Behavioral Models of Cocaine Use Disorder

BY

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LIST OF ABBREVIATIONS

BDD: behaviorally dependent dosing
CORT: corticosterone
DAT: dopamine transporter
EBLs: estimated brain levels
FR1: fixed ratio one
HD: hold down
HPA: hypothalamus pituitary adrenal
ICIs: inter-cluster intervals
ICSS: intra-cranial self-stimulation
IntA: intermittent access
IP: intraperitoneal
LgA: long access
NAc: nucleus accumbens
PFC: prefrontal cortex
Pmax: maximal price paid
PR: progressive ratio
SC: subcutaneous
ShA: short access
TH: threshold
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Forward

The order in which I conducted the experiments described in this manuscript does not match the order in which they were published. For example, the cocaine binge study (see chapter 4) was the first experiment I conducted during my rotation in the Roberts lab. However, those results were only recently written up and submitted for publication. I have opted against arranging the chapters in this dissertation based on chronological order of when the experiment was conducted or when the results were published. Instead, I have arranged all the chapters to optimize the flow of the overall story of results from my graduate work.

Based on the encouragement of my advisor, Dr. Roberts, the introduction (chapter one) and conclusion sections of this thesis have been written as a review of the literature, which is in preparation for publication.
ABSTRACT

Benjamin A. Zimmer

Behavioral Models of Cocaine Use Disorder

Dissertation under the direction of David C.S. Roberts, Ph.D.,

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The complex set of symptoms associated with substance use disorder has made creating animal models with high predictive validity difficult. One tactic to help translate results from animal studies to humans is to separate the symptoms of substance use disorder into two broad categories of dysregulated drug taking and dysregulated drug seeking. However, distinguishing the drug seeking or drug taking components in self-administration models can be difficult since the consumptive and appetitive responses are both directed towards the same operant. A variety of analysis methods, procedures, and schedules of reinforcement can be used to help separate the conflated consumptive vs appetitive aspects of self-administration, and distinguishing these characteristics represents a unique lens with which to examine results from self-administration studies.

In chapter one, this lens of appetitive vs consumptive changes is used to discuss results from many rodent procedures in the self-administration literature. That is, the effects of a variety of drug experiences on subsequent changes in drug-seeking and/or drug-taking is explored. It is emphasized that building a better
understanding of the underlying neurophysiological changes associated with either a change in drug-seeking, drug-taking or both provides a focus for targeted pharmacological interventions to treat various symptoms of substance use disorder. The literature reviewed in this chapter includes work from my own dissertation, and therefore places my work into the broader context of the self-administration literature.

In chapter two, a novel behaviorally dependent dosing (BDD) schedule was used to examine the relationship between doses of cocaine self-administered by rats and brain drug levels within a session. The BDD schedule used a hold-down response to activate a syringe pump. The length of time the lever was held down determined the duration that the syringe pump was activated. In the first experiment, rats self-administered cocaine for daily 3 h sessions and brain levels of cocaine were modeled using well-established parameters. Although analysis revealed that rats self-administered doses within a predicted range, one extremely large dose was consistently observed at the beginning of each session when brain levels of cocaine were low. In the second experiment, we introduced a range of timeout periods (10–25 min) in order to produce variability in brain-cocaine concentrations. Animals self-administered larger doses immediately following each timeout period and the dose size was inversely correlated with the length of the timeout. These results show that the dose of cocaine that rats self-administer within a session is inversely related to the amount of drug on board.

