Enk) (Badiani et al., 1999), possibly through corticostriatal glutamate (Ferguson et al., 2003). While faster rates of infusion were found to equally induce c-*fos* mRNA expression in both Enk+ and Enk- neurons (Samaha et al., 2004), the results of this study leave open the possibility that D2 expression may play a role in the induction of Fos protein as a result of faster rates of infusion.

The brain circuits strongly influenced by faster rates of drug infusion include the mesocorticolimbic circuits, which consist of parallel cortico-subcortical loops (Alheid and Heimer, 1988; Heimer and Van Hoesen, 2006). These systems are normally involved with reward processes such as incentive motivation (Berridge and Robinson, 1998; Berridge, 2004) and reward associated learning (Yin et al., 2004; Ostlund and Balleine, 2005; Yin et al., 2008); dopamine projections from neurons in the substantia nigra pars reticulata and ventral tegmental area to these regions have been strongly implicated in mediating the reinforcing and other effects of cocaine (Roberts et al., 1977; Roberts et al., 1980; Ritz et al., 1987; Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Camp et al., 1994). Furthermore, changes in these same systems as a result of exposure to drugs of abuse are thought to contribute to the development of addiction (Robinson and Berridge,

1993; Hyman and Malenka, 2001; Robinson and Berridge, 2003). Thus, the fact that rapidly delivered cocaine preferentially engages this circuitry may be one reason why drugs or formulations that enter the brain rapidly are more likely to produce addiction.

In summary, a diverse variety of long-term, persistent neuroadaptations in the mesocorticolimbic circuit can be mediated by increased striatal Fos expression. Since faster rates of infusions preferentially induce greater Fos expression and induce behavioral sensitization (Samaha et al., 2002; Samaha et al., 2004), faster rates of cocaine infusion should also have a strong impact on drug-taking behavior in the rat, especially under conditions that produce addition-like symptoms. This question is explored in the next chapter of this dissertation.

Fig. 2.1 Mean (± SEM) locomotor activity (number of quarter turns) during the first 12 minutes after a single acute i.v. injection of 2.0 mg/kg cocaine delivered over 5, 25 or 100 sec, or saline delivered over 5 sec.

Behavior During First 12 Minutes
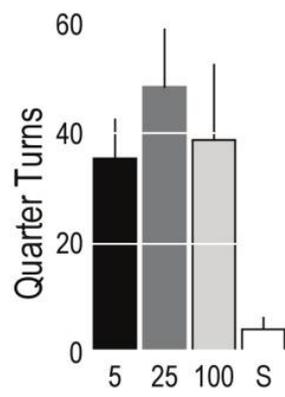
Fig. 2.2 Example images showing Fos positive neurons (labeled in green) in two subregions of the dorsal striatum across two rostrocaudal levels of bregma 2 hours after an acute infusion of 2.0 mg/kg cocaine given over 5 – 100 sec or saline given over 5 sec. Scale bar indicates 100 microns. Areas sampled in the experiment relative to other anatomical features are schematically indicated on the left. Areas depicted by images are shaded gray.

Dorsal Medial +0.0   Dorsal Medial +1.7

5

25

100

Saline

1.7

1.2

0.0

47
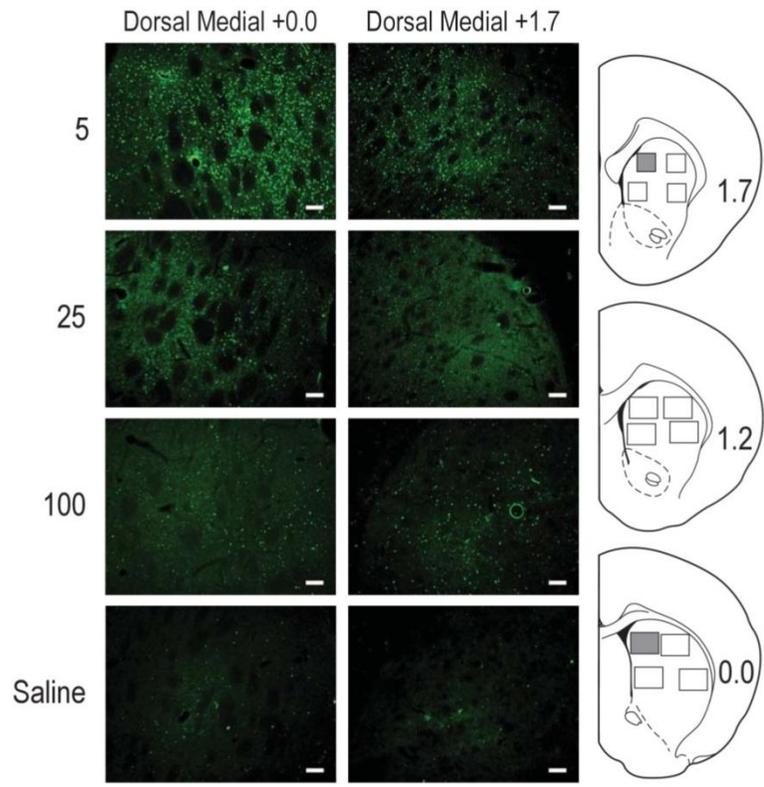
Fig. 2.3 Example images of Fos positive neurons (labeled in green) in the nucleus accumbens (NAc) and infralimbic cortex at 2.0 mm from bregma two hours after an acute infusion of 2.0 mg/kg cocaine given over 5 – 100 sec or saline over 5 sec. Scale bar indicates 100 microns. Areas sampled within the nucleus accumbens (core and shell subdivisions) relative to other anatomical features are indicated schematically on the left.

	IL	NAc
5		
25		
100		
Saline		
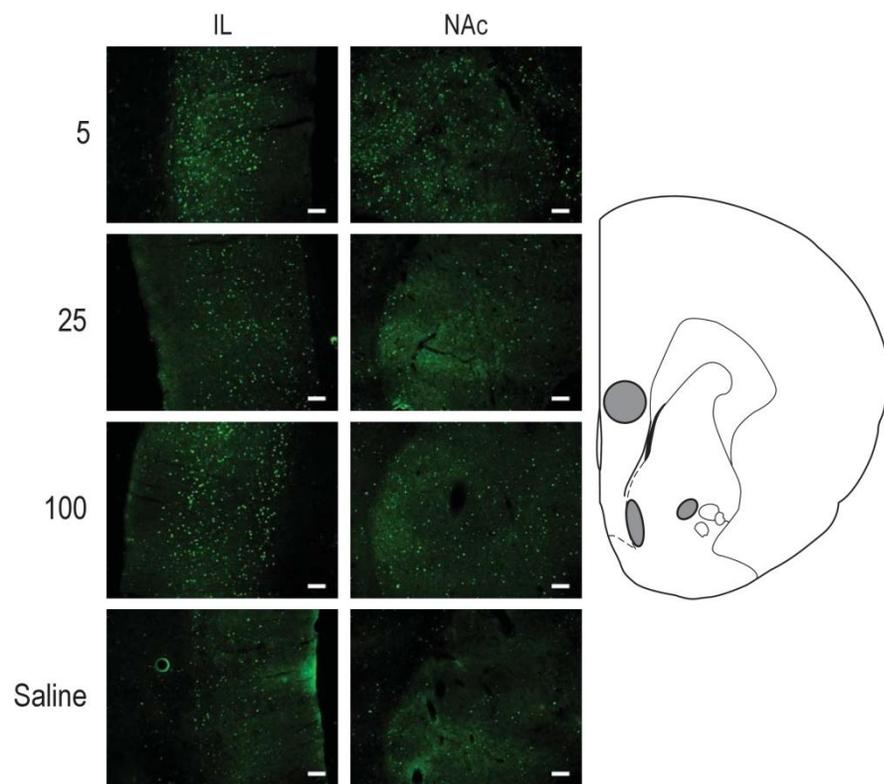
Fig. 2.4 Example images of the patch (identified as areas of high Mu opioid receptor immunoreactivity labeled in red) and matrix subcompartments where the distribution of Fos positive nuclei (labeled in green) was quantified in the dorsal medial and dorsal lateral subregions of the dorsal striatum +1.2 mm from bregma. Scale bar indicates 100 μm. Areas sampled in the experiment are indicated in the diagram on the left. Areas sampled in the experiment relative to other anatomical features are schematically indicated on the left. Areas depicted by images are shaded gray.

Dorsal Medial +1.2   Dorsal Lateral +1.2

5

25

100

Saline

1.7

1.2

0.0
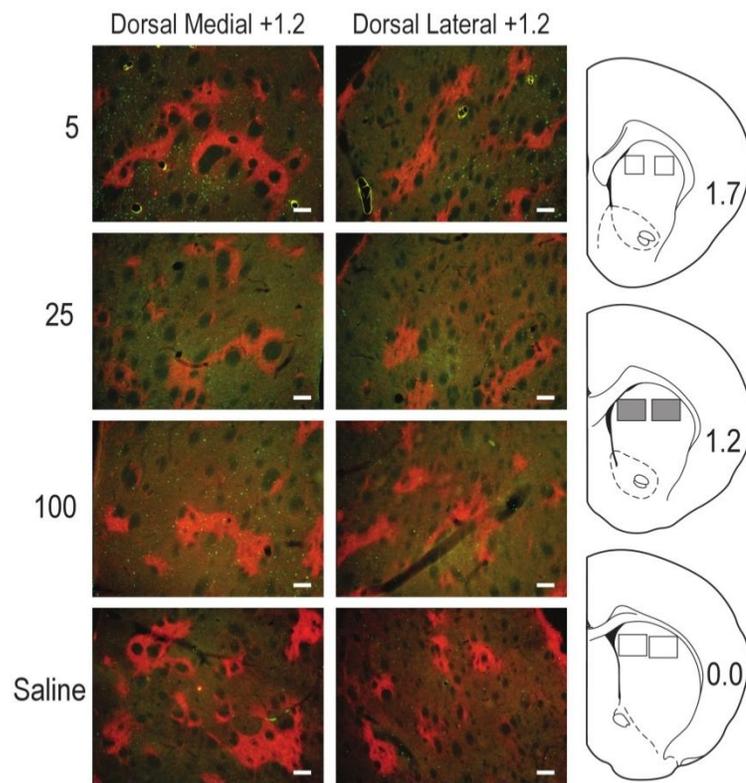
Fig. 2.5 The mean (±SEM) number of Fos positive nuclei per mm^2 in four subregions of the dorsal striatum across three rostrocaudal levels (+1.7, +1.2, +0.0mm from bregma) in rats given an acute infusion of saline or cocaine delivered over 5, 25 or 100 sec.

Dorsal Medial   Dorsal Lateral

Ventral Medial   Ventral Lateral

Fos Nuclei / mm^2

500

250

0

Posterior to Anterior   Posterior to Anterior

0.0   1.2 1.7   0.0   1.2 1.7

5s
25s
100s
Saline

53
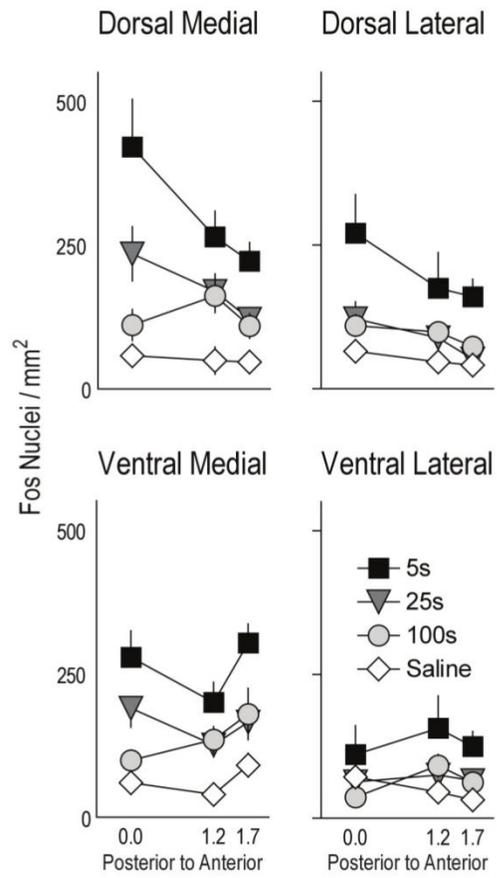
Fig. 2.6 The mean (± SEM) number of Fos positive nuclei per mm^2 in the nucleus accumbens shell and core subdivisions, and the infralimbic cortex (IL) sampled at +2.0mm from bregma, in rats given an acute infusion of saline or cocaine delivered over 5, 25 or 100 sec.
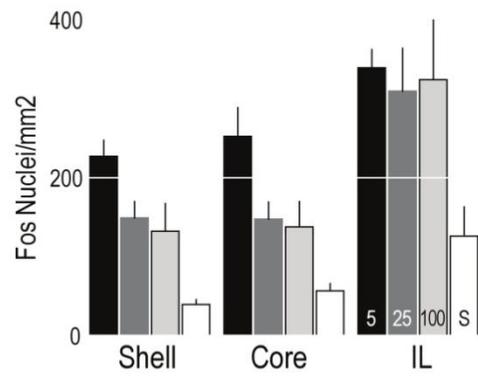
Fig. 2.7 Panel a: The mean (± SEM) number of Fos positive nuclei in the patch (P) or matrix (M) subcompartments of either the dorsal medial (DM) or dorsal lateral (DL) striatum in rats given saline (S), or an acute infusion of cocaine delivered over 5, 25 or 100 sec. The number of Fos positive cells was averaged across all rostrocaudal levels sampled (+1.7 mm, +1.2 mm, +0.0mm), as this factor did not influence the results. Panel b: The same data as in Panel a, but expressed as the mean (+ SEM) Index of Striasomal Matrix Predominance (ISMP). A value of 1 indicates no difference between the patch (striasome) and matrix. Values greater than 1 indicate more Fos nuclei in the patch subcompartment.

a) Patch & Matrix Fos Counts
Averaged Across Levels

b) Patch Matrix Ratio
Averaged Across Levels
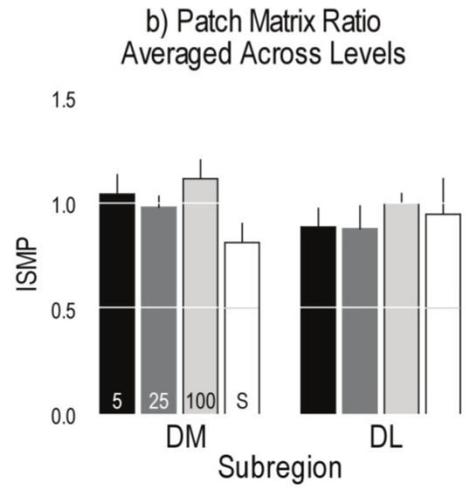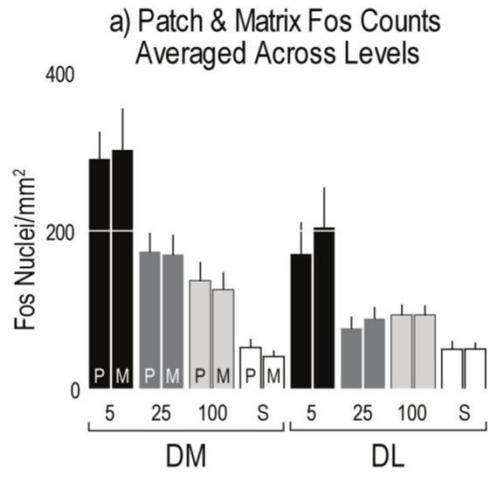
CHAPTER 3

THE INFLUENCE OF RATE OF INTRAVENOUS COCAINE DELIVERY ON SELF-ADMINISTRATION BEHAVIOR UNDER LIMITED VS. EXTENDED ACCESS CONDITIONS

Introduction

Cocaine use and its ability to foster addiction is a persistent and widespread public health problem, therefore there is considerable interest in understanding the factors that influence liability to addiction. One such factor is how quickly cocaine reaches the brain. The rate at which a drug reaches the brain largely depends on its formulation and route of administration into the body (Javaid et al., 1978; Javaid et al., 1983; Jones, 1990; Foltin and Fischman, 1991; Hatsukami and Fischman, 1996), and there is strong evidence that how rapidly cocaine reaches the brain is a major factor contributing to its abuse liability (Jones, 1990; Gossop et al., 1992, 1994; Hatsukami and Fischman, 1996; Ferri and Gossop, 1999). For example, subjects who initiate their use through smoking the rapidly absorbed "crack" cocaine base, rather than insufflating ("snorting") the more slowly absorbed cocaine hydrochloride, are more likely to develop symptoms of addiction or clinical dependence (Gorelick, 1998; Chen and Anthony, 2004; O'Brien and Anthony, 2005).

The rapid delivery of cocaine produces greater euphorigenic effects than slower delivery (Resnick et al., 1977; Fischman, 1984; Volkow et al., 2000; Abreu et al., 2001; Nelson et al., 2006), and this is a common explanation for faster rates of cocaine administration facilitating the transition to addiction (Gorelick, 1998). Consistent with this, there is some evidence from animal studies that increasing the rate of drug infusion increases the reinforcing properties of cocaine (Balster and Schuster, 1973; Kato et al., 1987; Panlilio et al., 1998; Woolverton and Wang, 2004). However, in some of these

58

studies, effects were seen only when rates of infusion differed to such an extent that they would result in different brain levels of the drug. Indeed, in rats, cocaine infused between 5 and 100 sec produced the same peak brain levels of cocaine, and there was no effect on self-administration behavior when animals were given limited access to the drug over a range of different schedules of reinforcement (Pickens et al., 1969; Crombag et al., 2008). Although rats can learn to discriminate and preferentially select faster rates of cocaine delivery when given a choice (Schindler et al., 2009), the evidence that it influences the reinforcing effects of cocaine is not very compelling.

However, in the preceding chapter, I reported that how rapidly cocaine reaches the brain has a large effect on its neurobiological impact on the mesocorticolimbic system. When cocaine reaches the brain rapidly it engages more cells and circuits than when it reaches the brain at a slightly slower rate. These neurobiological changes produced by faster rates of drug delivery may contribute to the transition from casual use to addiction (Samaha and Robinson, 2005). If so, faster rates of infusion should also strongly influence drug-taking and drug-seeking behavior in the rat, especially under conditions that can produce addiction-like symptoms.

Previous studies on the effects of rate of drug delivery on self-administration behavior mentioned above used relatively limited access self-administration procedures, and there are now a number of reports that extended access to cocaine is required for the development of addiction-like symptoms. When given extended access to cocaine, either by increasing the amount of time it is available each day or by making it available for months, rats begin to exhibit changes in behavior resembling the symptoms of human addicts, including a escalation of intake (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002), resistance to drug related punishment (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Pelloux et al., 2007; Belin et al., 2008), and an increased motivation for drug (Deroche-Gamonet et al., 2004; Ben-Shahar et al., 2008). Consistent with this, rats with a fairly extensive history of self-administration under a progressive ratio schedule of reinforcement gradually increase their breakpoints across successive sessions, but only if their drug is delivered rapidly (Liu et al., 2005). I

hypothesized, therefore, if the rate of cocaine delivery to the brain influences the propensity to addiction, it may influence self-administration behavior only if rats are given extended access to cocaine – even if the same rate has no effect on its reinforcing properties under limited access conditions. The purpose of this study was to test this hypothesis.

Material and Methods

Subjects and Housing

Male Wistar rats (Harlan, IN) weighing between 225 – 250 g upon arrival were used in this experiment. Rats were individually housed in a climate-controlled animal colony maintained on a 14:10 light/dark cycle (lights on at 0800), with food and water available *ad libitum*. Rats were given a minimum of 5 days to habituate to the animal colony. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Animals were included in the experiment only if they acquired and maintained stable self-administration behavior and completed all phases of testing. A total of 46 animals completed the study. Animals were removed from the experiment mainly because they did not acquire self-administration behavior or their catheters lost patency before completing the experiment. This did not impact one group more than the others.

Apparatus

Behavioral training and testing occurred in operant chambers located in a different room than the animal's home cage. The chambers measured 22 x 18 x 13 cm (Med-Associates, VT), and were located inside larger sound-attenuating cabinets, which were continuously ventilated by a fan providing background noise. Each operant chamber was outfitted with two illuminated infrared nose-poke ports surrounding a centrally located pellet dispenser and pellet cup, and a red houselight was positioned on the opposite wall. The floor of the chamber consisted of a grid floor with corncob bedding

below the grids, which was replaced daily. Chambers were controlled by a PC computer (Intel Core Duo E4500) running Med-PC Version IV.

Each operant chamber was equipped with an infusion apparatus consisting of a 10 mL syringe installed in a PHM-100 pump (Med-Associates, VT) attached via a length of Tygon microbore tubing to an Instech-Solomon liquid swivel (Plymouth Meeting, PA) mounted on a counterbalanced arm. On training and test days, animal's catheters were attached to the liquid swivel via another length of PE tubing inserted within a homemade spring. Rates of drug delivery were controlled by installing motors (Model R-DE, Med-Associates, VT) capable of delivering a 50 µL infusion over 4.5 sec (2 RPM, 0.661 mL/min), 45.4 sec (0.2 RPM, 0.066 mL/min), and 90.8 sec (0.1 RPM, 0.033 mL/min). In referring to these groups, the rates are "rounded" and I will refer to groups receiving cocaine over 5, 45 or 90 sec. Prior to each use, the apparatus was flushed with a 20% ethanol aqueous solution (V/V) to ensure adequate liquid flow through the swivel and lines. Additionally, every 20 days the infusion efflux delivered by the apparatus was volumetrically measured.

The rates of infusion used here were chosen because they represent a range similar to what addicts report using (Zernig et al., 2003), captures the variation between snorting and smoking cocaine (Jones, 1990), and a range that influences its subjective effects in humans (Abreu et al., 2001). Also, as demonstrated in the previous chapter, variation in cocaine delivery over this range influences the ability of cocaine to induce immediate early genes, even though they produce comparable peak levels of cocaine in the brain and the same amount of dopamine efflux in the striatum (Pan et al., 1991; Ferrario et al., 2008).

Pretraining

An illustration of the general experimental design is shown in Figure 3.1. To facilitate the acquisition of cocaine self-administration, rats underwent operant response training using a food reward prior to jugular catheterization. On the first day, rats were habituated to the operant chamber, and one food pellet (45 mg Banana flavored Dustless Precision Pellets, Bio-Serv, NJ) was delivered into the pellet cup at a pseudo-random

interval approximately every 30 sec during a 30 min session. On the next three days, rats were trained to nosepoke for food reward using a continuous schedule of reinforcement (FR1). At the start of session, the houselight turned on and the "active" nosepoke port was illuminated for 20 sec. Responses in the active port resulted in the delivery of one pellet. Responses in the "inactive" port were recorded but did not have any programmed consequences. Rats were then transitioned to a fixed ratio 2 (FR2) schedule for a minimum of three days to further aid in discrimination between the active and inactive nosepoke ports.

Catheter Construction and Surgical Procedures

Chronically indwelling jugular catheters were placed into rats using a similar procedure described elsewhere (Weeks and Davis, 1964; Weeks, 1972; Crombag et al., 1996; Samaha et al., 2002). Briefly, catheters were constructed from SILASTIC® tubing as described in Chapter 2. While not in use, catheters were capped with obdurators. All catheters had a dead volume of 24-26 μL.

Rats were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg, intraperitoneally (i.p.)). The backport was secured between the animal's shoulder blades, while the silicon end of the catheter was inserted into the left or right external jugular vein. Catheters were then filled with 0.1 mL solution of 10 mg/mL gentamicin sulfate (Vedco, St. Joseph, MO), in 0.9% sterile bacteriostatic saline to minimize infections and catheter occlusions.

Animals were allowed to recover for a minimum of 4 days. Starting the day after the surgery, catheters were flushed manually once per day with 0.2 mL of the gentamicin solution. Every 7 days animals were infused intravenously with 0.2 mL sodium thiopental solution (20 mg/mL, dissolved in sterile water, Hospira, Lake Forest, IL) to assess catheter patency. Animals that did not become ataxic within 5-10 sec were removed from the study. Rats did not undergo self-administration training on their catheter patency test days.

Drugs and self-administration training

Cocaine hydrochloride (National Institutes of Drug Abuse, MD) was prepared in a 0.9% sterile saline solution. During self-administration sessions, animals received infusions consisting of 0.4 mg/kg cocaine (weight of the salt) in a volume of 50 μL. The unit dose was adjusted every four days.

After recovery from surgery, animals were re-acclimatized to the operant chamber by an additional session with an FR2 response requirement for a food pellet. The following day all animals began daily cocaine self-administration training sessions. All training sessions were one hour long, and *in all training sessions all rats received cocaine over 5 sec*. Before each session, catheters were flushed with 0.2 mL of the gentamicin solution, and then attached to the infusion apparatus. Upon an experimenter-initiated command, the infusion pumps were activated to fill the dead volume of the catheters prior to the start of session. Once catheters were filled, the session began with the illumination of the houselight and a 20 sec illumination of the active nosepoke port. Initially, animals were trained on a continuous reinforcement schedule (FR1) receiving cocaine followed by an unsignaled 20 sec timeout interval, where further responses were recorded but had no consequences. Once animals were responding above a minimum training criterion (2 or more injections per session, twice as many active versus inactive nosepokes) for two consecutive days, the response requirement was increased to an FR2 schedule of reinforcement. After achieving stable performance above the minimum training criterion at this schedule for two consecutive days, the timeout interval in subsequent daily sessions was lengthened from 20 sec to 45 sec, then 63 sec, and finally 85 sec. The reason for increasing the final timeout period to 85 sec was so that in subsequent sessions using different rates of infusion, the total number of infusions possible for all subjects in all groups would remain the same. That is, regardless of infusion rate all rats could only take one injection every 90 sec (see Fig. 3.2).

Influence of Rate of Infusion: Short Access (1 hr) Sessions

After acquiring cocaine self-administration on a FR2 schedule of reinforcement during one hour sessions where cocaine was injected over 5 sec with an 85 sec timeout

interval (this is referred to as "Baseline"), animals were assigned to one of three groups that differed in rate of cocaine delivery. One group ("5 sec," n=14) continued to receive cocaine infusions at the training rate of infusion with the same timeout interval. A second group ("45 sec," n=18) received their infusions over 45.4 sec followed by a 45 sec timeout interval. The last group ("90 sec," n=14) was infused with cocaine delivered over 90.8 sec, with no timeout interval (Fig 3.2). Individual subjects were assigned in such a way that the mean number of infusions achieved during the last two days of baseline training was balanced between groups. Rats were tested for three daily sessions at their respective new rates during "short access" (1 hr) sessions.

Influence of Rate of Infusion: Long Access (6 hr) Sessions

From Day 4 to Day 20 of self-administration testing, the length of each session for all groups was increased to six hours/day, as this procedure is reported to lead to the development of addiction-like symptoms (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002). Once the sessions were extended, animals began self-administration testing at 1100 in order to reduce the influence of circadian rhythms on self-administration performance. Rats were removed from the study during long access sessions if they failed to maintain a median number of infusions greater than 2.5 and a median active/inactive nosepoke ratio greater than 1 throughout the experiment.

Statistics

Behavioral data during long access sessions was analyzed as bins comprised of the average of two contiguous daily sessions. Between group comparisons were analyzed using one-way analyses of variance (ANOVA) or two-way ANOVA, where appropriate. Data with repeated measures over sessions or time was analyzed using Linear Mixed Models analysis (LMM) using the SPSS 16 statistical package. For LMM analysis, the best fitting model of repeated measures covariance was determined by the lowest Akaike Information Criterion (AIC) score (West, 2007). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. If the LMM resulted

in significance, mixed model pairwise multiple comparisons of repeated measures means (Kowalchuk and Keselman, 2001) were used to test for group differences.

Results

Training

Fig 3.3 shows the number of active and inactive nosepokes (Panel a) and cocaine infusions (Panel b) during training sessions where rats met the criteria for self-administration (2 or more injections per session, twice as many active versus inactive nosepokes, for two consecutive days), and advanced through stages where the timeout interval during successive daily sessions was increased from 20, 45, 63, and finally 85 sec. During these training sessions, all rats self-administered 0.4 mg/kg/inf cocaine delivered over 5 sec. As expected, a linear mixed models analysis (LMM) with repeated effects of training session showed that there was no effect of differing timeout intervals on either active nosepoking behavior ($F(3,45)=1.667$ $p=0.187$) or the number of infusions during these sessions ($F(3,135)=1.247$ $p=0.295$).

Fig. 3.4 "Baseline" shows the mean number of active and inactive nosepokes (Panel a) and cocaine infusions (Panel b) averaged over the last two training sessions when all rats received cocaine over 5 sec (for these data the 3 groups represent the animals that on the next day will receive cocaine at different rates). A two-way ANOVA on the number of nosepokes during the session with group (rate of infusion, ROI) and nosepoke type ("Active" or "Inactive") as fixed factors showed a main effect of nosepoke type ($F(1,84)=54.68$ $p<0.0001$), but no effect of group ($F(2,84)=2.423$ $p=0.095$), and no significant group by nosepoke interaction ($F(2,84)=1.028$ $p=0.362$). A one-way ANOVA on the number of infusions with ROI as the factor revealed no significant difference in the number of infusions (Fig. 3.4b; $F(2,26)=0.622$ $p=0.54$). Thus, prior to being subjected to different conditions, there were no group differences in either discriminating nosepoke ports or number of infusions.

Short Access Sessions

Figure 3.4a also shows the number of nosepokes made during three daily one hour ("short access," ShA) sessions (ShA1-3) after rats were assigned to groups that received cocaine over 5, 45 or 90 sec. A LMM on the number of active nosepokes during the ShA sessions with ROI (group) as a fixed factor and session as a repeated measure revealed that there was no significant effect of group ($F_{(2,43.8)}=0.776$ p=0.465), session ($F_{(2,74.2)}=0.198$ p=0.819) or a significant interaction ($F_{(4,74.2)}=0.611$ p=0.655). Figure 3.4b shows that for number of infusions there were also no effects of group ($F_{(2,44)}=2.553$ p=0.88), session ($F_{(2,85)}=0.407$ p=0.666), or group by session interaction ($F_{(4,85)}=0.134$ p=0.96).

Figure 3.4c shows the number of infusions achieved by three representative subjects from each group during the last short access session. Subjects were chosen who represent the group averages. The length of the horizontal line represents the duration of one short access session, and each vertical line shows the time during the session the animal received an infusion of cocaine.

In summary, during ShA sessions there was no significant effect of varying the rate of cocaine infusion on self-administration behavior, as assessed by either the number of active nosepokes or total infusions received during the session. This is consistent with our more extensive previous studies on the effect of rate of cocaine or amphetamine delivery on self-administration behavior under limited access conditions (Crombag et al., 2008).

Long Access Sessions

Figure 3.5 shows the number of active nosepokes (Panel a) and cocaine infusions (Panel b) made by rats in each group after the sessions were lengthened to 6 hours/day ("long access," LgA). LgA session data were analyzed as blocks of two daily sessions, and the average for the ShA sessions is shown for comparison.

Not surprisingly, all groups increased both their total number of nosepokes (Panel a) and infusions (Panel b) when the test session was extended from 1 to 6 hrs/days. To determine whether active nosepokes differed by group during LgA sessions, an overall

LMM analysis with session blocks as a repeated measure was conducted on the number of active nosepokes during LgA sessions. There was an overall effect of group ($F_{(2,43)}$=3.562 p<0.038), but no effect of session ($F_{(7,43)}$=0.990 p=0.452), and no interaction ($F_{(14,43)}$=0.931 p=0.535). To determine how the groups differed from one another during LgA sessions, each group was compared using paired repeated effects LMM analysis. The 5 sec group made significantly more nosepokes than the 90 sec group (effect of group, $F_{(1,30)}$=7.625 p<0.011; interaction $F_{(7,39)}$=1.103 p=0.381). Likewise the 45 sec group made significantly more nosepokes than the 90 sec group ($F_{(1,33)}$=5.695 p<0.024; interaction $F_{(7,50)}$=0.471 p=0.851). Fig. 3.5a shows that there was a tendency for the 45 sec group to make more active nosepokes than the 45 sec group, but this difference was not statistically significant ($F_{(1,30)}$=1.245 p=0.273). The reason for the trend, however, is because animals in the 45 sec group made more unrewarded responses during the timeout period than animals in the 5 sec group.

Similar LMM tests were conducted to determine if there was an effect of group on the number of infusions obtained during LgA sessions. There was an overall effect of group ($F_{(2,45)}$=13.986, p<0.001), but no effect of session ($F_{(7,87)}$=1.842 p=0.089) and no group by session interaction ($F_{(14,87)}$=1.718 p=0.066). The 5 sec group obtained significantly more infusions than the 90 sec group (group, $F_{(1,27)}$=24.719 p<0.001; interaction ($F_{(7,49)}$=3.608 p<0.004), as did the 45 sec group ($F_{(1,30)}$=25.426 p<0.001). The 5 and 45 sec groups did not differ from one another (group, $F_{(1,31)}$=0.028, p=0.868; interaction ($F=_{(7,58)}$=1.556 p=0.167).

In summary, it is obvious from inspection of Fig. 3.5 that animals given cocaine over 5 or 45 sec markedly increased the number of nosepokes and their associated cocaine intake by comparable rates when given 6 hrs of access to cocaine each day. In striking contrast, animals given cocaine over 90 sec only modestly increased their drug intake relative to what they took when drug was only available for 1 hr/day. Indeed, animals in the 5 and 45 sec groups increased their cocaine intake about 8-fold when given a longer time to self-administer, but despite 6-fold more time available to take cocaine, animals in the 90 sec group only increased their intake about 2-fold.

Within Session Pattern of Self-Administration

Fig. 3.6a shows that during the LgA sessions, all animals in all groups discriminated between the active and inactive nosepoke ports. Although the ratio of active to inactive responses was highest in the 5 and 45 sec groups, the 90 sec group made 10-fold more responses in the active than in the inactive port, indicating they clearly continued to discriminate between the active and inactive ports.

Fig. 3.6 also illustrates the temporal pattern of self-administration behavior within the 6 hr sessions, first by plotting cumulative infusions in 30 min bins (averaged over the last 6 days of LgA testing). Fig. 3.6b shows that the 5 and 45 sec groups maintained a stable and high rate of self-administration for the duration of the 6 hr sessions. The cumulative infusions in the 90 sec group was much lower than in the other groups, but did progressively increase as the session progressed (effect of session bin, $F(11,46)=8.822$ ($p<0.001$), indicating that animals in the 90 sec group continued to take cocaine throughout the session. This is also illustrated in Fig. 3.6c, which shows representative data from individual subjects (also shown in Fig. 3.3c) during session 14 of LgA. The horizontal line represents the duration of a single six hour session and each vertical tick mark represents a single infusion received by the subject. Finally, the rate of self-administration (infusions/min) within sessions is shown in Fig. 3.6d, confirming that the 5 and 45 sec groups maintained a stable rate of self-administration for the entire duration of the session. Although the rate of self-administration in the 90 sec group was much lower as was expected, self-administration was not only maintained throughout the session, but the rate was even somewhat elevated in the last 3 hrs relative to the first 3 hrs of the session (effect of session bin, $F(10,13)=4.365$ $p<0.009$). This was consistent with the small upward deflection seen in cumulative infusions during the latter half of the session (see Panel b). Thus, all groups continued to self-administer cocaine throughout the 6 hr sessions.

Analysis of the First Hour of the LgA Sessions

It has been reported that when given extended access to cocaine, rats increase not only their total intake, but over time they escalate the amount of drug they take during the

first hour of each session (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002). Therefore, I also analyzed self-administration behavior during the first hour of the extended access sessions. When all subjects were taken into account, animals in neither 5 sec nor 45 sec groups significantly escalated their number of infusions during the first hour of LgA sessions as compared to ShA sessions (5 sec – $F_{(8,13)}=2.312$ p=0.086; 45 sec – $F_{(8,17)}=0.737$), and animals in 90 sec group actually decreased infusions in the first hour over days of testing ($F_{(8,104)}=10.429$ p<0.001).

There was, however, considerable individual variation in first hour intake. Therefore, I divided the animals within each group into "high performers" (top 50%) and "low performers" (bottom 50%), based on the total number of first hour infusions during the last 8 days of LgA testing. The low performers in all groups showed a small decline in 1st hr infusions across days of LgA testing (data not shown). In marked contrast, "high performers" in the 5 and 45 sec groups did not decrease 1st hr intake over time (Fig. 3.7). Indeed, animals in the 5 sec group tended to increase their 1st hr intake across days of testing ($F_{(8,37)}=2.122$ p=0.058), although there was no change across time in the 45 sec group ($F_{(8,26)}=0.519$, p=0.831). Even the high performers in the 90 sec group decreased their first hour intake over time (Fig. 3.7). The differences between the 5 and 45 sec groups evident in Fig. 3.6 were statistically significant (5 vs. 45 sec, effect of group, $F_{(1,30.8)}=4.187$ p<0.050; interaction non-significant; 5 vs. 90 sec, effect of group $F_{(1,16)}=34.735$ p<0.001, group by time interaction $F_{(7,67)}=2.210$ p<0.045). This analysis suggests, therefore, that at least a subset of animals ("high performers") given a 5 sec infusion of cocaine escalated their first hour cocaine intake to a greater extent than animals given cocaine over 45 or 90 sec.

Discussion

The purpose of this study was to test whether the rates of cocaine delivery shown in Chapter 2 to strongly influence Fos expression would also influence drug-taking behavior if rats were given extended access to cocaine – even if these rates do not alter the reinforcing effects of cocaine under more limited access conditions. The study

assessed whether varying the rate of i.v. cocaine injections (5, 45 or 90 sec injections) would influence cocaine self-administration behavior in rat under two different conditions. Rats were initially tested under limited access conditions (1 hr/day), then subsequently allowed more extended access (6 hrs/day). Self-administration under limited access conditions is thought to either model more casual use in humans (Deroche-Gamonet et al., 2004; Robinson, 2004), or tests the reinforcing qualities of cocaine (Headlee et al., 1955; Pickens et al., 1969). Indeed, one highly influential hypothesis as to why more rapid rates of drug infusion are addictive is that they are more pleasurable (Resnick et al., 1977; Fischman, 1984; Volkow et al., 2000; Abreu et al., 2001; Nelson et al., 2006), and therefore are more reinforcing (Gorelick, 1998). However, even though rats can distinguish between slower and faster rates of drug reward and prefer them when given a choice (Schindler et al., 2009), this study confirmed our previous findings that infusions between 5 sec and 90 sec do not significantly affect cocaine self-administration behavior in rats allowed only limited access to drug as infusions between 5 – 90 sec were equally reinforcing (also see Crombag et al., 2008).

However, when animals were given extended access to drug, a procedure previously shown to produce symptoms resembling addiction in rats, striking group differences in drug-taking behavior emerged. Animals that had their cocaine delivered over 5 or 45 sec increased their total intake about 8-fold. However, despite having 6-fold more time to take cocaine, when cocaine was delivered over 90 sec animals only increased their intake by about 2-fold. This was not due to group differences in the opportunity to self-administer the drug, because the experimental design was such that all animals had the same opportunity to take exactly the same number of injections by keeping the minimum inter-infusion interval the same across all groups.

n order to ensure that all groups had the same opportunity to self-administer cocaine, the timeout intervals were required to vary as a function of group. We considered, therefore, whether this influenced the reinforcing effects of cocaine, and thus self-administration behavior. I found that it did not. First, all rats were initially trained to self-administer cocaine (0.4 mg/kg/inf given over 5 sec) with each timeout interval used

in the latter portions of the study. During this stage of training, there was no influence of varying the timeout interval on self-administration behavior. Importantly, there was also no influence of rate of cocaine delivery (and thus also varying the timeout interval) when animals were tested under short access conditions. This is consistent with previous studies that used the same procedures as used here, even when animals were tested using a greater variety of schedules of reinforcement (Crombag et al., 2008). For example, Crombag et al. (2008) found no effect of varying the rate of cocaine or amphetamine delivery between 5 and 90 sec on performance on a progressive ratio schedule of reinforcement – all group achieved the same "breakpoints". These data suggest, therefore, that variation in the timeout interval itself does not alter self-administration behavior by altering either the reinforcing effects of cocaine, or motivation for cocaine. It seems unlikely, therefore, that this factor is responsible for the large group differences in the amount of cocaine intake seen when animals were tested under extended access conditions.