In chapter three, the regulation of cocaine intake during the maintenance phase was examined. It has long been observed that rats self-administer
psychostimulants in a highly regular pattern. The inverse relationship between dose and rate of drug intake has been interpreted as a titration phenomenon wherein brain-cocaine levels are maintained within a range. Most studies examining this phenomenon have used fixed, unit doses in which case the only titration strategy available to the animal is to adjust inter-infusion intervals. In this study, we examined whether selection of dose size could also be a factor in regulation of intake. We used a schedule of reinforcement, under which the dose can vary through a wide range and is determined by the behavior of the animal. Rats self-administered cocaine using a BDD schedule of reinforcement, under which the size of each dose was determined by the length of time the lever was held down. The concentration of cocaine was changed across sessions. Total pump-time self-administered decreased by 56% following each doubling of the concentration, which led to an average 11% increase in total intake. Similarly, estimated brain levels of cocaine increased by 12% for each doubling of concentration. These adjustments were the result of manipulation of both the size and spacing of infusions. In agreement with previous studies, the regular pattern of intake appears to be the result of a titration mechanism in which animals maintain brain levels of cocaine above some threshold. Compensatory regulation appeared to involve both the selection of dose size and inter-infusion intervals.

In chapter four, the acute effects of long-duration cocaine binges on appetitive responding was assessed. Human cocaine abusers typically develop a binge-abstinence pattern of use. Animals given unlimited access to cocaine also show a binge-abstinent pattern of use, and many studies have examined a variety of
factors that can influence intake and regularity within a cocaine binge. The goal of the this study was to examine the time-course of motivation within a 24 hour cocaine binge. Additionally, the influences of circadian cycles, the transience of binge-induced decreases in breakpoint, and the effects of dose within a binge were examined. Cocaine binges consisted of self-administration sessions lasting 6-24 hours, with a low response cost (FR1), and no limits on the number of earnable infusions. Two doses of cocaine (0.56 and 1.5 mg/kg/inf) within the binge were assessed. Following each binge session, a progressive ratio (PR) schedule was immediately run to assess the motivation to continue the binge. PR breakpoints were significantly decreased in a binge-length dependent manner, and time of day had no significant effect on these deficits. Breakpoints recovered to baseline levels within 48 hours, and decreasing the dose within a binge decreased intake but not breakpoints. This study demonstrated that within a high-intake cocaine binge, motivation to self-administer decreases in a time-dependent fashion. These data add to a growing literature examining the role of psychostimulant binge administration.

Chapter five examined the consequences of a history of intermittent access to cocaine. Recent attempts to model the addiction process in rodents have focused on cocaine self-administration procedures that provide extended daily access. Such procedures produce a characteristic loading phase during which blood levels rapidly rise and then are maintained within an elevated range for the duration of the session. This set of experiments tested the hypothesis that multiple fast rising spikes in cocaine levels contribute to the addiction process.
more robustly than constant, maintained drug levels. Here, we compared the
effects of various cocaine self-administration procedures that produced very
different patterns of drug intake and drug dynamics on Pmax, a behavioral
economic measure of the motivation to self-administer drug. Two groups
received intermittent access (IntA) to cocaine during daily 6-h sessions. Access
was limited to twelve 5-min trials that alternated with 25-min timeout periods,
using either a hold-down procedure or a fixed ratio 1 (FR1). Cocaine levels could
not be maintained with this procedure; instead the animals experienced 12 fast-
rising spikes in cocaine levels each day. The IntA groups were compared with
groups given 6-h FR1 long access and 2-h short access sessions and two other
control groups. Here, we report that cocaine self-administration procedures
resulting in repeatedly spiking drug levels produce more robust increases in
Pmax than procedures resulting in maintained high levels of cocaine. These
results suggest that rapid spiking of brain-cocaine levels is sufficient to increase
the motivation to self-administer cocaine.
CHAPTER ONE

Conflation of Cocaine Seeking and Cocaine Taking Responses in IV Self-administration Experiments in Rats: Implications on preclinical models of Substance Use Disorder