It is not surprising that a timeout interval of up to 90 sec has little effect on the rate of self-administration when one considers the typical interval between injections when rats are tested with much shorter timeout intervals – and therefore with greater opportunity to take drugs. For example, Mantsch and colleagues (2001) reported that when rats were given 10 hours of access to self-administer 0.5 mg/kg/inf cocaine over 5 sec with a 20 sec timeout interval, on average they took one infusion roughly every 6 minutes (360 sec). In addition, Belin and colleagues (2009) reported that rats given extended access to cocaine and showing addiction-like behaviors also develop "burst"-like patterns of self-administration. However, even in animals showing the most severe index of addiction-like behavior, they reported a mean interinfusion interval of 3 minutes (180 sec). Lastly, Ferrario and colleagues reported an average interinfusion interval of 108 sec in long access sessions with a 20 sec timeout interval and using conditioned stimuli associated with drug delivery, which facilitates even higher levels of drug-taking. Thus, rats in extended access conditions "self impose" a longer interval between injections than was required here. Finally, even if long timeout intervals constrained self-

administration behavior, the most likely outcome would have been a decrease in self-administration in groups with the 85 sec timeout interval (animals receiving cocaine over 5 sec) and an increase in self-administration in rats with no timeout interval (those receiving cocaine over 90 sec). This is clearly not the case; animals with the longest timeout interval (and fastest rate of infusion) increased their intake 8-fold while animals with no timeout interval only increased their intake 2-fold.

Obviously, if an individual does not increase their drug intake when they have the opportunity to do so, they will be less likely to abuse drugs and to develop an addiction. Perhaps this is one reason that drugs or formulations that enter the brain relatively slowly tend to be less addictive – they do not promote increased intake. The important question, therefore, is if rate of infusion does not markedly alter the reinforcing effects of cocaine under limited access conditions, why does it have such a profound effect on self-administration behavior under extended access conditions?

Perhaps this increase in drug intake reflects a greater neurobiological impact of faster drug delivery (Samaha and Robinson, 2005). I have shown in the previous chapter that Fos IEG expression after 90 sec infusions is significantly lower than when the same dose of drug is delivered over 5 sec. Recently, increased cocaine-induced Fos expression has been associated with other persistent biochemical and structural changes in the brain related to psychomotor sensitization (Zhang et al., 2005; Xu, 2008). Therefore, addiction-like behavior, such as a marked escalation of intake in the 5 and 45 sec groups during long access sessions, may reflect sensitized incentive motivational processes (Robinson and Berridge, 1993). Thus, while cocaine remains reinforcing when delivered over 90 sec, as indicated by the fact that animals continue to take the drug regularly throughout the session, it is possible that this rate of infusion does not induce neurobiological changes in the brain sufficient to sensitize incentive motivational processes. In support of this idea, a greater neurobiological impact specific to extended access and not limited access self-administration has been found. Ferrario and colleagues (2005) have shown that escalation of drug intake during long access is associated with greater psychomotor sensitization and a higher density of dendritic spines in the core of the nucleus

accumbens. Increases in dendritic spine density in the core of the nucleus accumbens is a structural modification reflecting possible sensitization-related synaptic reorganization within the mesocorticolimbic circuitry (Li et al., 2004; Robinson and Kolb, 2004; Ferrario et al., 2005).

One behavioral consequence of these drug-induced neurobiological changes in the mesocorticolimbic circuitry is a sensitized motivation for drug – an increased "wanting" for drug (Robinson and Berridge, 1993). While I have shown in Chapter 2 that faster rates of drug infusion have a greater neurobiological impact as indicated by Fos expression, and in this study I have shown that faster drug delivery strongly influenced rates of drug-taking only during conditions able to produce addiction-like behavior, many questions remain. For example, if the escalation in intake observed in rats receiving cocaine over 5-45 sec during extended access is due to addiction-related changes in the brain resulting in a sensitization of incentive motivation, then only rats with prior experience with rapid infusions during long access should develop other symptoms resembling addiction. Rats with rapid infusion experience should have a greater and persistent motivation for drug when compared to animals with a history of extended access to 90 sec cocaine infusions, particularly in models testing for the propensity to relapse. This hypothesis is examined in the next chapter.

Fig. 3.1 An illustration of the experimental design.

Pre-training ⟶ Implant ⟶

| 1 Hour | 6 Hours |

Self-Administration Training
(0.4 mg/kg over 5s) ⟶ Baseline Sessions

Short Access ⟶ Long Access
(0.4 mg/kg over 5s)

Short Access ⟶ Long Access
(0.4 mg/kg over 45s)

Short Access ⟶ Long Access
(0.4 mg/kg over 90s)
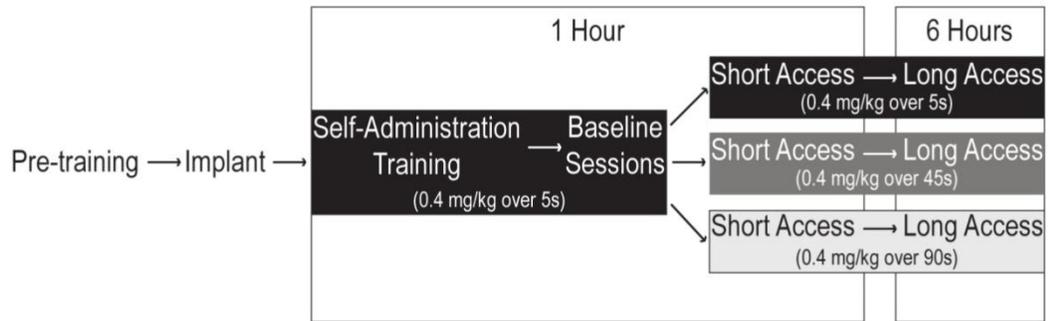
Fig. 3.2 A representation of a typical long access session for animals in the 5, 45 and 90 sec groups. In the bottom portion of the figure, the length of the session is represented by a horizontal line. Vertical tick marks represent nosepokes where animals received an infusion of cocaine. For clearer comparison, the inset circle shows the infusion lengths and timeout intervals of the three groups in the experiment. The duration of the infusion and following timeout interval is indicated for each group. Note that the number of opportunities to self-administer an injection is exactly the same in all groups because the timeout periods varied accordingly.
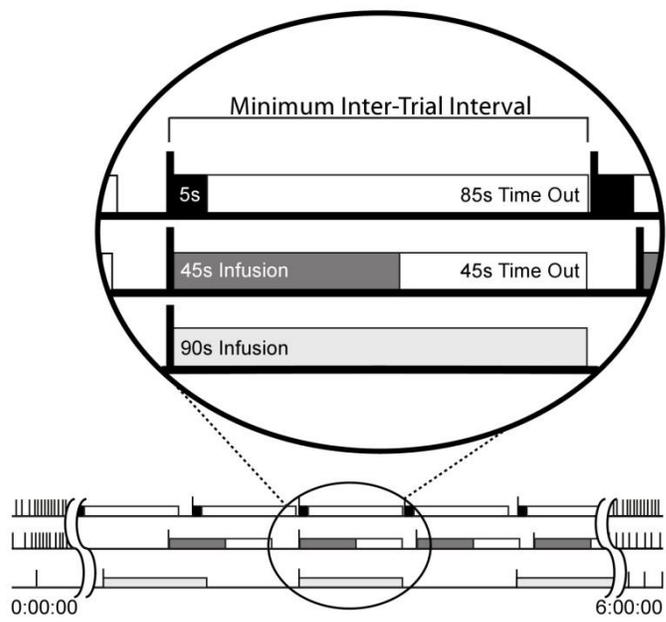
Fig 3.3 Panel a: The mean (±SEM) number of active and inactive nosepokes made by all rats used in this experiment during self-administration training sessions. Panel b: The mean number of infusions made by all subjects during self-administration training sessions. During these training sessions all rats received the same rate of cocaine infusion (5 sec), and the timeout lengths were lengthened each time a rat reached criterion performance for self-administration. Rats began training with a 20 sec timeout interval and were stepped to intervals of 45, 63, and 85 sec in subsequent sessions. Data presented in the 85 sec timeout sessions in this figure is the average of all subjects comprising the "Baseline" data point presented in Fig. 3.3. Thus to reduce possible confounding contributions of timeout intervals on self-administration behavior, all rats had prior experience with all possible timeout intervals used in subsequent portions of this study.

Timeout Interval Training

a

Nosepokes

Active

Inactive

b

Infusions

20s   45s   63s   85s

Timeout Interval

79
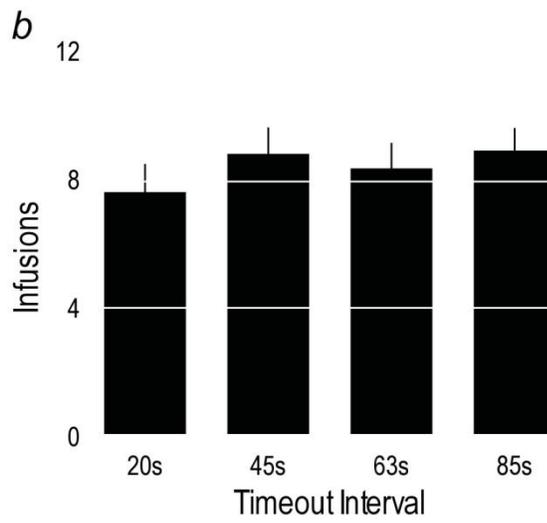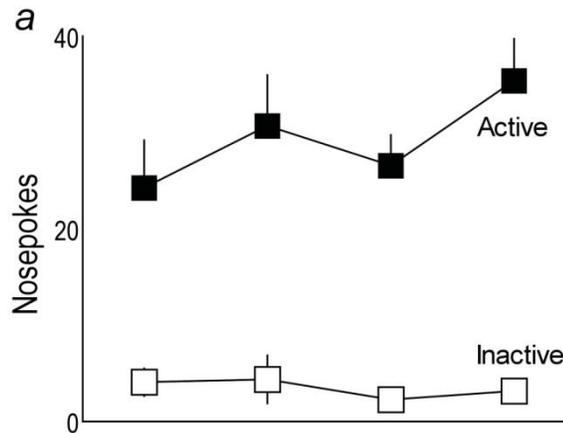
Fig. 3.4 Panel a: The mean (±SEM) number of active and inactive nosepokes made during baseline training sessions, when all rats received cocaine at the same rate (5 sec), and during short access (ShA) sessions when rats were assigned to groups that received cocaine over either 5, 45, or 90 sec. Baseline data are averaged over 2 sessions. Panel b: The mean (± SEM) number of infusions received during the same baseline training and ShA sessions as panel a. The pattern of self-administration behavior in 3 representative rats during ShA session 3. The horizontal line represents the duration of the session, and vertical marks indicate the time during the session when an animal injected itself with cocaine.

Short Access Sessions
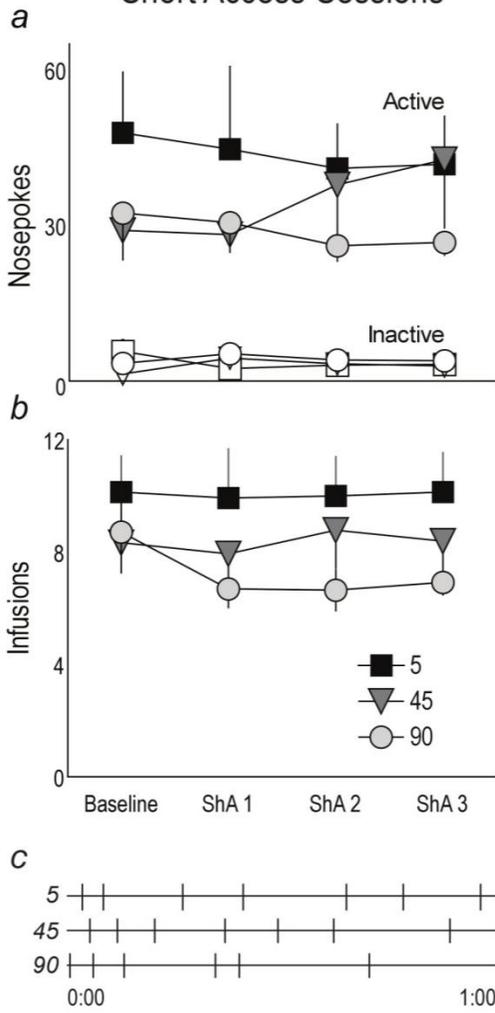
Fig. 3.5 shows the mean (± SEM) number of active nosepokes (Panel a) and cocaine infusions (Panel b) made by rats receiving 0.4 mg/kg/inf cocaine over 5, 45 or 90 sec after self-administration sessions were lengthened from 1 hour to 6 hours/day ("long access", LgA). LgA session data were analyzed as blocks of two daily sessions, and the average for the ShA sessions is shown for comparison.

Long Access Sessions

a

Active Nosepokes

4096
1024
256
64
16

45
5
90

b

Infusions

100
75
50
25
0

45
5
90

ShA 1 2 3 4 5 6 7 8

Sessions Blocks

83
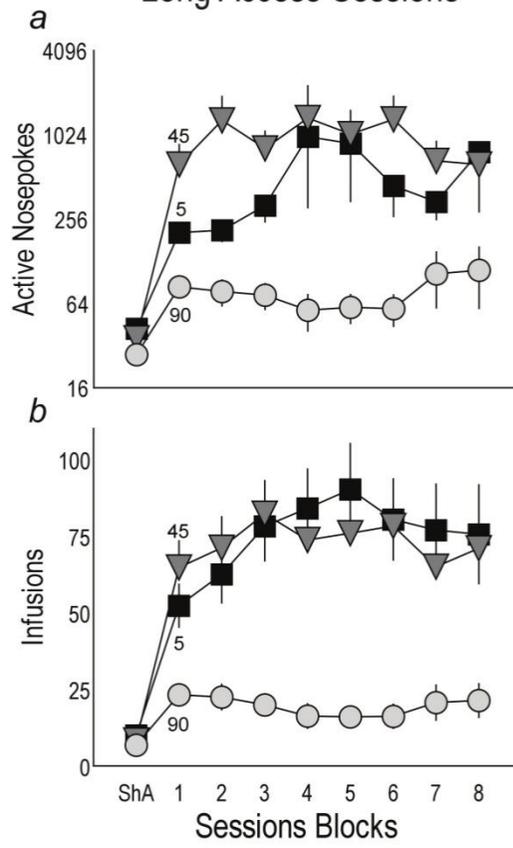
Fig. 3.6 Panel a: The mean (+SEM) ratio of active to inactive nosepokes made by groups during long access sessions. All groups discriminated between the active and inactive ports. Panels b-d show how self-administration behavior was distributed within the last 6 LgA sessions. Panel b: Mean (±SEM) cumulative infusions and total dose of cocaine self-administered by rats receiving cocaine over 5, 45, 90 sec during 6 hr LgA sessions. Panel c: Infusions during LgA session 14 in the same 3 representative rats as shown in Fig 3.3c. Panel d: The mean (±SEM) rate of self-administration (infusions/min) within LgA sessions.

Behavior during LgA Sessions

85
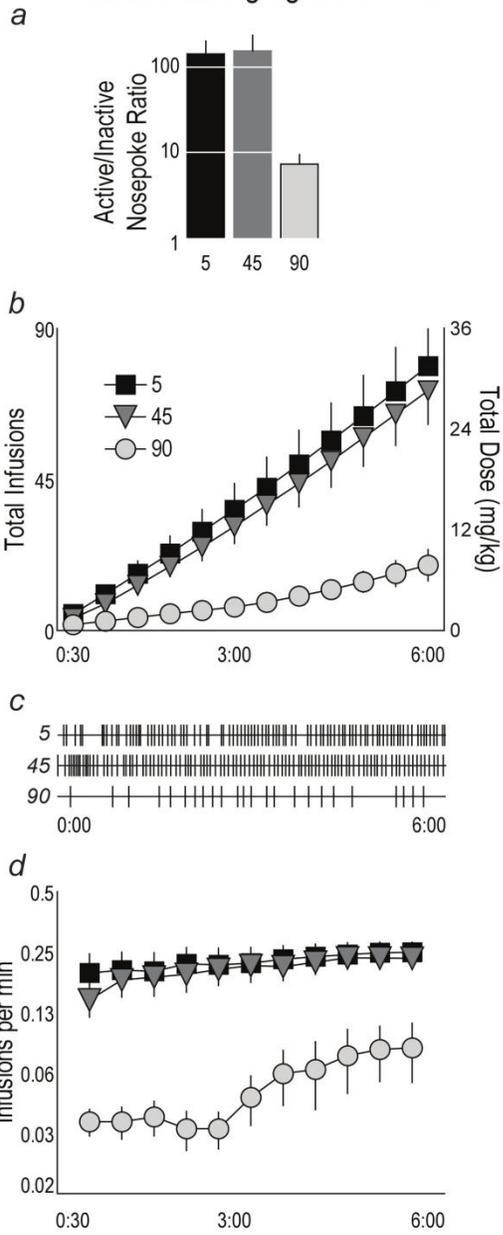
Fig. 3.7 The mean (±SEM) number of infusions during the first hour of each session in "high performers" in each group, plotted over days of testing. "High performers" in each group were those that took above the median number of injections (top 50%) during the first hour (averaged across all LgA sessions).

High Performers

87
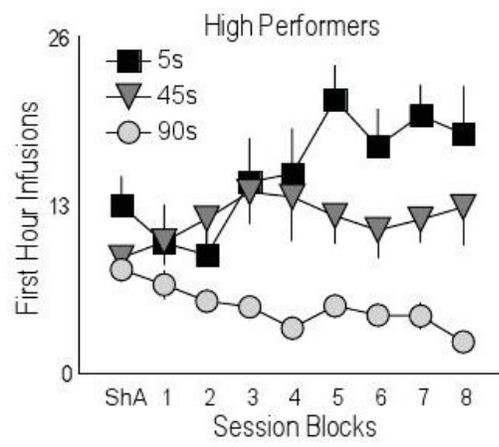
CHAPTER 4

DRUG-PRIMED REINSTATEMENT OF DRUG-SEEKING AND NEUROBIOLOGICAL ADAPTATIONS PERSISTS IN RATS WITH A HISTORY OF EXTENDED ACCESS TO RAPID (5 SEC) BUT NOT SLOW (45-90 SEC) INFUSIONS DURING COCAINE SELF-ADMINISTRATION.

Introduction

In the previous experiment (Chapter 3), I showed that faster (5 – 45 sec) but not slow (90 sec) rates of cocaine infusion are associated with marked increases in drug intake during extended access self-administration sessions. This is despite the fact that varying the rate of infusion over this range has no effect on total amount of drug that reaches the brain (Pan et al., 1991; Samaha et al., 2004) or the amount of dopamine overflow in the striatum (Ferrario et al., 2008). Although one possible explanation for this finding is that faster rates of cocaine are more reinforcing (Gorelick, 1998), an alternative hypothesis is that faster rates of drug infusion preferentially induce long lasting changes in the brain, resulting in the sensitized incentive motivation (Samaha and Robinson, 2005). Sensitized motivation that induces pathological "wanting" of drug and drug-related cues could underlie drug relapse, which remains an enduring and persistent symptom of drug addiction in humans even after prolonged periods of abstinence (Robinson and Berridge, 1993; O'Brien, 1997; American Psychiatric, 2000; Hyman, 2001; Hyman and Malenka, 2001). In support of this hypothesis, intense motivation for drug in humans can be provoked by many drug associated stimuli including re-exposure to small amounts of drug itself (Ehrman et al., 1992; O'Brien et al., 1992).

In rats and other animals, one well established way to model relapse is by drug-primed reinstatement of extinguished drug responding (Gerber and Stretch, 1975; de Wit and Stewart, 1981). This tests the ability of a small amount of drug to reinstate drug-seeking behavior in rats with a history of self-administration training, and is a measure of

heightened motivation for drug (de Wit and Stewart, 1981; Markou et al., 1993; Shalev et al., 2002). While previous work by Crombag and colleagues (2008) in our laboratory has shown that the degree of reinstatement is not influenced by the rapidity of priming infusions, in those studies animals only had limited access to drug during self-administration testing. I reasoned that the effect of rate of infusion on extinction and reinstatement may only appear in rats with a history of self-administration using procedures that foster the development of addiction-like behaviors (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Pelloux et al., 2007; Belin et al., 2008; Ben-Shahar et al., 2008). Others have shown that rats with a history of extended access to cocaine show a greater persistence for drug-seeking during extinction, a greater degree of reinstatement when re-exposed to the drug, and have differing levels of gene expression (Mantsch et al., 2004; Ferrario et al., 2005; Ahmed and Cador, 2006; Kippin et al., 2006; Knackstedt and Kalivas, 2007).

I hypothesized, therefore, if the rate of drug delivery to the brain influences the propensity to addiction, rats with a history of fast rates of infusion during extended access self-administration would persist in seeking drug during extinction and reinstate their drug-seeking behavior when challenged with cocaine to a greater degree than animals with similar experience in self-administration but at slower rates of drug delivery. I also hypothesized that rats with a history of rapid infusions during extended access self-administration sessions may have enduring neurobiological adaptations as a consequence of their experience. I tested this latter hypothesis by measuring the ability of the cocaine priming injection used to reinstate drug-seeking behavior to also induce the immediate early gene, c-*fos*, as assessed by counting the number of cells positive for Fos protein using immunohistochemistry in the nucleus accumbens.

Materials and Methods

Male Wistar rats (Harlan, IN) weighing between 225 – 250 g upon arrival were used in this experiment. Rats were individually housed in a climate-controlled animal

colony maintained on a 14/10 light/dark cycle (lights on at 0800), with food and water available *ad libitum*. Rats were given a minimum of 5 days to habituate to the animal colony. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA). A total of 61 rats were used in this experiment. Of these, 46 had previously been allowed to self-administer cocaine delivered over 5 (n=14), 45 (n=18), or 90 sec (n=13) during daily 6 hr sessions for a total of 16 days (these are the same rats described in Chapter 3). One animal in the 90 sec group died during the course of the 30 day withdrawal and was removed from the analysis. A subset of these animals were used for Fos immunochemistry (5 sec, n=6; 45 sec, n=10; 90 sec, n=8), and an additional 15 drug naïve rats were used as controls for the immunohistochemistry portion of this study.

Apparatus

Behavioral training and testing occurred in operant chambers located in a different room than the animal's home cage, and were identical to the chambers used to train animals on extended access self-administration (Chapter 3). Each operant chamber was equipped with an infusion apparatus consisting of a 10 mL syringe installed in a PHM-100 pump located outside of the chamber (Med-Associates, VT) and attached via a length of Tygon microbore tubing to an Instech-Solomon liquid swivel (Plymouth Meeting, PA) mounted on a counterbalanced arm. On extinction training days, animal's catheters were attached to the liquid swivel via another length of PE tubing inserted within a homemade spring, but the syringes attached to those lines were empty.

Control animals for the immunohistochemistry portion of the experiment were transported daily at the same time as experimental animals were put into the operant chambers, and placed into holding chambers consisting of red plastic buckets 25 cm in diameter and 36 cm in height. They were left there for the same amount of time experimental animals were in the operant boxes. The room housing these chambers was located in a different room from either the home colony or from the operant testing facility. The habituation room for the duration of the experiment was illuminated with a

red light. The purpose of this was to control for daily handling and transport from the home cage.

Groups and Procedures

An illustration of the general design of this experiment is shown in Fig. 4.1. After their last long access self-administration session, animals underwent 10 days of extinction training, using procedures similar to those described previously (De Vries et al., 1998; Shalev et al., 2002; Crombag et al., 2008). During these sessions, animals were taken from the animal colony, placed into test chambers and attached to the infusion apparatus. The session proceeded in an identical fashion to self-administration sessions, except that a nosepoke in the active nosepoke port activated the infusion pump without delivery of drug. Each extinction session was one hour in length. Following extinction sessions, animals underwent three one hour pre-reinstatement sessions where the animals were not connected to the infusion apparatus, and the operant chamber remained unlit for the entire session. Responses to either nosepoke port were recorded but had no other consequences. These sessions were conducted to further reduce the contribution of possible conditioned stimuli associated with drug delivery on nosepoking (De Vries et al., 1998), and reduce possible contributions of novelty introduced by the operational changes between extinction sessions and reinstatement sessions.

The following day (14 days after the last self-administration session), rats were injected with saline IP and immediately placed in the self-administration chamber for 2 hrs. Similar to pre-reinstatement sessions, stimuli associated with drug delivery such as the attachment to the infusion line, houselight, and infusion pump that might confound or potentiate reinstatement of drug-seeking behavior were not present for the duration of the session. Responses to the previously active and inactive nose-pokes were recorded, but had no other consequence. The next day (15 days after last LgA session), animals were tested for the reinstatement of drug-seeking behavior by receiving a 10 mg/kg i.p. injection of cocaine prior to placement into the test chamber. Thus, the first reinstatement test took place 15 days after the last self-administration session. Active and inactive nosepokes were recorded, although they had no consequence. The animals were then left

undisturbed for an additional 30 days, after which time the cocaine priming reinstatement test was repeated as described above. Thus, the second test for reinstatement took place 45 days after the last self-administration session.

Control rats were moved daily from the home colony room to holding chambers concurrently with animals undergoing self-administration training, where they were left undisturbed for 6 hrs a day for an average of 10 days. Control rats were matched with animals or groups of animals undergoing their final reinstatement test. During these days, control animals spent two hours in their transport chambers, mirroring the length of the reinstatement session. All control animals received saline injections on the same day as animals undergoing reinstatement testing. The following day, some control animals received a second saline IP injection (n=5), while others (n=10) received an acute 10 mg/kg IP injection of cocaine. In this way, these control animals experienced the general effects of handing, transport, and time outside the animal colony in a distinctly different environment similar to self-administration animals (Ferrario et al., 2005). The control animals remained either drug naïve or experienced a single acute exposure to cocaine equivalent to the dose and route of administration as the cocaine priming injection used to reinstate cocaine seeking behavior in rats that had undergone extinction training.

Immediately after the end of the second drug-primed reinstatement session or 2 hours after injecting a transport control animal, rats were taken to a different room and deeply anesthetized with sodium pentobarbital (390 mg/kg i.p., Vortech, Dearborn MI), and transcardially perfused with 500 mL ice cold perfusion rinse (73 mM sucrose, 18 mM procaine hydrochloride, 139 mM sodium chloride in 0.1 M sodium phosphate buffer (SPB), pH 7.4) followed by 250 mL ice cold paraformaldehyde rinse (4% (W/V) paraformaldehyde dissolved in 0.1 M SPB buffer containing 73 mM sucrose, pH 7.4). Brains were removed and placed in 4% formaldehyde overnight at 4 degrees Celsius, and then transferred to 30% (W/V) sucrose for 3 days at the same temperature. Brains were coronally sectioned to a 40 μm thickness with a freezing microtome. Series of sections were collected 120 or 160 μm apart. Sections that were processed for

immunohistochemistry within 2 days were stored in 0.1 M SPB, otherwise sections were stored at -20 Celsius in liquid cryoprotectant.

Immunohistochemistry

Sections were processed for Fos and Mu-opioid receptor protein immunofluorescence in a similar manner to Reynolds and Berridge (2007), and as described in Chapter 2.

Visualization

Sections were visualized at 100x total magnification with a Leica DM6000B (Wetzlar, Germany) microscope with a Leica EL6000 external fluorescent light source coupled to a Q-Imaging monochrome 12 bit 1.4 megapixel digital camera (Surrey, Canada). AlexaFlor 488 was fluoresced using a Leica L5 bandpass filter cube, and AlexaFlor 594 was fluoresced using a TX2 bandpass filter cube. The same section and region was imaged for Fos and Mu opioid immunoreactivity. Separate Fos and Mu opioid images were captured as LZW lossless compression TIFF files to minimize image compression artifacts in dark-field areas of the image and were analyzed using the MCID Core 7.0/Analysis (Cambridge, UK) software package.

Sampling Areas

The number of Fos positive cells in the core and shell of the nucleus accumbens was quantified, and data from the left and right hemisphere was summed together. All brain regions were identified by Paxinos and Watson (1998). Areas sampled are indicated by the grey areas in Fig. 4.2. The nucleus accumbens (NAc) shell and core were sampled at +2.0 mm from bregma. Mu opioid receptor expression was used to delineate the boundaries of the subregions of the nucleus accumbens (Heimer et al., 1997). An elliptical sampling area of 300 μm x 650 μm was used to sample the shell, and a smaller sampling area of 300 μm x 450 μm was used for the core. To better follow the contours of the shell and the core, sampling ellipses were angled. Representative images are shown in Fig. 4.2.

Quantification

The number of Fos positive cells was quantified as described in Chapter 2. In all cases the person quantifying the images was blind to the experimental conditions of each image. While still blind to the conditions, each image was also qualitatively rated for the amount of artifact and nonspecific binding on a fixed scale (1 "none" – 3 "moderate"). For Fos immunoreactivity, there was a range of nonspecific binding in the Fos images across all experimental groups (5, 45, 90 sec, Acute Cocaine Transport Control, Acute Saline Transport Control). Therefore, to minimize possible contributions of nonspecific binding and artifact to the experiment, the means of each nonspecific binding grade group regardless of experimental manipulation were normalized to the grand mean of the entire study. Normalized Fos counts within each brain region and subregions were then compared.

Statistics

Extinction data were analyzed as bins comprised of the average of two contiguous daily sessions. Data with repeated measures over time were analyzed using Linear Mixed Models analysis (LMM) using the SPSS 16 statistical package. For each analysis, the best fitting model of repeated measures covariance was determined by the lowest Akaike Information Criterion (AIC) score (West, 2007). Between-group comparisons were made by one-way analyses of variance (ANOVA) or two-way ANOVAs, followed by Fisher's LSD post-hoc tests or one tailed t-tests, when appropriate.

Results

Extinction

Fig. 4.3 shows the number of active and inactive nosepokes by animals in each group during the 10 days of extinction training. Active nosepokes are illustrated by symbols, while inactive nosepokes are shown as lines. A LMM analysis on inactive nosepokes revealed no group ($F_{(2,44)}=1.672$ $p=0.200$), session ($F_{(4,44)}=1.676$ $p=0.173$) or interaction effects ($F_{(8,44)}=1.135$ $p=0.359$). There was a comparable decrease in

active nosepokes over time in all groups (effect of session $F_{(4,43)}=5.598$ p<0.006; effect of group, $F_{(2,43)}=2.056$ p=0.140; interaction, $F_{(8,43)}=1.169$ p=0.340). There were no significant group differences on the last extinction session bin, indicating that all animals had extinguished their level of response to the same degree ($F_{(2,43)}=1.427$ p=0.2511).

First Reinstatement Test (15 days after the last self-administration session)

Fig. 4.4 (Panel a) shows the number of nosepokes after an IP priming injection of either saline or cocaine given 15 days after the last self-administration session and extinction training. Active nosepokes are represented by bars and inactive nosepokes are represented by inset bars. Animals in all groups reinstated responding following a cocaine priming injection, as indicated by a significant increase in the number of active nosepokes, and there were no significant group differences in the degree of reinstatement (main effect of treatment $F_{(1,84)}=9.403$ p<0.004; no effect of rate of infusion (ROI) history, $F_{(2,84)}=0.371$, p=0.691; no treatment x ROI history interaction, $F_{(2,84)}$ p=0.094). There were no effects of treatment (saline vs. cocaine priming injection), ROI history or interaction on inactive nosepoke responding (treatment $F_{(1,84)}=0.5937$ p=0.2159; ROI history $F_{(2,84)}= 1.31$ p=0.5546; Treatment x ROI history interaction $F_{(2,84)}=3.67$ p=0.194).

Second Reinstatement Test (45 days after the last self-administration session)

Fig. 4.4 (Panel b) shows the number of nosepokes during the test for reinstatement conducted 45 days after the last self-administration session, and 30 days after the first reinstatement session. On this test there were significant group differences in reinstatement. For active nosepokes, a two-way ANOVA resulted in a significant main effect of treatment ($F_{(1,84)}=6.081$ p<0.017) and of ROI history ($F_{(2,84)}=3.991$ p<0.023), and the treatment by ROI history interaction was almost significant ($F_{(2.84)}=2.967$ p=0.057). To determine which groups reinstated responding, active nosepokes following the cocaine prime were compared with those produced by an injection of saline. Only animals that previously received cocaine over 5 sec showed significant reinstatement ($t_{(13)}=2.383$ p<0.0167). In this test animals in both the 45 and 90 sec groups did not

significantly increase active nosepokes, relative to that seen following a saline injection (45 sec group, t(17)=1.070, p=0.149; 90 sec group, t(12)=1.256, p=0.1166).

Similar to the first reinstatement test, there were no significant effects of treatment or ROI history on the number of inactive nosepokes (treatment, F(1,84)=0.1261 p=0.7234; ROI history, F(2,84)=1.859 p=0.1621; treatment x ROI interaction, F(2,84)=0.0022 p=0.9978).

Fos Immunoreactivity After Cocaine Priming

Representative images showing Fos positive cells and areas sampled within the nucleus accumbens core and shell are shown in Fig 4.2.

Fig. 4.5 illustrates the number of Fos positive nuclei per mm^2 in the core and shell of the nucleus accumbens two hours after an IP priming injection of cocaine on the second reinstatement test day. There were significant effects of drug history on the ability of a cocaine injection to induce Fos in the nucleus accumbens.

In the NAc shell, a one-way ANOVA on the number of Fos positive nuclei revealed a main effect of group (F(4,34)=3.901, p<0.011). Relative to animals given saline, cocaine increased the number of Fos positive cells in all groups, except in the 5 sec group (5 sec group, p=0.363; 45 sec group, p<0.004; 90 sec, p<0.007; acute cocaine control group, p<0.008). The 45 sec, 90 sec and acute cocaine control groups did not differ from one another, and all showed significantly greater Fos expression than the 5 sec group (5 sec vs. 45 sec, p<0.028; vs. 90 sec, p<0.047; vs. acute cocaine control, p=0.059).

For the NAc core, a one-way ANOVA also indicated there were significant group differences in the number of Fos positive cells (F(4,34)=8.168 p<0.001). Compared to the saline control group, a cocaine injection increased the number of Fos positive cells in the 45 sec group (p<0.001), the 90 sec group and the acute cocaine control group (p<0.003), and these groups did not differ from one another. In marked contrast, cocaine failed to increase Fos expression in the 5 sec group (5 sec vs. saline, p=0.357), and the 5 sec group had significantly fewer Fos positive cells than all the other groups given cocaine (p's <0.02).

In summary, in animals with a history of self administering cocaine delivered over 5 sec, the ability of a cocaine challenge injection to induce Fos 45 days after the last self-administration session was markedly suppressed, relative to animals with a history of self administering cocaine delivered over either 45 or 90 sec. There appears to be, therefore, long term alterations in the nucleus accumbens produced by a previous history with self administered cocaine when it is administered rapidly (over 5 sec) that are not evident when it is administered more slowly (45 – 90 sec).

Discussion

In the previous chapters, I reported that faster rates of cocaine delivery have a greater neurobiological impact on the brain as indicated by IEG expression, and this is associated with marked increases in drug intake only when rats are allowed extended access to cocaine. Although one possible explanation for this finding remains that faster rates of cocaine are more reinforcing (Gorelick, 1998), this study tested our hypothesis that the escalation of intake in animals receiving drug rapidly during long access sessions was due to sensitized incentive motivational processes as a result of faster drug delivery having a greater neurobiological impact on the brain (Samaha and Robinson, 2005).

Sensitized motivation that induces pathological "wanting" of drug and drug related cues could underlie drug relapse, an enduring and persistent hallmark of drug addiction in humans, even after prolonged periods of abstinence (Robinson and Berridge, 1993; O'Brien, 1997; American Psychiatric, 2000; Hyman, 2001; Hyman and Malenka, 2001). Indeed, recent human studies have supported the idea that intense, pathological desire for drug precedes and increases prior to cocaine taking events, and remains elevated during periods of use (Preston et al., 2009). In addition, diagnosed addicts report a greater desire for cocaine than casual users when exposed to i.v. cocaine in the laboratory, show a greater motivation for drug, and are more susceptible to tests measuring relapse liability (Walsh et al., 2009).

In the rat, experience with extended access to cocaine self-administration is associated with many symptoms of addiction-like behavior. Importantly for this

discussion, such history can facilitate an increase in drug-seeking behavior when drug is not available (Ferrario et al., 2005), and can potentiate the reinstatement of extinguished drug-seeking behavior when challenged with a small priming dose of drug (Mantsch et al., 2004; Ferrario et al., 2005; Ahmed and Cador, 2006; Kippin et al., 2006; Knackstedt and Kalivas, 2007). Both have been interpreted as a measure for increased motivation for drug and drug related cues (Markou et al., 1993; Leri and Stewart, 2001). Extended access experience also potentiates the reinstatement of drug-seeking behavior when challenged with a small priming dose of drug. Increases in cocaine reinstatement after long access experience is also associated with enduring neurobiological changes in the mesocorticolimbic circuits, such as differences in striatal preproenkephalin neuropeptide gene expression (Mantsch et al., 2004) and dendritic spine density (Ferrario et al 2005). These neurobiological changes can be accompanied by increases in psychomotor sensitization (Ferrario et al 2005), a behavioral symptom of the sensitized incentive motivational processes associated with the neurobiological changes induced by repeated exposure to drug (Robinson & Berridge 1993).

In the present study, I found that different rates of infusion during extended access self-administration had no effect on extinction learning, indicating that early after the cessation of self-administration there was no effect on continued drug-seeking under these extinction conditions. Furthermore, when animals were tested for drug-induced reinstatement soon after extinction training, all groups reinstated cocaine-seeking behavior to a similar extent. This reinstatement was not the result of a generalized behavioral activation as evidenced by little or no increases in responding to the nosepoke port that was previously inactive (Shalev et al., 2002). These findings resemble those of Crombag and colleagues (2008) in rats given only limited access to drug and tested immediately after extinction training.

In contrast, when animals were tested for reinstatement 45 days after the last extended access self-administration session, and 30 days after the first test for reinstatement, only animals with a history of 5 sec infusions showed significant reinstatement of drug-seeking following a priming injection of cocaine. This is

98

particularly striking when taking into account the history of the 45 sec group – they had taken similar amounts of drug as the 5 sec group during extended access self-administration sessions. This suggests that a high level of total cocaine intake during self-administration alone was not sufficient to render animals liable to reinstatement after prolonged withdrawal.

An examination of Fos expression induced by the cocaine priming injection also yielded evidence that a history of rapidly administered cocaine (5 sec) had different, long-lasting neurobiological effects than a history of cocaine delivered more slowly (45 – 90 sec). In both the core and shell of the accumbens, the ability of a cocaine priming injection to induce Fos expression was significantly attenuated in animals with a history of extended access to 5 sec infusions during self-administration. Desensitization of Fos expression in response to a cocaine challenge as a consequence of chronic treatment has previously been reported (Hope et al., 1992; Couceyro et al., 1994; Daunais and McGinty, 1994; Daunais et al., 1995; Moratalla et al., 1996; Willuhn et al., 2003). Similar attenuated expression in the NAc was also seen in rats after 30 days of abstinence with extended but not limited access cocaine self-administration (Ben-Shahar et al., 2004). It should be noted that similar attenuation of Fos expression occurred despite the fact that the rats underwent extinction training, which has previously been shown to effect experience dependent plasticity in the nucleus accumbens shell (Sutton et al., 2003). Also, repeated rapid infusions of nicotine are more effective in suppressing c-*fos* gene expression in the mesocorticolimbic circuit than slower infusions (Samaha et al., 2005).