Benjamin A. Zimmer and David C.S. Roberts

The following manuscript is in preparation
1. Introduction

The *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; American Psychiatric Association, 2013) acknowledges substance abuse as a spectrum disorder with 11 unique symptoms. The challenge for basic research scientists is to develop animal models of substance use disorder that have predictive validity despite the complexity of this disorder. Therefore, developing parallels between human addicts and findings from animal studies is paramount. There are, however, some symptoms that are specific to humans that would be difficult to model in a rodent study (e.g. substance use interferes with major obligations). One strategy to simplify the complex nature of these symptoms for rodent models is to categorize the symptoms into disorders of consumption (examples: substance is taken in larger quantities or for longer durations than intended; tolerance to the effects of the drug such that more is required to achieve the same effects) and disorders of appetitive behavior (examples: important activities are reduced because of substance use; large amount of time, energy, and effort is devoted to procuring using or recovering from the substance). In this manner, rodent procedures that produce changes in drug seeking (appetitive) or drug taking (consumptive) behavior may be more readily translated to the human disease.

Distinguishing between appetitive and consumptive behaviors is a simple task in standard operant procedures. A typical operant experiment involves an instrumental-consummatory response chaining. For example, in a food experiment the subject would perform an operant response (e.g. lever press or
nose poke) to fulfill the schedule requirements and then receive a physical
reward (e.g. food pellet), which would be consumed via a separate set of
behaviors (e.g. chewing or swallowing). The appetitive behavior is directed
toward instrumental responding whereas the consumptive behavior is directed
toward consuming the food. In this way, designing experiments that focus
exclusively on consumption of a particular reward involves simply providing the
reward without limits and measuring intake. Indeed, food consumption studies
have followed this design (e.g. Hanlon et al. 2004). Similarly, designing
experiments to assess appetitive, reward seeking behavior can involve
manipulation of the operant schedule requirements. The clear separation of
appetitive- and consumptive-oriented behaviors makes distinguishing between
these two classes of behavior simple and direct in most cases.

However, distinguishing between drug seeking and drug taking behavior in IV
self-administration experiments is difficult because it represents a special case of
operant procedures in which consumptive and appetitive behaviors are conflated
(Roberts et al. 2013). This conflation occurs because both the consumptive and
appetitive responses are directed towards the same operant. That is, the
appetitive operant response to fulfill the schedule requirements (e.g. lever
pressing) is the same as the consumptive behavior (e.g. pressing the same lever
to activate the syringe pump) to consume the reward. This simple difference
between IV drug and other operant experiments - that consuming the reward
involves an operant response - completely removes the distinction between drug
seeking- and taking-oriented behaviors. Therefore drug self-administration is one
of the few, if not only, cases in which operant responding can be a measure of reward-consumption behavior or reward-seeking behavior depending on the experimental setup.

Several procedures, schedules of reinforcement and methods of analysis can help to separate these conflated aspects of self-administration behavior. The extent various procedures reflect consumptive vs appetitive responding was the subject of detailed discussion in our previous review (Roberts et al. 2013). Briefly, to measure appetitive responding free of the drug taking regulation mechanisms, procedures such as extinction and reinstatement can be used, in which no drug is given (Shaham et al., 2003). Self-administration schedules of reinforcement with very high response requirements such as progressive ratio (PR) also appear to follow rules of appetitive responding (e.g. increasing dose response function with increasing doses –Richardson and Roberts, 1996; Roberts et al., 2013). To measure drug taking free from the influences of drug seeking mechanisms, schedules of reinforcement with very low response requirements are typically used (Roberts et al., 2013). For example, responding for psychostimulants under a fixed ratio one (FR1) schedule of reinforcement follows classical consumption rules such as decreased responding as doses are increased (Gerber and Wise, 1989; Pettit and Justice, 1991; Pickens and Thompson, 1968; Wilson et al., 1971). A recently developed procedure termed the within-session threshold (TH) schedule provides a method of measuring both appetitive and consumptive behaviors based on behavioral economic principles (Bentzley et al. 2013; Oleson and Roberts, 2009). This procedure involves
responding under FR1 conditions, and every 10 minutes the dose of drug is reduced in an exponential fashion. Responding early in the session - when the dose is large - is primarily governed by consumption regulation mechanisms whereas late in the session - when the doses are miniscule - responding follows drug seeking rules (Bentzley et al., 2013; Oleson and Roberts, 2009). Therefore, both consumptive and appetitive responding can be assessed within the same session using behavioral economic principles.