Desensitization of Fos expression can be a result of the long-term neuroadaptations initially induced by Fos serving as a negative feedback mechanism for its further activation (Zhang et al., 2006; Renthal et al., 2008; Xu, 2008). One candidate transcription factor is ΔFosB. ΔFosB is a modified 35-37 kD Fos transcription factor from the *fosB* gene homologous with c-*fos* (Morgan and Curran, 1991) and can also form functional AP-1 subunits, influencing the transcription of downstream targets (Chen et al., 1997; Hiroi et al., 1998). But in contrast to c-*fos*, ΔFosB has a long half-life (Chen et al., 1997; Nestler, 2008) and accumulates in the nucleus accumbens as a result of repeat

exposure drugs such as cocaine. The over-expression of ΔFosB in the nucleus accumbens in transgenic mice is associated with greater psychomotor activation after acute or chronic treatment with cocaine (Kelz et al., 1999), and greater sensitivity to the rewarding effects of cocaine during self-administration (Colby et al., 2003; Nestler, 2008). Recently, ΔFosB has been shown to suppress the transcription of c-*fos* mRNA through chromatin remodeling; high levels of ΔFosB result in the deactylation of histones surrounding the c-*fos* gene, resulting in a tighter association of the DNA containing the c-*fos* gene with histones and deactivating the c-*fos* gene (Renthal et al., 2008).

Thus, suppressed Fos expression can be viewed as an indicator of more persistent drug-induced neuroadaptations in animals with a history of exposure to rapidly administered cocaine. That is, it appears that a history of 5 sec infusions during extended access facilitates the persistence of neuroadaptations in the nucleus accumbens, and this is associated with an increased propensity to reinstate drug-seeking behavior after prolonged abstinence. These neuroadaptations and vulnerability towards drug reinstatement can occur independently of the total amount of drug consumed by the animal, highlighting the importance of the rapidity of drug delivery to the brain as a factor in its addictive liability.

Fig. 4.1 An illustration of the experimental design. Icons depict the number of sessions for each condition.

History of 5s LgA
■■■■■■■■■■■■■■■■

History of 45s LgA → Extinction → Pre-Reinstatement → | Reinstatement 15 | 30d | Reinstatement 45 | → Perfusion
▦▦▦▦▦▦▦▦▦▦▦   □□□□□□□□□   ○○○ | Saline IP → Cocaine IP | ⊪⊪ | Saline IP → Cocaine IP |
                                                       | ▽   ▼ | | ▽   ▼ |

History of 90s LgA
□□□□□□□□□□□□□□□□□

Fig. 4.2 Examples of Fos positive nuclei in the nucleus accumbens +2.0 from bregma (labeled in green) at the end of the reinstatement session 2 hours after a 10 mg/kg i.p. priming injection of cocaine. Core and shell divisions sampled within the nucleus accumbens are indicated by the white ellipses, and correspond to gray areas marked on the atlas figure. Scale bar indicates 100 microns.

NAc Shell   NAc Core

5

45

90

Acute
Cocaine

Acute
Saline
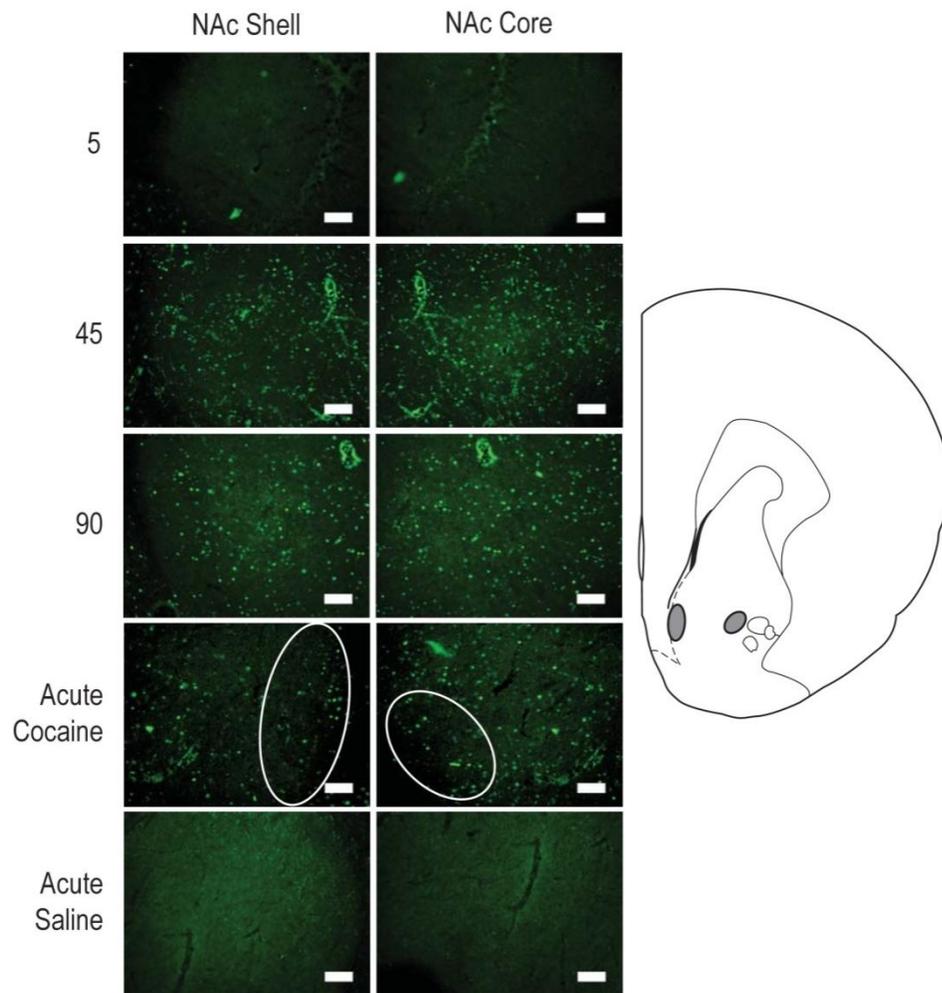
Fig. 4.3 The mean (±SEM) number of nosepokes made by animals in each group during extinction training when cocaine reward was not available. Active nosepokes are shown using symbols and inactive nosepokes are depicted as lines. Bins are an average of two sessions.

106
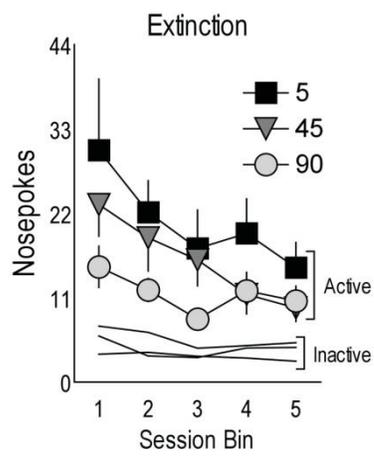
Fig. 4.4 Panel a shows the mean (+SEM) number of nosepokes during the first reinstatement test, conducted 14-15 days after the last self-administration session, and 3 days after extinction training was completed. On Day 14 all animals received an injection of saline IP (Sal) and the next day (Day 15) an injection of cocaine IP (Coc). Panel b shows data from the second reinstatement test, which was conducted 45 days after the last self-administration session and 30 days after the first reinstatement test. The number of active nosepokes are represented by thick bars, and inactive nosepokes by thin inset bars.

a Reinstatement 15 Days After SA
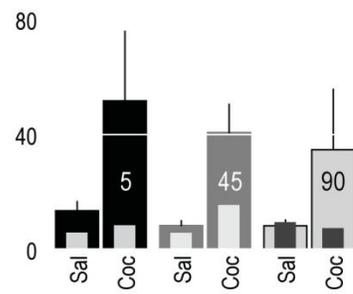
b Reinstatement 45 Days After SA

108
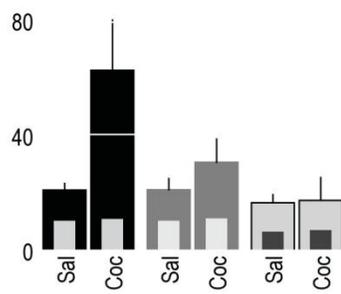
Fig. 4.5 The mean (+SEM) number of Fos positive nuclei in the nucleus accumbens (+2.0 mm from bregma) core and shell subdivisions two hours after an IP cocaine priming injection (10 mg/kg) in rats with a history of cocaine self-administration, when cocaine was delivered IV over 5, 45 or 90 sec, relative to control animals with no history of cocaine self-administration and given an IP injection of either saline (S) or cocaine for the first time (C). This test was conducted 45 days after the last self-administration session.
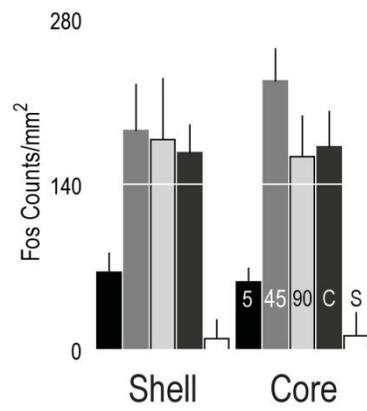
CHAPTER 5

DISCUSSION

In the preceding chapters, I explored whether relatively rapid rates of i.v. cocaine delivery, which promotes psychomotor sensitization (Samaha et al., 2002), also has a greater neurobiological impact on the brain, and on drug-taking behavior. In the first study (Chapter 2), I found that varying the rate of an acute infusion of cocaine had marked effects on the ability of cocaine to increase expression Fos in the dorsal and ventral striatum – fast rates (5 sec) induced greater levels of Fos expression than slower rates (25 – 100 sec) and this effect was equally evident in the patch and matrix subcompartments of the dorsal striatum, suggesting that fast rates of infusion had a widespread impact on this structure. In a second study (Chapter 3), I showed that faster rates of cocaine infusion (5 – 45 sec) facilitated an escalation in overall drug intake when rats were given extended access to cocaine, a procedure modeling one aspect of human addiction. In contrast, slow rates of cocaine infusion (90 sec) in similar conditions only modestly increased drug-taking behavior. In a third study (Chapter 4), I found that fast (5 sec) rates of cocaine infusion during extended access self-administration were associated with a persistent susceptibility to reinstate drug-seeking behavior in response to a drug induced priming injection, and that this was accompanied by a persistent change in the ability of cocaine to induce Fos protein expression in the nucleus accumbens.

Functional implications of Fos expression induced by rapid cocaine delivery

In the first study, (Chapter 2) I found that the neurobiological impact of cocaine in the striatum was greater when the drug was delivered more rapidly (5 sec), as assessed by its ability to induce the IEG, Fos. Some skepticism has persisted about the relevance of measuring Fos induction in neurons due to the multiplicity of stimuli that can induce its

111

expression and questions surrounding its functional role (Hyman et al., 1993). However, functional roles of Fos in mediating the brain's response to cocaine have recently been discovered in several key studies, suggesting that increased Fos expression resulting from rapid rates of cocaine delivery is associated with long-term and persistent changes in the brain.

In one example, transgenic mice with targeted c-*fos* gene deletions in D1 receptors expressing striatal MSNs show marked differences in their behavioral and neurobiological response to cocaine, while still expressing D1 receptors and otherwise appearing normal (Zhang et al., 2006; Xu, 2008). Given acutely, these mutant mice show an attenuated psychomotor response to a high dose of cocaine in addition to a reduced expression of Fos. Given chronically, these mice show attenuated psychomotor sensitization. These mutants also show marked reductions in the neurobiological impact of cocaine compared to wild-type controls, which is evident in the decreased levels of AP-1 binding protein, which is the functional transcription factor formed by the dimerization of Fos protein with Jun (Morgan and Curran, 1991). These mutants also show attenuated expression of many other downstream proteins including the transcription factor ΔFosB, which is associated with psychomotor activation after acute or chronic treatment with cocaine (Kelz et al., 1999), and greater sensitivity to the rewarding effects of cocaine during self-administration (Colby et al., 2003; Nestler, 2008). Other proteins which exhibit lower levels of expression after chronic treatment in these mutants including Cdk5, a transcription factor important in neuronal structural changes (Bibb et al., 2001), and the NMDA NR1 glutamate receptor subunit, a neurotransmitter receptor subunit implicated in stimulant-induced sensitization (White and Kalivas, 1998; Wolf, 1998). These mutants also exhibit less dendritic density and branching after treatment, important markers for structural plasticity associated with psychomotor sensitization (Robinson and Kolb, 2004). In this way, faster rates of cocaine delivery resulting in greater Fos expression in D1 expressing MSNs may result in enduring neurobiological adaptations in these neurons.

Increased Fos expression as a result of faster rates of infusion may also functionally impact D2 receptor expressing MSNs, since varying the rate of an acute cocaine infusion has similar effects on c-*fos* mRNA expression in enkephalin (Enk+) positive and enkephalin negative (Enk-) neurons in the dorsomedial striatum (Samaha et al., 2004). While Enk- MSNs predominantly express dynorphin and D1 receptors, Enk+ neurons co-express dopamine D2 receptors, and comprise a functionally distinct "indirect" striatopallidal pathway in the striatum (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990). While injections of acute cocaine itself can induce Fos activation in D2 expressing MSNs (Dilts et al., 1993; LaHoste et al., 1993; Ruskin and Marshall, 1994), this effect is strongly mediated by environmental factors associated with drug (Badiani and Robinson, 2004). For example, when drugs such as cocaine or amphetamine are given in novel environments distinct from the homecage, Fos expression is potentiated in the striatum primarily in D2 receptor expressing MSNs (Badiani et al., 1998, 1999). This difference in Fos expression is also associated with a facilitation in psychomotor sensitization. Novel environments and their cues can increase the ability of cocaine and amphetamine to induce psychomotor sensitization – the same dose of drug that can induce psychomotor sensitization fails to do so in the homecage (Badiani et al., 1995; Badiani et al., 1995). A similar functional role for Fos expression in the nucleus accumbens in mediating this kind of specific psychomotor sensitization has recently been demonstrated. Selectively targeting and inhibiting the activity of Fos-expressing and presumably D2 receptor expressing MSNs in the nucleus accumbens through a novel c-*fos*-LacZ daunorubicin system attenuated context-dependent cocaine sensitization (Koya et al., 2009). Thus, in addition to having effects on D1 receptor expressing MSNs, faster rates of cocaine infusion could also precipitate long lasting neurobiological adaptations in D2 expressing MSNs.

To the extent that Fos expression in the striatum after an acute infusion of cocaine is largely dependent on D1 dopamine receptors, the results of my experiments strongly suggest that D1 dopamine receptors in the striatum may be exquisitely sensitive to the temporal dynamics of dopamine occupancy. After all, while the only difference in

dopamine between 5 – 100 sec is the time to peak levels (Ferrario et al., 2008), these rates have strikingly different effects on Fos expression. Stimulation of striatal dopamine D1 receptors *in vivo*, either by the administration of direct or indirect dopamine agonists such as SKF-82958 or amphetamine initiates a complex series of events mediated by G-protein coupled interactions (e.g. Seeman and Van Tol, 1994), with one consequence leading to the desensitization of the dopamine receptor via internalization and intercellular trafficking to endosomes prior to recycling back into the synaptic membrane (Dumartin et al., 1998). While Dumartin and colleagues (1998) examined the effect of an acute striatal injection of SKF-82958 delivered over 2 minutes, the results of my experiment suggest this and other intracellular processes may be even more sensitive to the rate of receptor occupancy. To my knowledge few if any researchers have examined the effects of dopamine receptor dynamics at such a short time frame, and this should be examined. After all, effects on dopamine receptor dynamics over these very rapid time frames are not outside the realm of possibility. Other neurotransmitter receptor systems such as AMPA receptors in the brain have recently been shown to have even subsecond responses to repeated stimuli – AMPA receptors can quickly move in and out of the active zone of a synapse by moving laterally (Heine et al., 2008).

As well as post-synaptic alterations in the nucleus accumbens mediated by Fos expression, faster rates of cocaine delivery could also result in a long-term increase in dopamine release. Although the rate of an acute infusion of cocaine does not influence the peak levels or total amount of dopamine in the striatum (Ferrario et al., 2008), chronic treatment of drugs such as cocaine and amphetamine that result in behavioral sensitization also results in an enhanced release of dopamine in the mesocorticolimbic system (medial prefrontal cortex, dorsal striatum and nucleus accumbens) following a challenge injection, particularly after prolonged periods of withdrawal (Robinson and Becker, 1982, 1986; Kalivas and Stewart, 1991; Vezina, 2004). Thus one plausible locus for enhanced dopamine release in the nucleus accumbens is potentiated release from neurons projecting from the ventral tegmental area. For example, animals sensitized by direct injections of amphetamine into the ventral tegmental area show enhanced

motivation for the same drug during progressive ratio schedules of self-administration and increased dopamine in the nucleus accumbens (Vezina et al., 2002). While dopaminergic cells in the ventral tegmental area that project to the nucleus accumbens themselves do not express D1 dopamine receptors, Vezina (2004) has proposed that the local somatodentritic release of dopamine from ventral tegmental area dopamine neurons to D1 dopamine receptors expressed on the pre-synaptic afferents of GABAergic and glutamatergic neurons form a local feedback loop that can influence the activity of dopaminergic afferents to the nucleus accumbens. Thus changes in local dopamine neurotransmission in the ventral tegmental area can alter the release of dopamine in the ventral striatum. Whether varying the rate of drug delivery directly into the ventral tegmental area influences the development of sensitization and induces greater dopamine release into the nucleus accumbens is an interesting question worth examining in the future.

In addition to glutamate projections to midbrain dopamine neurons, corticostriatal glutamate neurotransmission has also previously been implicated in the expression of psychomotor sensitization (Pierce et al., 1996). More recently, real-time measurements of striatal glutamate have revealed that chronic treatment of cocaine rapidly increases extracellular glutamate levels over successive treatments (Lee et al., 2008). Indeed, cortical glutamate neurotransmission and its neuromodulation due to nonsynaptically released glutamate by the cystine/glutamate antiporter within the nucleus accumbens undergoes extensive neuroadaptation in response to chronic cocaine treatment (Kalivas, 2009). In addition, pharmacological inhibition of ventral medial prefrontal cortex neurons including those in the infralimbic cortex can attenuate extinction responding 30 days after self-administration sessions, demonstrating a functional role of the infralimbic cortex (Koya et al., 2009). However, while I observed elevated Fos expression in the infralimbic cortex after an acute infusion of cocaine, there was no effect of rate of infusion – all rates of cocaine equally induced Fos expression. This differed from the results of Samaha and colleagues (2004) where 5 sec rates of cocaine infusion induced much more c-*fos* mRNA expression in the infralimbic cortex than rates over 25 – 100 sec. While it is possible that

post-transcriptional regulation of c-*fos* mRNA on factors influencing its translation may have played a role in influencing Fos protein expression (Rahmsdorf et al., 1987), interpretation of Fos expression in cortical areas is also complicated by the fact that Fos expression can be induced either by neuronal excitation or inhibition (Farivar et al., 2004; Zahm et al., 2009). In addition, while faster rates of acute cocaine infusion induced distinct patterns of striatal Fos expression depending on the subregion of the striatum, this effect was equally evident in the patch and matrix subcompartments, indicating that Fos expression was equally upregulated by rapid rates of cocaine infusion in functionally distinct subcompartments (White and Hiroi, 1998; Lawhorn et al., 2009). Additionally, work by Venton and colleagues (2006) with highly sensitive microdialysis coupled to capillary electrophoresis with laser-induced fluorescence detection techniques showed that acute 5 sec infusions of cocaine do not detectably increase levels of glutamate in the striatum.

In summary, varying the rate of cocaine delivery can have a profound and widespread impact on Fos expression in the striatum. This upregulation in Fos expression can have functional consequences related to the development of psychomotor sensitization and the initiation of gene cascades which can persistently alter the processes mediating incentive motivational attribution to drug related cues (Robinson and Berridge, 1993; Samaha and Robinson, 2005). How faster rates of acute drug delivery can preferentially upregulate Fos expression is still virtually unknown. Many different pre- or post-synaptic mechanisms systems could contribute towards this effect. Nevertheless, the results reported here raise a challenge the field of neuroscience and pharmacology to further examine the influence of small variation in the temporal dynamics of receptor occupancy on the intracellular signaling cascades that alter gene expression.

Faster rates of drug infusion facilitate an escalation in overall drug intake – increased cocaine reinforcement, tolerance or incentive sensitization?

In Chapter 3 of this dissertation, I showed that faster rates of cocaine infusion have a greater neurobiological impact and facilitate an escalation in overall intake while

the reinforcing effects of cocaine remain unchanged. I came to this conclusion based on several key points. While previously conducted work in the laboratory suggested that faster rates of cocaine infusion had a profound impact on the brain and could induce psychomotor sensitization (Samaha et al., 2002; Samaha et al., 2004), extensive testing using self-administration and related paradigms under limited access conditions failed to find differences in cocaine self-administration behavior (Crombag et al., 2008). This was evident even though rats can differentiate between the rates of cocaine infusion and prefer faster rates over slower ones when given a choice (Schindler et al., 2009).