It is important to separate between drug seeking and drug taking behaviors in self-administration experiments because they are fundamentally distinct behaviors with separate underlying neurobiological mechanisms. There is ample evidence that consumptive and appetitive responding reflects separate classes of behavior and are regulated by distinct neurobiological mechanisms (Roberts et al. 2013). This evidence can be condensed into the finding that manipulations that affect one of these classes of behavior does not necessarily affect the other. For example, experiments have shown that administration of glutamate antagonists or GABA agonists into the shell of the nucleus accumbens dramatically increased feeding when no operant requirements were imposed to gain access to the food (Hanlon et al. 2004; Zhang et al. 2003). However, the same pharmacological manipulation had no effects on operant responding for food. Similarly, depleting dopamine in the nucleus accumbens has been shown to decrease responding under a PR schedule of reinforcement, but did not change intake under free-feeding conditions (Aberman and Salamone 1999).

With regard to IV self-administration experiments, various treatments have been
shown to produce disparate consequences on drug seeking vs drug taking responding. For example, a number of experimental manipulations such as lesions (Hubner and Koob, 1990; Koob et al. 1987; Roberts et al. 1977) or fluoxetine treatment (Carroll et al. 1990; Richardson et al. 1991) can make both appetitive and consumptive responding change in the same direction. Other manipulations can produce changes in one class of behavior but not the other, such as intra-striatal injections of a D1 receptor antagonist (McGregor and Roberts 1995), the female estrous cycle (Roberts et al. 1989), or orexin antagonist treatment (Aston-Jones et al. 2009; Borgland et al. 2009; Espana et al. 2010; Smith et al. 2009), while other treatments can produce opposite effects in consumptive vs appetitive responding such as haloperidol, clozapine or flupenthixol treatment (Loh et al. 1992; Richardson et al. 1994; Roberts et al. 1989; Roberts and Vickers 1984). Taken together, these findings provide strong evidence that consumptive and appetitive behaviors are not correlated and have distinct underlying neurobiological mechanisms.

This distinction between consumptive and appetitive responding represents a unique way to examine self-administration results. In our previous review, the validity of separating drug seeking and drug taking responses into distinct categories was argued (Roberts et al., 2013). In this review we will discuss many rodent procedures that have been developed in the drug addiction field with an emphasis on what effects the procedures have on subsequent drug seeking and drug taking.


2. Principles of drug taking

Results from operant experiments are normally interpreted as appetitive, seeking behavior. As discussed above, in most operant paradigms the operant instrumental response is followed by a distinct reward consumption phase. Thus, the operant response itself reflects seeking of the reward and is uncontaminated by behaviors directed at interacting with and consuming the reward. Therefore, the tendency with drug self-administration experiments is to interpret the operant response as drug seeking. However, as discussed previously (Roberts et al. 2013) the influences of simple schedules of reinforcement with very low response costs are primarily controlled by mechanisms of drug consumption. This section will examine the current understanding of the regulation of psychostimulant consumption that has been revealed by the extensive literature using FR1 and other simple schedules of reinforcement.

Many studies examining psychostimulant intake have led to the development of a sophisticated model of drug taking that can make predictions about why certain patterns of consumption occur. Psychostimulant intake is self-administered in a highly regular pattern, and the rate of infusions is inversely related to the size of the dose (Gerber and Wise, 1989; Pettit and Justice, 1991; Pickens and Thompson, 1968; Wilson et al., 1971). Additionally, total intake across sessions are typically shown to be relatively stable regardless of large changes in the size of the unit dose available, and several studies have demonstrated that levels of psychostimulants such as amphetamine and cocaine as well as dopamine levels in the NAc are maintained at a relatively constant level throughout the duration of
the self-administration session (Ranaldi et al., 1999; Tsibulsky and Norman, 1999; Wise et al., 1995; Yokel and Pickens, 1974). It appears that subjects maintain these constant levels by combining multiple infusions within a short “loading” period at the beginning of the session to quickly elevate drug levels, and then regularly spacing infusions to maintain the elevated levels in the “maintenance” phase which lasts for the remainder of the session (Ettenberg et al., 1982; Pickens and Thompson, 1968; Wilson et al., 1971; Wise et al., 1995).