During limited access sessions, cocaine infusions were equally reinforcing across 5 – 90 sec. Only when rats were allowed extended access to cocaine self-administration did they begin to show marked differences in drug-taking. This finding supports the underlying reasoning behind my initial hypothesis: testing the reinforcing effects of a drug under limited access sessions may not adequately model all of the factors influencing its addiction liability. Only under conditions amenable to the development of addiction-like behaviors will the influence of some factors mediating the addictive liability of a drug emerge. In contrast to limited access conditions, extended access self-administration is associated with the development of many addiction-like behaviors (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002; Ahmed and Cador, 2006), such as greater motivation for drug both when available (Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Belin et al., 2008) and not (Deroche-Gamonet et al., 2004; Robinson, 2004), resistance to adverse consequences associated with drugs, (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Pelloux et al., 2007; Belin et al., 2008), changes in the pattern of drug-taking (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002; Ahmed and Cador, 2006; Belin et al., 2009), and a persistence to reinstate drug seeking behavior (Deroche-Gamonet et al., 2004; Ferrario et al., 2005; Knackstedt and Kalivas, 2007; Belin et al., 2009). Extended access conditions have also been shown to induce psychomotor sensitization and structural plasticity, both symptoms of adaptations in the brain as a result of repeated drug administration (Ferrario et al., 2005).

Similar to human addiction where the condition has many symptoms (Robinson and Berridge, 1993; Deroche-Gamonet et al., 2004; Robinson, 2004) with a complex diagnostic criteria (American Psychiatric, 2000), the severity of reinstatement in rats allowed extended access to cocaine can predict the severity of other associated addiction-like behaviors (Deroche-Gamonet et al., 2004; Belin et al., 2009).

This correlates with results from the acute infusion study in Chapter 2, where I found that while the intermediate rate of infusion (25 sec) induced similar amounts of low Fos expression as the 90 sec group, drug-taking in rats receiving a comparable rate (45 sec) resembled that of the 5 sec group in amount of drug consumed over the extended access sessions. These results would be confusing if one considers drug-taking in the extended access condition as the singular behavioral measure of addictive liability. However, considering the totality of each group's behavior, including their first hour intake and propensity to reinstate their cocaine seeking behavior, perhaps the results of the three experiments will become clearer.

Although the 45 sec group consumed very similar amounts of drug over the entire session as the 5 sec group, their behavior differed from the 5 sec group in two ways. One such measure was quantity of drug consumed in the first hour (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002). Although there was a large contribution of individual differences in both 5 and 45 sec groups, "high performers" in the 5 sec group differed significantly from those in the 45 sec group by escalating the amount of drug consumed over the extended access sessions. "High performers" from the 45 sec group did not. The second measure where the 5 sec and 45 sec rats differed was during reinstatement – only animals with a history of 5 sec infusions during extended access reinstated their cocaine seeking behavior 45 days after their last self-administration session.

If reinstatement was considered as the sole behavioral measure of addiction-like symptoms, results from the first reinstatement test would seem to conflict with the results from the acute infusion study in Chapter 2. The pattern and overall intake of drug during extended access sessions, particularly in light of the persistence to reinstate drug seeking at 45 days after their last self-administration session, would also seem contradictory.

However, taking into account all behaviors, the data suggests that the 5 sec animals overall manifest the most severe addiction-like symptoms: 1) a tendency to escalate intake rapidly over the first hour of extended access (Ahmed and Koob, 1998, 1999; Ahmed et al., 2002), 2) marked increases in overall drug intake and 3) a persistence to seek drug during reinstatement tests (Deroche-Gamonet et al., 2004; Belin et al., 2009). Animals in the 5 sec group also had enduring neuroadaptations in the nucleus accumbens, reflecting a change in systems in the brain mediating incentive motivational attribution toward drug related cues (Samaha and Robinson, 2005).

Of course, this is not meant to reduce the contribution of drug-reinforcement towards addiction. It is critical to consider that addiction is a multi-symptom disease and may have many contributing mechanisms towards its etiology (Robinson and Berridge, 1993; Jentsch and Taylor, 1999). After all, the reinforcing effects of cocaine and crack cocaine at these rates can be subjectively different in humans (Abreu et al., 2001), and drug euphorogenesis is likely to play a role in initiating drug use; after all, heuristically it seems unlikely that people initiating crack cocaine use do so with the express goal of becoming addicted. In addition to reinforcement and incentive sensitization, tolerance is also considered to play important roles in the mechanisms of addiction.

One view suggests that tolerance towards the negative properties of drugs (Lasagna et al., 1955) mediates the escalation in drug intake. Indeed, cocaine can produce potent aversive anxiogenic effects concomitant with its positive subjective effects in both humans and rats (Mulvaney et al., 1999; Ettenberg, 2009). From this perspective, drug addicts take greater and greater amounts of drugs to experience greater subjective effects because they can tolerate the unpleasant or even life-threatening negative effects of the drug (Robinson and Berridge, 1993). For example, when rats are trained on a runway model of self-administration where they are required to traverse a runway from a "start box" to a "goal box" where upon entry they can receive an infusion of cocaine, rats pretrained with limited access cocaine self-administration show progressively slower "run times" towards the goal box (a measure of motivation for drug) and increased numbers of retreats from the threshold of the goal box to the start box (a measure of the

anxiogenic effects of the drug since this behavior can be mitigated by antianxiety drugs, see Ettenberg and Bernadi, 2006). In contrast, rats with a previous history of extended access self-administration show faster runtimes towards the "goal box", showing greater motivation for cocaine, and a decreased number of retreats (Ben-Shahar et al., 2008). Although this suggests that extended access self-administration experience results in tolerance towards the anxiogenic effects of cocaine, it should be noted that when a separate of animals were trained on long access self-administration and tested on an Elevated Plus Maze (a different test of anxiety) limited and extended access animals performed similarly, although these results may be the result of procedural differences. Regardless, tolerance towards the anxiogenic effects of cocaine remains an interesting alternate hypothesis for the effects of rate of infusion on extended access self-administration, mainly because there is some recent evidence to suggest that the anxiogenic effects of cocaine are dissociable neurobiologically from either the incentive motivational or reinforcing effects (Guzman et al., 2009). Whether varying the rate of cocaine delivery during extended access self-administration influences the anxiogenic effects of cocaine is not yet known.

Alternatively, the "hedonic allostasis" view suggests that negative affective states associated with the symptoms of drug withdrawal, such as dysphoria, irritability, and anxiety during abstinence (Gawin and Kleber, 1988) provides the motivation for drug-taking in addiction (Koob and Le Moal, 2001). The hedonic allostasis hypothesis of drug addiction (Koob and Le Moal, 2001) posits that the development of drug addiction stems from dysregulation of normal homeostatic mechanisms in the brain that regulate reward. In this view, the normal drug naïve brain has two automatic and opposing processes (Solomon and Corbit, 1974) that normally regulate the impact of positive reinforcers – a positive hedonic process which is opposed by a negative hedonic process (Koob and Le Moal, 1997). These negative processes are slower and take longer to act than the positive processes, and ultimately serve to return the brain to a "set point" reflecting balanced homeostasis. In this view, the initial euphoric effects of drugs like cocaine are mitigated by the subsequent negative hedonic process of withdrawal. Changes in the positive and

negative opponent processes regulating reward, or "hedonic allostasis" have been proposed as an explanation for addiction. Thus, repeated exposure to drug causes a chronic deviation to gradually develop in all of the regulatory systems regulating drug reward from the normal state of operation (allostatic state), masking the initial positive hedonic effects of the drug. In other words, with each drug treatment, drug withdrawal does not balance the "high" or "rush" and results in a persistent state of withdrawal even after a prolonged period of abstinence.

A recent study using increased intracranial self stimulation thresholds (ICSS) as a measure of decreased reward sensitivity (i.e. increased negative hedonic processes due to withdrawal) between limited access and LgA rats (Ahmed et al., 2002) concluded that increased escalation of cocaine intake was associated with persistent decreases in brain reward function. That is, increased cocaine intake is a compensatory response to the brain's lowered hedonic "set-point." Thus, one interpretation of our results with the reward allostasis hypothesis would be that faster rates of cocaine administration can induce greater symptoms of withdrawal. However, there is some evidence from studies of opiate replacement therapies to suggest that slower rates of drug administration (methadone) are associated with more severe and long-lasting withdrawal symptoms than heroin (Gossop and Strang, 1991), a drug that can also induce escalation in intake during extended access self-administration (Ahmed et al., 2000). If so, I would have expected in our study that slower rates of cocaine infusion might lead to similar or greater escalation in intake. However, I do not know if slow rates of cocaine delivery could induce greater withdrawal symptoms in rats as the impact of varying rates of cocaine infusion on ICSS threshold is not known. This question would merit further study.

The hedonic allostasis hypothesis proposes that the mechanism underlying relapse in humans is a persistent state of withdrawal (Koob and Le Moal, 2001), reflected in long term tolerance in the mesocorticolimbic system to the effects of cocaine. Behaviorally, the hedonic allostasis hypothesis would suggest that rats given extended access to 5 sec infusions of cocaine would develop a dysregulated reward system resulting in a state of persistent withdrawal and a tolerance towards the rewarding effects of cocaine. Thus

given a priming injection, rats with a history of rapid infusion rates would experience greater anhedonia, and attempt to alleviate those symptoms by seeking drug even when not available. On the other hand, if longer rates of cocaine infusions were more effective in inducing withdrawal states (similar to methadone and heroin), one might expect the propensity to reinstate reversed. However, it seems unlikely that an increased state of withdrawal accounts for drug primed reinstatement behavior. While both rats and primates can robustly reinstate their cocaine seeking behavior after a priming injection of cocaine (Gerber and Stretch, 1975; de Wit and Stewart, 1981), inducing withdrawal in rats through the administration of antagonists does not reinstate their drug seeking behavior (Shaham and Stewart, 1995; Shaham et al., 1996).

According to Ben-Shahar and colleagues (2004), one of the neurobiological manifestations of this heightened anhedonia after extended access self-administration is a persistent suppression of Fos expression. They demonstrated that in rats experienced with long access self-administration of cocaine, Fos immunoreactivity in the core and shell divisions of the nucleus accumbens was suppressed in comparison to animals with only limited access experience to cocaine self-administration after a 14 day period of forced withdrawal. Based on the importance of D1 dopamine transmission in facilitating Fos expression during an acute injection of cocaine, this loss of Fos expression was interpreted as a tolerance of D1 receptor dopamine neurotransmission.

While there are experimental differences between the studies in this dissertation and with those of Ben-Shahar and colleagues (2004), I also found an enduring suppression of Fos expression in rats with a history of rapid rates of cocaine infusion compared to those with a history of extended access to slower rates of infusion during self-administration. While I agree that persistent Fos expression is an indicator for enduring neurobiological adaptations, evidence suggesting that this suppression is due to D1 receptor mediated tolerance is not clear. Desensitization of Fos expression in response to a cocaine challenge as a consequence of chronic treatment has previously been reported (Hope et al., 1992; Couceyro et al., 1994; Daunais and McGinty, 1994; Daunais et al., 1995; Moratalla et al., 1996; Willuhn et al., 2003; Zahm et al., 2009). In contrast, it

has also been reported that prior cocaine experience can *enhance* Fos expression in the nucleus accumbens produced by a drug challenge, although this was only seen after a long period of drug abstinence (Crombag et al., 2002; Todtenkopf et al., 2002) and only if cocaine was administered in association with environmental novelty (Hope et al., 2006). However, this hypersensitivity is not always seen (e.g. Ostranader et al., 2003; Willuhn et al., 2003), and it is not clear exactly what conditions lead to a sensitization vs. desensitization of the Fos response, even following extended abstinence.

It is also important to note that beyond the nucleus accumbens, the pattern of Fos expression varies widely in other corticothalamic systems in the brain (Zahm, 2006). Fos expression or suppression in these regions can be influenced by many experimental factors, including the specific area of the brain, whether cocaine was administered contingently through self-administration or by the experimenter, the amount of experience the animal had with respect to drug self-administration, and finally the degree to which the animal was experienced with the operant task and task environment without any drug experience (i.e. groups experienced only in saline self-administration) (Ben-Shahar et al., 2004; Zahm et al., 2009). While a summary of the impact of all of the experimental manipulations in all 43 regions surveyed by Zahm and colleagues (2009) is beyond the scope of the current discussion, there are several key points from this study that have relevance to this dissertation. First, consistent with many other studies and with my own results, acute cocaine experience (1 day of self-administration) induces greater Fos expression than chronic cocaine experience in specific subregions of the dorsal and ventral striatum. Thus in my study, the persistence of accumbal Fos suppression only in rats with a history of rapid rates of delivery during extended access self-administration suggests two things. First, varying the rate of drug delivery during extended access self administration introduces a complex temporal dynamic in Fos suppression or expression. My results along with data from other experiments (Crombag et al., 2002; Todtenkopf et al., 2002) suggests that striatal Fos expression undergoes a biphasic time course consisting of an initial suppression, then a return to initial levels of inducibility, and followed by a period of hyperinducibility. Although it is clear from my results that at 45

days after their last self-administration session, animals with a history of 5 sec drug delivery had a persistent suppression in the ability of cocaine to induce Fos expression in comparison to animals with a history of 45 and 90 sec drug delivery, it remains to tested whether 5 sec animals evince hyperinducibility at an even later timepoint. Secondly, my results leave open the possibility that Fos inducibility in other regions of the brain is also impacted by varying the rate of drug delivery during chronic extended access experience. Many other brain regions such other parts of the mesocorticolimbic system and those involved in other brain circuits such as the extended amygdala show altered Fos inducibility (Ben-Shahar et al., 2004; Zahm et al., 2009). For example, although not surveyed in my extended access experiment, it is reasonable to predict that faster rates of drug delivery during extended access self-administration will lead to preferential expression of Fos in striatal neurons in the patch subcompartment, either by an enduring suppression of the inducibility of Fos in the matrix compartment, or by increased hyperinducibility in the patch compartment.

One possible mechanism of suppressed Fos expression in the NAc after extended access experience might be that Fos expression is suppressed by the increased expression of ΔFosB. ΔFosB is a modified 35-37 kD Fos transcription factor from the *fosB* gene that has homology with c-*fos* (Morgan and Curran, 1991) and can also form functional AP-1 subunits, influencing the transcription of downstream targets (Chen et al., 1997; Hiroi et al., 1998). In contrast to c-*fos*, ΔFosB has a long half-life (Chen et al., 1997; Nestler, 2008) and accumulates in the nucleus accumbens as a result of repeated exposure drugs such as cocaine. The increased expression of ΔFosB in neurons as a result of repeated cocaine exposure has recently been shown to suppress Fos expression by a histone deacetylase 1 (HDAC1) chromatin remodeling mechanism (Renthal et al., 2008). These studies strongly support the hypothesis that suppression of Fos activation due to extended access to fast rates of cocaine infusion is a result of many long-term neuroadaptations related to psychomotor sensitization and do not necessarily imply either a tolerance of the D1 receptor in the striatum or changes in the neurophysiological activity of these same neurons.

Although some have proposed that changes in Fos activation and suppression can be correlated with changes in neurophysiological activity and therefore also with behavior (e.g. Zahm et al., 2009), such inferences on the basis of Fos expression are fraught with complications. First and foremost, Fos can be both a marker for intracellular biochemical activation in response to a stimulus and a transcription factor initiating long term neuroadaptations. Thus once a stimulus such as drug administration is repeated, it immediately becomes difficult to distinguish Fos expression from its distinct two roles. Secondly, while the spatial sensitivity of Fos activation is high, the temporal resolution of Fos activation is extremely low (Kovacs, 2008), thus making strong correlations with specific operant behaviors becomes extremely problematic. In order to address further questions relating the activity of specific neurons in relationship to behavior, it is critical to use other methods of observation with a much higher temporal resolution, such as awake-behaving electrophysiology, *in vivo* voltammetry, or microdialysis coupled to capillary electrophoresis with laser-induced florescence detection.

Conclusions

I have shown through the studies in this dissertation that faster rates of cocaine infusion have a greater neurobiological impact on parts of the brain associated with drug addiction. These faster rates also facilitate the development of drug-taking behaviors that resemble those seen in human addicts. Experiencing faster rates of drug infusion during this experience leads to a persistent vulnerability to relapse, and a heightened desire for drug after prolonged abstinence. I have also shown that the behavioral effects associated with fast rates of cocaine delivery also facilitate persistent neuroadaptations in brain areas associated with addiction. This may be one reason why routes of administration that result in the rapid entry of cocaine into the brain preferentially promote the transition from casual use to addiction.

125

BIBLIOGRAPHY

Abreu ME, Bigelow GE, Fleisher L, Walsh SL (2001) Effect of intravenous injection speed on responses to cocaine and hydromorphone in humans. Psychopharmacology (Berl) 154:76-84.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Ahmed SH, Koob GF (1999) Long-lasting increase in the set point for cocaine self-administration after escalation in rats. Psychopharmacology (Berl) 146:303-312.

Ahmed SH, Cador M (2006) Dissociation of psychomotor sensitization from compulsive cocaine consumption. Neuropsychopharmacology 31:563-571.

Ahmed SH, Walker JR, Koob GF (2000) Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 22:413-421.

Ahmed SH, Kenny PJ, Koob GF, Markou A (2002) Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. Nat Neurosci 5:625-626.

Akimoto K, Hamamura T, Otsuki S (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. Brain Res 490:339-344.

Alheid GF, Heimer L (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27:1-39.

American Psychiatric A (2000) Diagnostic and statistical manual of mental disorders: DSM-IV-TR. Washington, DC: American Psychiatric Association.

Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol 15:327-339.

Badiani A, Browman KE, Robinson TE (1995) Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of cocaine and amphetamine. Brain Res 674:291-298.

Badiani A, Anagnostaras SG, Robinson TE (1995) The development of sensitization to the psychomotor stimulant effects of amphetamine is enhanced in a novel environment. Psychopharmacology (Berl) 117:443-452.

Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1998) Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: modulation by environmental novelty. J Neurosci 18:10579-10593.

Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1999) Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. Behav Brain Res 103:203-209.

Balfour D (1990) Nicotine as the basis of the tabacco smoking habit. London: Pergamon press.

Balster RL, Schuster CR (1973) Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. J Exp Anal Behav 20:119-129.

Becker JB (2009) Sexual differentiation of motivation: a novel mechanism? Horm Behav 55:646-654.

Belin D, Balado E, Piazza PV, Deroche-Gamonet V (2009) Pattern of intake and drug craving predict the development of cocaine addiction-like behavior in rats. Biol Psychiatry 65:863-868.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320:1352-1355.

Bell DS (1971) The precipitants of amphetamine addiction. Br J Psychiatry 119:171-177.

Ben-Shahar O, Ahmed SH, Koob GF, Ettenberg A (2004) The transition from controlled to compulsive drug use is associated with a loss of sensitization. Brain Res 995:46-54.

Ben-Shahar O, Posthumus EJ, Waldroup SA, Ettenberg A (2008) Heightened drug-seeking motivation following extended daily access to self-administered cocaine. Prog Neuropsychopharmacol Biol Psychiatry 32:863-869.

Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314-347.

Berridge KC (2004) Motivation concepts in behavioral neuroscience. Physiol Behav 81:179-209.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26:507-513.

Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. Curr Opin Pharmacol 9:65-73.

Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410:376-380.

Bonate PL, Swann A, Silverman PB (1997) Context-dependent cross-sensitization between cocaine and amphetamine. Life Sci 60:PL1-7.

Briand LA, Blendy JA (2009) Molecular and genetic substrates linking stress and addiction. Brain Res.

Briand LA, Flagel SB, Garcia-Fuster MJ, Watson SJ, Akil H, Sarter M, Robinson TE (2008) Persistent alterations in cognitive function and prefrontal dopamine D2 receptors following extended, but not limited, access to self-administered cocaine. Neuropsychopharmacology 33:2969-2980.

Browman KE, Badiani A, Robinson TE (1998) The influence of environment on the induction of sensitization to the psychomotor activating effects of intravenous cocaine in rats is dose-dependent. Psychopharmacology (Berl) 137:90-98.

Brown J, Bullock D, Grossberg S (1999) How the basal ganglia use parallel excitatory and inhibitory learning pathways to selectively respond to unexpected rewarding cues. J Neurosci 19:10502-10511.

Brown LL, Feldman SM, Smith DM, Cavanaugh JR, Ackermann RF, Graybiel AM (2002) Differential metabolic activity in the striosome and matrix compartments of the rat striatum during natural behaviors. J Neurosci 22:305-314.

Brown PL, Kiyatkin EA (2005) Brain temperature change and movement activation induced by intravenous cocaine delivered at various injection speeds in rats. Psychopharmacology (Berl) 181:299-308.

Cador M, Bjijou Y, Stinus L (1995) Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience 65:385-395.

Camp DM, Browman KE, Robinson TE (1994) The effects of methamphetamine and cocaine on motor behavior and extracellular dopamine in the ventral striatum of Lewis versus Fischer 344 rats. Brain Res 668:180-193.

Canales JJ (2005) Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. Neurobiol Learn Mem 83:93-103.

Canales JJ, Graybiel AM (2000) A measure of striatal function predicts motor stereotypy. Nat Neurosci 3:377-383.

Canales JJ, Graybiel AM (2000) Patterns of gene expression and behavior induced by chronic dopamine treatments. Ann Neurol 47:S53-59.

Canales JJ, Capper-Loup C, Hu D, Choe ES, Upadhyay U, Graybiel AM (2002) Shifts in striatal responsivity evoked by chronic stimulation of dopamine and glutamate systems. Brain 125:2353-2363.

Cardinal RP, JA Hall, J Everitt, BJ (2003) The contribution of the amygdala, nucleus accumbens, and prefrontal cortex to emotion and motivated behaviour. In: Cognition and emotion in the brain. Selected topics of the International Symposium on Limbic and Association Cortical Systems, pp 347-370: International Congress Series.

Carroll E (1977) Coca: the plant and its use. NIDA Res Monogr Series 13:35-45.

Carta AR, Gerfen CR, Steiner H (2000) Cocaine effects on gene regulation in the striatum and behavior: increased sensitivity in D3 dopamine receptor-deficient mice. Neuroreport 11:2395-2399.

Caulkins JP (1997) Is crack cheaper than (powder) cocaine? Addiction 92:1437-1443.

Chang SL, Squinto SP, Harlan RE (1988) Morphine activation of c-fos expression in rat brain. Biochem Biophys Res Commun 157:698-704.

Chen CY, Anthony JC (2004) Epidemiological estimates of risk in the process of becoming dependent upon cocaine: cocaine hydrochloride powder versus crack cocaine. Psychopharmacology (Berl) 172:78-86.

Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. J Neurosci 17:4933-4941.

Clarke PB, Kumar R (1983) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharmacol 78:329-337.

Clarke PB, Pert A (1985) Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348:355-358.

Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW (2003) Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. J Neurosci 23:2488-2493.

Couceyro P, Pollock KM, Drews K, Douglass J (1994) Cocaine differentially regulates activator protein-1 mRNA levels and DNA-binding complexes in the rat striatum and cerebellum. Mol Pharmacol 46:667-676.

Crombag HS, Badiani A, Robinson TE (1996) Signalled versus unsignalled intravenous amphetamine: large differences in the acute psychomotor response and sensitization. Brain Res 722:227-231.

Crombag HS, Ferrario CR, Robinson TE (2008) The rate of intravenous cocaine or amphetamine delivery does not influence drug-taking and drug-seeking behavior in rats. Pharmacol Biochem Behav 90:797-804.

Crombag HS, Mueller H, Browman KE, Badiani A, Robinson TE (1999) A comparison of two behavioral measures of psychomotor activation following intravenous amphetamine or cocaine: dose- and sensitization-dependent changes. Behav Pharmacol 10:205-213.

Crombag HS, Jedynak JP, Redmond K, Robinson TE, Hope BT (2002) Locomotor sensitization to cocaine is associated with increased Fos expression in the accumbens, but not in the caudate. Behav Brain Res 136:455-462.

Curran EJ, Akil H, Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. Neurochem Res 21:1425-1435.

Curran T, Teich NM (1982) Candidate product of the FBJ murine osteosarcoma virus oncogene: characterization of a 55,000-dalton phosphoprotein. J Virol 42:114-122.

Curran T, MacConnell WP, van Straaten F, Verma IM (1983) Structure of the FBJ murine osteosarcoma virus genome: molecular cloning of its associated helper virus and the cellular homolog of the v-fos gene from mouse and human cells. Mol Cell Biol 3:914-921.

Curran T, Peters G, Van Beveren C, Teich NM, Verma IM (1982) FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J Virol 44:674-682.

Daunais JB, McGinty JF (1994) Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. Synapse 18:35-45.

Daunais JB, Roberts DC, McGinty JF (1995) Short-term cocaine self administration alters striatal gene expression. Brain Res Bull 37:523-527.

Day HE, Badiani A, Uslaner JM, Oates MM, Vittoz NM, Robinson TE, Watson SJ, Jr., Akil H (2001) Environmental novelty differentially affects c-fos mRNA expression induced by amphetamine or cocaine in subregions of the bed nucleus of the stria terminalis and amygdala. J Neurosci 21:732-740.

De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ (1998) Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following

long-term extinction is associated with expression of behavioural sensitization. Eur J Neurosci 10:3565-3571.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75:134-143.

de Wit H, Bodker B, Ambre J (1992) Rate of increase of plasma drug level influences subjective response in humans. Psychopharmacology (Berl) 107:352-358.

Deneau GA, Seevers MH (1964) Pharmacological Aspects Of Drug Dependence. Adv Pharmacol 3:267-283.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for Addiction-like Behavior in the Rat. Science 305:1014-1017.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274-5278.

Dilts RP, Jr., Helton TE, McGinty JF (1993) Selective induction of Fos and FRA immunoreactivity within the mesolimbic and mesostriatal dopamine terminal fields. Synapse 13:251-263.

Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397-403.

Downs DA, Woods JH (1974) Codeine- and cocaine-reinforced responding in rhesus monkeys: effects of dose on response rates under a fixed-ratio schedule. J Pharmacol Exp Ther 191:179-188.

Dumartin B, Caille I, Gonon F, Bloch B (1998) Internalization of D1 dopamine receptor in striatal neurons in vivo as evidence of activation by dopamine agonists. J Neurosci 18:1650-1661.

Dunn J, Laranjeira RR (1999) Transitions in the route of cocaine administration--characteristics, direction and associated variables. Addiction 94:813-824.

Eblen F, Graybiel AM (1995) Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. J Neurosci 15:5999-6013.

Ehrman RN, Robbins SJ, Childress AR, O'Brien CP (1992) Conditioned responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology (Berl) 107:523-529.

Eikelboom R, Stewart J (1982) Conditioning of drug-induced physiological responses. Psychol Rev 89:507-528.

Enoch MA, Goldman D (1999) Genetics of alcoholism and substance abuse. Psychiatr Clin North Am 22:289-299, viii.

Ettenberg A (2009) The runway model of drug self-administration. Pharmacol Biochem Behav 91:271-277.

Ettenberg A, Bernardi RE (2006) Anxiolytic-like actions of buspirone in a runway model of intravenous cocaine self-administration. Pharmacol Biochem Behav 85:393-399.

Fallon JH, Moore RY (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545-580.

Fantegrossi WE (2008) In vivo pharmacology of MDMA and its enantiomers in rhesus monkeys. Exp Clin Psychopharmacol 16:1-12.

Farivar R, Zangenehpour S, Chaudhuri A (2004) Cellular-resolution activity mapping of the brain using immediate-early gene expression. Front Biosci 9:104-109.

Ferguson SM, Thomas MJ, Robinson TE (2004) Morphine-induced c-fos mRNA expression in striatofugal circuits: modulation by dose, environmental context, and drug history. Neuropsychopharmacology 29:1664-1674.

Ferguson SM, Norton CS, Watson SJ, Akil H, Robinson TE (2003) Amphetamine-evoked c-fos mRNA expression in the caudate-putamen: the effects of DA and NMDA receptor antagonists vary as a function of neuronal phenotype and environmental context. J Neurochem 86:33-44.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751-759.

Ferrario CR, Shou M, Samaha AN, Watson CJ, Kennedy RT, Robinson TE (2008) The rate of intravenous cocaine administration alters c-fos mRNA expression and the temporal dynamics of dopamine, but not glutamate, overflow in the striatum. Brain Res 1209:151-156.

Ferri CP, Gossop M (1999) Route of cocaine administration: patterns of use and problems among a Brazilian sample. Addict Behav 24:815-821.

Fischman MW (1984) The behavioral pharmacology of cocaine in humans. NIDA Res Monogr 50:72-91.

Fischman MW, Schuster C.R. (1984) Injection duration of cocaine in humans. Fed Proc 43:570.

Flaherty AW, Graybiel AM (1995) Motor and somatosensory corticostriatal projection magnifications in the squirrel monkey. J Neurophysiol 74:2638-2648.

Foltin RW, Fischman MW (1991) Smoked and intravenous cocaine in humans: acute tolerance, cardiovascular and subjective effects. J Pharmacol Exp Ther 257:247-261.

Freud S (1884) Classics revisited. Uber Coca. By Sigmund Freud. J Subst Abuse Treat 1:206-217.

Garcia MM, Brown HE, Harlan RE (1995) Alterations in immediate-early gene proteins in the rat forebrain induced by acute morphine injection. Brain Res 692:23-40.

Gawin FH, Kleber HD (1988) Evolving conceptualizations of cocaine dependence. Yale J Biol Med 61:123-136.

Gawin FH, Ellinwood EH, Jr. (1989) Cocaine dependence. Annu Rev Med 40:149-161.

Gay GR, Inaba DS, Sheppard CW, Newmeyer JA (1975) Cocaine: history, epidemiology, human pharmacology, and treatment. a perspective on a new debut for an old girl. Clin Toxicol 8:149-178.

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055-1061.

Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461-464.

Gerfen CR (1985) The neostriatal mosaic. I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. J Comp Neurol 236:454-476.

Gerfen CR (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science 246:385-388.

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285-320.

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. J Neural Transm Suppl 36:43-59.

Gerfen CR, Young WS, 3rd (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161-167.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.

Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G (1985) Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res 348:201-203.

Geter RS (1994) Drug user settings: a crack house typology. Int J Addict 29:1015-1027.

Giros B, Caron MG (1993) Molecular characterization of the dopamine transporter. Trends Pharmacol Sci 14:43-49.

Glees P (1944) The anatomical basis of cortico-striate connexions. J Anat 78:47-51.

Goldberg ST, Kelleher RT (1976) Behavior controlled by scheduled injections of cocaine in squirrel and rhesus monkeys. J Exp Anal Behav 25:93-104.

Goodman A (2008) Neurobiology of addiction. An integrative review. Biochem Pharmacol 75:266-322.

Gorelick DA (1992) Progression of dependence in male cocaine addicts. Am J Drug Alcohol Abuse 18:13-19.

Gorelick DA (1998) The rate hypothesis and agonist substitution approaches to cocaine abuse treatment. Adv Pharmacol 42:995-997.

Gossop M, Strang J (1991) A comparison of the withdrawal responses of heroin and methadone addicts during detoxification. Br J Psychiatry 158:697-699.

Gossop M, Griffiths P, Powis B, Strang J (1992) Severity of dependence and route of administration of heroin, cocaine and amphetamines. Br J Addict 87:1527-1536.

Gossop M, Griffiths P, Powis B, Strang J (1994) Cocaine: patterns of use, route of administration, and severity of dependence. Br J Psychiatry 164:660-664.

Graybiel AM, Ragsdale CW, Jr. (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci U S A 75:5723-5726.

Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci U S A 87:6912-6916.

Graybiel AM, Ragsdale CW, Jr., Yoneoka ES, Elde RP (1981) An immunohistochemical study of enkephalins and other neuropeptides in the striatum of the cat with evidence that the opiate peptides are arranged to form mosaic patterns in register with the striosomal compartments visible by acetylcholinesterase staining. Neuroscience 6:377-397.

Griffiths RR, Findley JD, Brady JV, Dolan-Gutcher K, Robinson WW (1975) Comparison of progressive-ratio performance maintained by cocaine, methylphenidate and secobarbital. Psychopharmacologia 43:81-83.

Guzman D, Moscarello JM, Ettenberg A (2009) The effects of medial prefrontal cortex infusions of cocaine in a runway model of drug self-administration: evidence of reinforcing but not anxiogenic actions. Eur J Pharmacol 605:117-122.

Harlan RE, Garcia MM (1998) Drugs of abuse and immediate-early genes in the forebrain. Mol Neurobiol 16:221-267.

Hatsukami DK, Fischman MW (1996) Crack cocaine and cocaine hydrochloride. Are the differences myth or reality? Jama 276:1580-1588.

Headlee CP, Coppock HW, Hichols JR (1955) Apparatus and technique involved in a laboratory method of detecting the addictiveness of drugs. J Am Pharm Assoc Am Pharm Assoc (Baltim) 44:229-231.

Heikkila RE, Orlansky H, Cohen G (1975) Studies on the distinction between uptake inhibition and release of (3H)dopamine in rat brain tissue slices. Biochem Pharmacol 24:847-852.

Heimer L, Van Hoesen GW (2006) The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. Neurosci Biobehav Rev 30:126-147.

Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS (1997) The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci 9:354-381.

Heine M, Groc L, Frischknecht R, Beique JC, Lounis B, Rumbaugh G, Huganir RL, Cognet L, Choquet D (2008) Surface mobility of postsynaptic AMPARs tunes synaptic transmission. Science 320:201-205.

Herkenham M, Pert CB (1980) In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways. Proc Natl Acad Sci U S A 77:5532-5536.

Herkenham M, Pert CB (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. Nature 291:415-418.

Hiroi N, Marek GJ, Brown JR, Ye H, Saudou F, Vaidya VA, Duman RS, Greenberg ME, Nestler EJ (1998) Essential role of the fosB gene in molecular, cellular, and behavioral actions of chronic electroconvulsive seizures. J Neurosci 18:6952-6962.

Hooks MS, Jones GH, Liem BJ, Justice JB, Jr. (1992) Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intra-cranial amphetamine infusions. Ann N Y Acad Sci 654:444-447.

Hooks MS, Jones GH, Hemby SE, Justice JB, Jr. (1993) Environmental and pharmacological sensitization: effects of repeated administration of systemic or intra-nucleus accumbens cocaine. Psychopharmacology (Berl) 111:109-116.

Hope B, Kosofsky B, Hyman SE, Nestler EJ (1992) Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci U S A 89:5764-5768.

Hope BT, Simmons DE, Mitchell TB, Kreuter JD, Mattson BJ (2006) Cocaine-induced locomotor activity and Fos expression in nucleus accumbens are sensitized for 6 months after repeated cocaine administration outside the home cage. Eur J Neurosci 24:867-875.

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37:707-711.

Hser YI, Huang D, Brecht ML, Li L, Evans E (2008) Contrasting trajectories of heroin, cocaine, and methamphetamine use. J Addict Dis 27:13-21.

Hughes P, Dragunow M (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. Pharmacol Rev 47:133-178.

Hyman SE (2001) A 28-year-old man addicted to cocaine. Jama 286:2586-2594.

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2:695-703.

Hyman SE, Kosofsky BE, Nguyen TV, Cohen BM, Comb MJ (1993) Everything activates c-fos--how can it matter? NIDA Res Monogr 125:25-38.

Javaid JI, Fischman MW, Schuster CR, Dekirmenjian H, Davis JM (1978) Cocaine plasma concentration: relation to physiological and subjective effects in humans. Science 202:227-228.

Javaid JI, Musa MN, Fischman M, Schuster CR, Davis JM (1983) Kinetics of cocaine in humans after intravenous and intranasal administration. Biopharm Drug Dispos 4:9-18.

Jedynak JP (2009) Visualizing dendritic morphology in the striatum following repeated treatment with methamphetamine. In: Neuroscience Graduate Program, pp 145. Ann Arbor: University of Michigan.

Jekel JF, Allen DF, Podlewski H, Clarke N, Dean-Patterson S, Cartwright P (1986) Epidemic free-base cocaine abuse. Case study from the Bahamas. Lancet 1:459-462.

Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology 146:373.

Johanson CE (1984) Assessment of the dependence potential of cocaine in animals. NIDA Res Monogr 50:54-71.

Johanson CE, Balster RL (1978) A summary of the results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull Narc 30:43-54.

Jones RT (1984) The pharmacology of cocaine. NIDA Res Monogr 50:34-53.

Jones RT (1990) The pharmacology of cocaine smoking in humans. NIDA Res Monogr 99:30-41.

Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 10:561-572.

Kalivas PW, Weber B (1988) Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J Pharmacol Exp Ther 245:1095-1102.

Kalivas PW, Duffy P (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48-58.

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223-244.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Karch SB (1999) Cocaine: history, use, abuse. J R Soc Med 92:393-397.

Kato S, Wakasa Y, Yanagita T (1987) Relationship between minimum reinforcing doses and injection speed in cocaine and pentobarbital self-administration in crab-eating monkeys. Pharmacol Biochem Behav 28:407-410.

Keller RW, Jr., Maisonneuve IM, Carlson JN, Glick SD (1992) Within-subject sensitization of striatal dopamine release after a single injection of cocaine: an in vivo microdialysis study. Synapse 11:28-34.

Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. Nature 401:272-276.

Kemp JM, Powell TP (1971) The synaptic organization of the caudate nucleus. Philos Trans R Soc Lond B Biol Sci 262:403-412.

Kiba H, Jayaraman A (1994) Nicotine induced c-fos expression in the striatum is mediated mostly by dopamine D1 receptor and is dependent on NMDA stimulation. Brain Res Mol Brain Res 23:1-13.

Kippin TE, Fuchs RA, See RE (2006) Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 187:60-67.

Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res 447:346-352.

Knackstedt LA, Kalivas PW (2007) Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization. J Pharmacol Exp Ther 322:1103-1109.

Konradi C, Cole RL, Heckers S, Hyman SE (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 14:5623-5634.

Koob GF (1992) Neural mechanisms of drug reinforcement. Ann N Y Acad Sci 654:171-191.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52-58.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129.

Kovacs KJ (2008) Measurement of immediate-early gene activation- c-fos and beyond. J Neuroendocrinol 20:665-672.

Kowalchuk RK, Keselman HJ (2001) Mixed-model pairwise multiple comparisons of repeated measures means. Psychol Methods 6:282-296.

Koya E, Uejima JL, Wihbey KA, Bossert JM, Hope BT, Shaham Y (2009) Role of ventral medial prefrontal cortex in incubation of cocaine craving. Neuropharmacology 56 Suppl 1:177-185.

Koya E, Golden SA, Harvey BK, Guez-Barber DH, Berkow A, Simmons DE, Bossert JM, Nair SG, Uejima JL, Marin MT, Mitchell TB, Farquhar D, Ghosh SC, Mattson BJ, Hope BT (2009) Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization. Nat Neurosci 12:1069-1073.

Ksir C, Hakan R, Hall DP, Jr., Kellar KJ (1985) Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [3H]acetylcholine to nicotinic receptors. Neuropharmacology 24:527-531.

LaHoste GJ, Yu J, Marshall JF (1993) Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci U S A 90:7451-7455.

Lasagna L, Von Felsinger JM, Beecher HK (1955) Drug-induced mood changes in man. I. Observations on healthy subjects, chronically ill patients, and postaddicts. J Am Med Assoc 157:1006-1020.

Lawhorn C, Smith DM, Brown LL (2009) Partial ablation of mu-opioid receptor rich striosomes produces deficits on a motor-skill learning task. Neuroscience 163:109-119.

Le Moine C, Tison F, Bloch B (1990) D2 dopamine receptor gene expression by cholinergic neurons in the rat striatum. Neurosci Lett 117:248-252.

Lee DK, Bian S, Rahman MA, Shim YB, Shim I, Choe ES (2008) Repeated cocaine administration increases N-methyl-d-aspartate NR1 subunit, extracellular signal-regulated kinase and cyclic AMP response element-binding protein phosphorylation and glutamate release in the rat dorsal striatum. Eur J Pharmacol 590:157-162.