Three hypotheses have been offered to account for the regularity of cocaine intake. (1) High levels of cocaine produce inhibiting effects on responding such that subjects are unable to respond until cocaine has cleared by a sufficient amount (Pickens and Thompson, 1968). 2) Cocaine levels are actively titrated below an aversive threshold or (3) above a satiety threshold (Pickens and Thompson, 1968; Tsibulsky and Norman, 1999; Wilson et al., 1971; Wise et al., 1995). The first hypothesis was largely ruled out by a study in which concurrent access was provided for intracranial self-stimulation (ICSS) and amphetamine self-administration (Wise et al., 1977). Results showed that amphetamine self-administration produced an augmentation of ICSS responding during the amphetamine post-infusion pauses, suggesting rats are fully capable of responding during this period. Because of the similarities between hypotheses 2 and 3 (an aversive ceiling effect or a satiety floor effect), parsing differences between these mechanisms is difficult. Psychostimulants such as cocaine have demonstrable anxiogenic properties (Booth et al., 1977; DeVries and Pert, 1998; Ettenberg and Geist, 1991, 1993; Goudie et al., 1978). However, the finding that
animals consistently choose large doses of psychostimulants over smaller ones in choice procedures (Johanson and Schuster, 1975; Llewellyn et al., 1976; Lynch et al., 1998; Ward et al., 2005) suggests a satiety threshold is more likely (Ranaldi et al., 1999; Wise et al., 1995). That is, if titration was driven by avoidance of an upper aversive threshold it would be expected that more frequent small doses should be preferred (Ranaldi et al., 1999).

A novel self-administration procedure has provided unique insight into regulation of intake. The hold-down procedure was developed to address a large discrepancy between the dosing strategies between human addicts and research animals. Human drug users have precise control over their drugs when they consume them. That is, the size of the puff, gulp, line or injection are all under the discretion of the user (Ashton et al., 1979; Bernosky-Smith et al., 2012; DeGrandpre et al., 1992; McMorrow and Foxx, 1983; Schaefer et al., 1971; Sobell et al., 1972). In contrast, in research settings the dose size preselected by the experimenter is automatically administered upon completion of the response requirement. However, under the hold-down contingency the duration of time the lever is depressed directly determines the volume of drug that is infused, making the dose size a dependent variable under the control of the subject. This is accomplished by simply activating the syringe pump when the lever is pressed and inactivating it when the lever is released. It was demonstrated that rats were capable of self-administering cocaine under this contingency, and total intake was similar to intake under FR1 conditions (Morgan et al., 2009). The majority of selected cocaine dose sizes under these conditions fell well within the expected
range (Zimmer et al., 2011, 2013). Surprisingly, most hold-down sessions were also associated with a few unprecedentedly large doses of cocaine typically at the beginning of the session in the range of roughly 4 mg/kg self-administered within 20 seconds (Zimmer et al., 2011). These findings suggested that the dose rats preferred changes within a single session with very large "loading" doses being selected at the beginning of the session and smaller “maintenance” doses during the remainder of the session.