Leri F, Stewart J (2001) Drug-induced reinstatement to heroin and cocaine seeking: a rodent model of relapse in polydrug use. Exp Clin Psychopharmacol 9:297-306.

Lett BT (1989) Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology (Berl) 98:357-362.

Li Y, Acerbo MJ, Robinson TE (2004) The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 20:1647-1654.

Liu J, Nickolenko J, Sharp FR (1994) Morphine induces c-fos and junB in striatum and nucleus accumbens via D1 and N-methyl-D-aspartate receptors. Proc Natl Acad Sci U S A 91:8537-8541.

Liu Y, Roberts DC, Morgan D (2005) Sensitization of the reinforcing effects of self-administered cocaine in rats: effects of dose and intravenous injection speed. Eur J Neurosci 22:195-200.

Lorrain DS, Arnold GM, Vezina P (2000) Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. Behav Brain Res 107:9-19.

Magos L (1969) Persistence of the effect of amphetamine on stereotyped activity in rats. Eur J Pharmacol 6:200-201.

Mantsch JR, Ho A, Schlussman SD, Kreek MJ (2001) Predictable individual differences in the initiation of cocaine self-administration by rats under extended-access conditions are dose-dependent. Psychopharmacology (Berl) 157:31-39.

Mantsch JR, Yuferov V, Mathieu-Kia AM, Ho A, Kreek MJ (2004) Effects of extended access to high versus low cocaine doses on self-administration, cocaine-induced reinstatement and brain mRNA levels in rats. Psychopharmacology (Berl) 175:26-36.

Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993) Animal models of drug craving. Psychopharmacology (Berl) 112:163-182.

Marsch LA, Bickel WK, Badger GJ, Rathmell JP, Swedberg MD, Jonzon B, Norsten-Hoog C (2001) Effects of infusion rate of intravenously administered morphine on physiological, psychomotor, and self-reported measures in humans. J Pharmacol Exp Ther 299:1056-1065.

Matta SG, Foster CA, Sharp BM (1993) Nicotine stimulates the expression of cFos protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions. Endocrinology 132:2149-2156.

Matthews RT, German DC (1984) Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience 11:617-625.

McFarlane DK, Martonyi BJ, Robinson TE (1992) An Inexpensive Automated-System For The Measurement Of Rotational Behavior In Small Animals. Behavior Research Methods Instruments & Computers 24:414-419.

McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503-537.

Meredith GE, Farrell T, Kellaghan P, Tan Y, Zahm DS, Totterdell S (1999) Immunocytochemical characterization of catecholaminergic neurons in the rat striatum following dopamine-depleting lesions. Eur J Neurosci 11:3585-3596.

Mereu G, Gessa GL (1985) Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect? Brain Res 360:325-330.

Mieczkowski T (1990) The operational styles of crack houses in Detroit. NIDA Res Monogr 103:60-91.

Miller DK, Wilkins LH, Bardo MT, Crooks PA, Dwoskin LP (2001) Once weekly administration of nicotine produces long-lasting locomotor sensitization in rats via a nicotinic receptor-mediated mechanism. Psychopharmacology (Berl) 156:469-476.

Miura M, Saino-Saito S, Masuda M, Kobayashi K, Aosaki T (2007) Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor

in the mouse striatum with green fluorescent protein-expressing dopamine islands. J Neurosci 27:9721-9728.

Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147-156.

Morency MA, Beninger RJ (1986) Dopaminergic substrates of cocaine-induced place conditioning. Brain Research 399:33.

Morgan JI, Curran T (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci 14:421-451.

Mucha RF, Van Der Kooy D, O'Shaughnessy M, Bucenieks P (1982) Drug reinforcement studied by the use of place conditioning in rat. Brain Research 243:91.

Mulvaney FD, Alterman AI, Boardman CR, Kampman K (1999) Cocaine abstinence symptomatology and treatment attrition. J Subst Abuse Treat 16:129-135.

Nelson RA, Boyd SJ, Ziegelstein RC, Herning R, Cadet JL, Henningfield JE, Schuster CR, Contoreggi C, Gorelick DA (2006) Effect of rate of administration on subjective and physiological effects of intravenous cocaine in humans. Drug Alcohol Depend 82:19-24.

Nestler EJ (2001) Molecular neurobiology of addiction. Am J Addict 10:201-217.

Nestler EJ (2008) Review. Transcriptional mechanisms of addiction: role of DeltaFosB. Philos Trans R Soc Lond B Biol Sci 363:3245-3255.

Niemann A (1860) Ueber eine neue organische Base in den Cocablättern. Archiv der Pharmazie 153:129-155.

O'Brien CP (1997) Progress in the science of addiction. Am J Psychiatry 154:1195-1197.

O'Brien CP, Childress AR, McLellan AT, Ehrman R (1992) Classical conditioning in drug-dependent humans. Ann N Y Acad Sci 654:400-415.

O'Brien MS, Anthony JC (2005) Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000-2001. Neuropsychopharmacology 30:1006-1018.

Ostlund SB, Balleine BW (2005) Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. J Neurosci 25:7763-7770.

Ostrander MM, Badiani A, Day HE, Norton CS, Watson SJ, Akil H, Robinson TE (2003) Environmental context and drug history modulate amphetamine-induced c-fos mRNA expression in the basal ganglia, central extended amygdala, and associated limbic forebrain. Neuroscience 120:551-571.

Pan HT, Menacherry S, Justice JB, Jr. (1991) Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J Neurochem 56:1299-1306.

Panlilio LV, Goldberg SR, Gilman JP, Jufer R, Cone EJ, Schindler CW (1998) Effects of delivery rate and non-contingent infusion of cocaine on cocaine self-administration in rhesus monkeys. Psychopharmacology (Berl) 137:253-258.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport 14:2229-2232.

Paxinos G (1998) The rat brain in stereotaxic coordinates, 4th ed. Edition. San Diego: Academic Press.

Pelloux Y, Everitt BJ, Dickinson A (2007) Compulsive drug seeking by rats under punishment: effects of drug taking history. Psychopharmacology (Berl) 194:127-137.

Persico AM, Schindler CW, O'Hara BF, Brannock MT, Uhl GR (1993) Brain transcription factor expression: effects of acute and chronic amphetamine and injection stress. Brain Res Mol Brain Res 20:91-100.

Pert CB, Kuhar MJ, Snyder SH (1976) Opiate receptor: autoradiographic localization in rat brain. Proc Natl Acad Sci U S A 73:3729-3733.

Perugini M, Vezina P (1994) Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. J Pharmacol Exp Ther 270:690-696.

145

Petersen RC (1977) History of cocaine. NIDA Res Monogr Series 13:17-34.

Pettit HO, Pan HT, Parsons LH, Justice JB, Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J Neurochem 55:798-804.

Phillips AG, Di Ciano P (1996) Behavioral sensitization is induced by intravenous self-administration of cocaine by rats. Psychopharmacology (Berl) 124:279-281.

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

Pickens R, Dougherty J, Thompson T (1969) Effects of volume and duration of infusion on cocaine reinforcement with concurrent activity recording. In: Minutes of the Meeting of the Committee on Problems of Drug Dependence, pp 5805-5811. Washington, D.C.: NAS-NRC.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 16:1550-1560.

Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in the rat. Nature 260:731-732.

Preston KL, Vahabzadeh M, Schmittner J, Lin JL, Gorelick DA, Epstein DH (2009) Cocaine craving and use during daily life. Psychopharmacology (Berl).

Rahmsdorf HJ, Schonthal A, Angel P, Litfin M, Ruther U, Herrlich P (1987) Posttranscriptional regulation of c-fos mRNA expression. Nucleic Acids Res 15:1643-1659.

Renthal W, Carle TL, Maze I, Covington HE, 3rd, Truong HT, Alibhai I, Kumar A, Montgomery RL, Olson EN, Nestler EJ (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J Neurosci 28:7344-7349.

Resnick RB, Kestenbaum RS, Schwartz LK (1977) Acute systemic effects of cocaine in man: a controlled study by intranasal and intravenous routes. Science 195:696-698.

Reynolds SM, Zahm DS (2005) Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala. J Neurosci 25:11757-11767.

Reynolds SM, Berridge KC (2008) Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. Nat Neurosci 11:423-425.

Ribak CE, Vaughn JE, Roberts E (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. J Comp Neurol 187:261-283.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219-1223.

Robbins TW, Everitt BJ (1996) Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6:228-236.

Roberts DC, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615-620.

Roberts DC, Koob GF, Klonoff P, Fibiger HC (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol Biochem Behav 12:781-787.

Robins LN, Helzer JE, Davis DH (1975) Narcotic use in southeast Asia and afterward. An interview study of 898 Vietnam returnees. Arch Gen Psychiatry 32:955-961.

Robinson TE (1984) Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology (Berl) 84:466-475.

Robinson TE (2004) Neuroscience. Addicted rats. Science 305:951-953.

Robinson TE, Becker JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur J Pharmacol 85:253-254.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396:157-198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95 Suppl 2:S91-117.

Robinson TE, Berridge KC (2003) Addiction. Annu Rev Psychol 54:25-53.

Robinson TE, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 47 Suppl 1:33-46.

Rosen JB, Chuang E, Iadarola MJ (1994) Differential induction of Fos protein and a Fos-related antigen following acute and repeated cocaine administration. Brain Res Mol Brain Res 25:168-172.

Ruskin DN, Marshall JF (1994) Amphetamine- and cocaine-induced fos in the rat striatum depends on D2 dopamine receptor activation. Synapse 18:233-240.

Sagar SM, Sharp FR, Curran T (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240:1328-1331.

Samaha AN, Robinson TE (2005) Why does the rapid delivery of drugs to the brain promote addiction? Trends Pharmacol Sci 26:82-87.

Samaha AN, Li Y, Robinson TE (2002) The rate of intravenous cocaine administration determines susceptibility to sensitization. J Neurosci 22:3244-3250.

Samaha AN, Yau WY, Yang P, Robinson TE (2005) Rapid delivery of nicotine promotes behavioral sensitization and alters its neurobiological impact. Biol Psychiatry 57:351-360.

Samaha AN, Mallet N, Ferguson SM, Gonon F, Robinson TE (2004) The rate of cocaine administration alters gene regulation and behavioral plasticity: implications for addiction. J Neurosci 24:6362-6370.

Schenk S, Snow S, Horger BA (1991) Pre-exposure to amphetamine but not nicotine sensitizes rats to the motor activating effect of cocaine. Psychopharmacology (Berl) 103:62-66.

Schindler CW, Panlilio LV, Thorndike EB (2009) Effect of rate of delivery of intravenous cocaine on self-administration in rats. Pharmacol Biochem Behav 93:375-381.

Seeman P, Van Tol HH (1994) Dopamine receptor pharmacology. Trends Pharmacol Sci 15:264-270.

Segal DS, Mandell AJ (1974) Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. Pharmacol Biochem Behav 2:249-255.

Sesack SR, Pickel VM (1990) In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. Brain Res 527:266-279.

Shaham Y, Stewart J (1995) Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology (Berl) 119:334-341.

Shaham Y, Rajabi H, Stewart J (1996) Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. J Neurosci 16:1957-1963.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54:1-42.

Sharp BM, Beyer HS, McAllen KM, Hart D, Matta SG (1993) Induction and Desensitization of the c-Fos mRNA Response to Nicotine in Rat Brain. Mol Cell Neurosci 4:199-208.

Sheng M, Greenberg ME (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron 4:477-485.

Shippenberg TS, Heidbreder C (1995) Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther 273:808-815.

Shou M, Ferrario CR, Schultz KN, Robinson TE, Kennedy RT (2006) Monitoring dopamine in vivo by microdialysis sampling and on-line CE-laser-induced fluorescence. Anal Chem 78:6717-6725.

Siegel RK (1984) Changing patterns of cocaine use: longitudinal observations, consequences, and treatment. NIDA Res Monogr 50:92-110.

Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13:259-265.

Solomon RL, Corbit JD (1974) An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol Rev 81:119-145.

Song DD, Harlan RE (1994) Genesis and migration patterns of neurons forming the patch and matrix compartments of the rat striatum. Brain Res Dev Brain Res 83:233-245.

Steiner H, Gerfen CR (1993) Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci 13:5066-5081.

Stolerman IP, Fink R, Jarvik ME (1973) Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacologia 30:329-342.

Sutton MA, Schmidt EF, Choi KH, Schad CA, Whisler K, Simmons D, Karanian DA, Monteggia LM, Neve RL, Self DW (2003) Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. Nature 421:70-75.

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

Szucs RP, Frankel PS, McMahon LR, Cunningham KA (2005) Relationship of cocaine-induced c-Fos expression to behaviors and the role of serotonin 5-HT2A receptors in cocaine-induced c-Fos expression. Behav Neurosci 119:1173-1183.

Tan A, Moratalla R, Lyford GL, Worley P, Graybiel AM (2000) The activity-regulated cytoskeletal-associated protein arc is expressed in different striosome-matrix patterns following exposure to amphetamine and cocaine. J Neurochem 74:2074-2078.

150

Tanaka C, Ishikawa M, Shimada S (1982) Histochemical mapping of catecholaminergic neurons and their ascending fiber pathways in the rhesus monkey brain. Brain Res Bull 9:255-270.

Taylor JR, Horger BA (1999) Enhanced responding for conditioned reward produced by intra-accumbens amphetamine is potentiated after cocaine sensitization. Psychopharmacology (Berl) 142:31-40.

Todtenkopf MS, Mihalakopoulos A, Stellar JR (2002) Withdrawal duration differentially affects c-fos expression in the medial prefrontal cortex and discrete subregions of the nucleus accumbens in cocaine-sensitized rats. Neuroscience 114:1061-1069.

Torres G, Rivier C (1993) Cocaine-induced expression of striatal c-fos in the rat is inhibited by NMDA receptor antagonists. Brain Res Bull 30:173-176.

Totterdell S, Smith AD (1989) Convergence of hippocampal and dopaminergic input onto identified neurons in the nucleus accumbens of the rat. J Chem Neuroanat 2:285-298.

Ungerstedt U (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand Suppl 367:1-48.

Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res 24:485-493.

Uslaner J, Badiani A, Day HE, Watson SJ, Akil H, Robinson TE (2001) Environmental context modulates the ability of cocaine and amphetamine to induce c-fos mRNA expression in the neocortex, caudate nucleus, and nucleus accumbens. Brain Res 920:106-116.

Uslaner J, Badiani A, Norton CS, Day HE, Watson SJ, Akil H, Robinson TE (2001) Amphetamine and cocaine induce different patterns of c-fos mRNA expression in the striatum and subthalamic nucleus depending on environmental context. Eur J Neurosci 13:1977-1983.

Uslaner JM, Crombag HS, Ferguson SM, Robinson TE (2003) Cocaine-induced psychomotor activity is associated with its ability to induce c-fos mRNA expression in the subthalamic nucleus: effects of dose and repeated treatment. Eur J Neurosci 17:2180-2186.

Uslaner JM, Norton CS, Watson SJ, Akil H, Robinson TE (2003) Amphetamine-induced c-fos mRNA expression in the caudate-putamen and subthalamic nucleus: interactions between dose, environment, and neuronal phenotype. J Neurochem 85:105-114.

van der Kooy D, Fishell G (1987) Neuronal birthdate underlies the development of striatal compartments. Brain Res 401:155-161.

Vanderschuren LJ, Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305:1017-1019.

Vanderschuren LJ, Schoffelmeer AN, Van Leeuwen SD, Hof L, Jonker AJ, Voorn P (2002) Compartment-specific changes in striatal neuronal activity during expression of amphetamine sensitization are the result of drug hypersensitivity. Eur J Neurosci 16:2462-2468.

Venton BJ, Robinson TE, Kennedy RT (2006) Transient changes in nucleus accumbens amino acid concentrations correlate with individual responsivity to the predator fox odor 2,5-dihydro-2,4,5-trimethylthiazoline. J Neurochem 96:236-246.

Vezina P (1996) D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. J Neurosci 16:2411-2420.

Vezina P (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neuroscience & Biobehavioral Reviews 27:827.

Vezina P, Stewart J (1990) Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Res 516:99-106.

Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N (2002) Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. J Neurosci 22:4654-4662.

Volkow ND, Wang GJ, Fischman MW, Foltin R, Fowler JS, Franceschi D, Franceschi M, Logan J, Gatley SJ, Wong C, Ding YS, Hitzemann R, Pappas N (2000) Effects of route of administration on cocaine induced dopamine transporter blockade in the human brain. Life Sci 67:1507-1515.

Wallach MB, Gershon S (1971) Sensitization to amphetamines. Psychopharmacol Bull 7:30-31.

Walsh SL, Donny EC, Nuzzo PA, Umbricht A, Bigelow GE (2009) Cocaine abuse versus cocaine dependence: Cocaine self-administration and pharmacodynamic response in the human laboratory. Drug Alcohol Depend.

Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95:532-544.

Webster KE (1965) The Cortico-Striatal Projection In The Cat. J Anat 99:329-337.

Weeks J (1972) Long-term intravenous infusions. London: Academic Press.

Weeks JR, Davis JD (1964) Chronic Intravenous Cannulas For Rats. J Appl Physiol 19:540-541.

Weiss SR, Post RM, Pert A, Woodward R, Murman D (1989) Context-dependent cocaine sensitization: differential effect of haloperidol on development versus expression. Pharmacol Biochem Behav 34:655-661.

West BW, KB Galecki, AT (2007) Linear Mixed Models: A Practical Guide Using Statistical Software, 2nd Edition. Boca Raton: Chapman & Hill/CRC.

White FJ, Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. Drug Alcohol Depend 51:141-153.

White NM (1989) A functional hypothesis concerning the striatal matrix and patches: mediation of S-R memory and reward. Life Sci 45:1943-1957.

White NM, Hiroi N (1998) Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum. Proc Natl Acad Sci U S A 95:6486-6491.

Willuhn I, Sun W, Steiner H (2003) Topography of cocaine-induced gene regulation in the rat striatum: relationship to cortical inputs and role of behavioural context. Eur J Neurosci 17:1053-1066.

Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular inject of horseradish peroxidase. J Comp Neurol 194:599-615.

Wilson MC, Hitomi M, Schuster CR (1971) Psychomotor stimulant self administration as a function of dosage per injection in the rhesus monkey. Psychopharmacologia 22:271-281.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.

Wolf ME (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog Neurobiol 54:679-720.

Woods J (1977) Behavioral effects of cocaine in animals. NIDA Res Monogr Series 13:63-95.

Woolverton WL, Wang Z (2004) Relationship between injection duration, transporter occupancy and reinforcing strength of cocaine. Eur J Pharmacol 486:251-257.

Xu M (2008) c-Fos is an intracellular regulator of cocaine-induced long-term changes. Ann N Y Acad Sci 1139:1-9.

Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181-189.

Yin HH, Ostlund SB, Balleine BW (2008) Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. Eur J Neurosci 28:1437-1448.

Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513-523.

Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. Proc Natl Acad Sci U S A 88:1291-1295.

Zahm DS (2006) The evolving theory of basal forebrain functional-anatomical 'macrosystems'. Neurosci Biobehav Rev 30:148-172.

Zahm DS, Becker ML, Freiman AJ, Strauch S, Degarmo B, Geisler S, Meredith GE, Marinelli M (2009) Fos After Single and Repeated Self-Administration of Cocaine and Saline in the Rat: Emphasis on the Basal Forebrain and Recalibration of Expression. Neuropsychopharmacology.

Zapata A, Chefer VI, Ator R, Shippenberg TS, Rocha BA (2003) Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after intravenous cocaine self-administration in mice. Eur J Neurosci 17:590-596.

Zernig G, Giacomuzzi S, Riemer Y, Wakonigg G, Sturm K, Saria A (2003) Intravenous drug injection habits: drug users' self-reports versus researchers' perception. Pharmacology 68:49-56.

Zhang D, Zhang L, Tang Y, Zhang Q, Lou D, Sharp FR, Zhang J, Xu M (2005) Repeated Cocaine Administration Induces Gene Expression Changes through the Dopamine D1 Receptors. Neuropsychopharmacology 30:1443.

Zhang J, Zhang L, Jiao H, Zhang Q, Zhang D, Lou D, Katz JL, Xu M (2006) c-Fos facilitates the acquisition and extinction of cocaine-induced persistent changes. J Neurosci 26:13287-13296.