The question remained what factors were involved in regulating the selection of these loading and maintenance doses. To examine the loading doses, a series of forced timeout periods (range of 10-25 minutes) were introduced throughout normal 6 hour hold-down sessions (Zimmer et al., 2011). These timeout periods caused estimated brain-cocaine concentrations to decline by a predictable amount, and once access to cocaine was reinitiated rats could select a dose of any size to compensate for the decline. In effect, this protocol produced a session with multiple loading phases. Results showed that forced timeout periods produced much larger doses to be selected, and the size of each dose was significantly negatively correlated with the extent of the decline of brain-cocaine levels (Zimmer et al., 2011). To examine the regulation of maintenance doses, a detailed analysis of selected dose size and inter-dose intervals was performed during hold-down sessions with a variety of cocaine concentrations (range of 1.25 – 10.0 mg/ml). The total time the syringe pump was activated declined in near proportional manner with the change in concentration such that the total amount of cocaine was relatively constant regardless of concentration (Zimmer et
Interestingly, the size of each self-administered dose was highly correlated with the subsequent, but not previous, inter-dose interval. That is, the size of the selected dose determined how long the animal waited before responding for another one.

Taken together, studies using simple schedules have provided a clear understanding of the mechanisms that regulate intake of psychostimulants. The size of the selected loading dose depends on the length of time since the previous infusion and the size of the selected maintenance dose determines the length of the subsequent post-infusion pause. At first glance these two principles can appear contradictory. However, these results can be explained by the hypothesis that rats titrate cocaine levels above a satiety threshold. When brain-cocaine concentrations fall below the satiety threshold a loading dose is selected in order to elevate levels above the threshold, the size of which is determined by how far below the threshold they fell. When brain levels are being maintained above the threshold however, the size of each maintenance dose determines the length of time before brain levels will once again fall to the satiety threshold, and therefore the post-infusion pause is dependent on the size of each maintenance dose.

3. Binge models of self-administration

One of the unique characteristics of psychostimulant addiction is the emergence of binge-abstinence patterns of intake (Gawin, 1991). This pattern consists of high intake episodes that can last from several hours to many days (Gawin,
1989, 1991; Gawin and Ellinwood, 1989). Rats and non-human primates given unlimited access to cocaine for 24 hr/day develop a similar binge-abstinence pattern (Balster and Schuster, 1973; Bozarth and Wise, 1985; Johanson et al., 1976). This similarity has made studying binge self-administration of psychostimulants a common procedure. However, it has been shown that high-intake episodes can ultimately lead to deteriorating health and eventually death (Aigner and Balster, 1978; Bozarth and Wise, 1985; Johanson et al., 1976). Therefore, to avoid toxicity many procedures have modeled binge-like intake of cocaine by placing constraints such as limits on dose (Carroll and Lac, 1987; Carroll et al., 1989), number of reinforcers per hour (Fitch and Roberts, 1993; Roberts et al., 2002), and session length (Ahmed and Koob, 1998, 1999). Other studies have provided cocaine access for long durations with no limits on the number of injections, but have given multiple days off in between each binge episode (Covington and Miczek, 2001, 2005; Quadros and Miczek, 2009; Zimmer and Roberts, 2013).

3A. Long-duration (12-48 hours) binge sessions

Most long-duration binge access protocols involve either providing access to a single very long binge session (12-48 hours) or multiple binge sessions separated by recovery periods to prevent toxicity, and these studies have provided information on both the consumptive (what regulates or terminates a binge) and appetitive (what are the consequences of binge intake on subsequent drug craving/seeking?) aspects.
In regard to the consequences of long-duration binges on consumptive behavior, studies examining rodent psychostimulant long-duration binge self-administration have described a number of factors that increase the probability or intensity of a cocaine binge. Procedures that produce sensitization to the locomotor-activating effects of cocaine, have been shown to significantly increase responses within a 24 hr binge (Covington and Miczek, 2001). Stress following social confrontation and defeat has also been shown to increase responding and intake within a binge (Covington et al., 2005; Covington and Miczek, 2001, 2005; Cruz et al., 2011; Quadros and Miczek, 2009), and these effects lasted at least 2 months after the stressful incident indicating that stress can increase long-term vulnerability to cocaine binge self-administration (Covington et al., 2005).

The consequences of long-duration binge self-administration of cocaine and withdrawal following these sessions has been the focus of many animal studies. One study found decreased metabolism in the striatum, olfactory tubercle, and somatosensory and motor cortices 6 or 72 hours after a 12 hr cocaine binge (Hammer et al., 1993). Interestingly, a significant negative correlation was found between cocaine intake within the binge and the extent of the metabolism decrease. Withdrawal following a 12 hour binge has also been associated with marked decreases in extracellular levels of serotonin and dopamine in the NAc (Parsons et al., 1995). Neuronal activity (as measured by zif268 mRNA) was shown to be significantly decreased in the hippocampus and VTA following a 16 hr binge and in the dorsal and ventral striatum, hippocampus and basolateral amygdala 24 hours following the long-duration binge relative to yoked controls.
(Mutschler et al., 2000). Long-duration cocaine binge self-administration is also associated with disruption of homeostatic mechanisms and behavioral patterns including regulation of cocaine self-administration itself (Fowler et al., 2007; Tornatzky and Miczek, 2000). It is also thought that motivational processes related to cocaine seeking also become disrupted following high-intake binges, as PR breakpoints were significantly decreased in a binge length dependent fashion (Zimmer and Roberts, 2013). This reduction in drug seeking may be one factor that leads to the ultimate termination of a binge.

Studies suggest that cocaine binge self-administration also produces increases in stress measures. Both 12 and 48 hour cocaine binges were associated with increases in startle-induced ultrasonic vocalizations (commonly considered a measure of stress in rodents) as well as sensitization of the startle reflex (Mutschler et al., 2001; Mutschler and Miczek, 1998b). In addition, withdrawal following cocaine binges also produced robust increases in ultrasonic vocalizations (Mutschler and Miczek, 1998a). Taken together, these studies suggest that stress increases the likelihood and severity of cocaine binges, and the high-intake binges significantly increase stress. Thus, it is suggested that a cycle emerges that increases cocaine consumption despite severe negative health symptoms, and ultimately this cycle leads to the death of the subject (Aigner and Balster, 1978; Bozarth and Wise, 1985; Johanson et al., 1976).

3B. Discrete trials
Another method that has been used to analyze binge intake of psychostimulants is to impose a limit on the number of infusions available per hour. In these studies access is typically provided 24 hr/day, and the dose and number of injections allowed are manipulated depending on the research question being asked.

Manipulating the number of infusions available can dramatically alter consumptive behavior. Under conditions of 1 or 2 injections per hour, it has been shown that rats self-administer in a highly circadian pattern with a high probability of self-administering cocaine only during the dark/active cycle, whereas when the number of available infusions was increased, subjects began self-administering well into the light cycle as well (Fitch and Roberts, 1993; Roberts et al., 2002). Likewise, smaller doses of cocaine increased the probability of a circadian pattern emerging, whereas larger cocaine doses typically led to a disruption of these circadian influences (Fitch and Roberts, 1993; Roberts et al., 2002). Under circumstances of large doses or higher frequency, rats typically responded for every opportunity for more than 48 hrs (Roberts et al., 2002).

Self-administration under discrete trial binges also produces profound increases in subsequent appetitive responding. Exposure to cocaine under these binge-inducing circumstances coupled with a withdrawal period was shown to significantly increase subsequent drug-seeking as measured by PR breakpoints (Morgan et al., 2002; Morgan et al., 2005b).

3C. Long access self-administration (6 hr)
One popular model, originally described by Ahmed and Koob (Ahmed and Koob, 1998, 1999), involves increasing access to cocaine from the normal training session length (usually 1-2 hours) to a longer period (LgA; usually 6 hours), which essentially is providing access to repeated short-duration binge sessions. This paradigm results in a robust escalation of cocaine intake over the course of roughly two weeks relative to rats that continue self-administering in the shorter training-length sessions (ShA – short access). In addition to cocaine, this escalation of intake following LgA self-administration has been replicated in studies examining other psychostimulants such as amphetamine (Gipson and Bardo, 2009), methamphetamine (Anker et al., 2012; Kitamura et al., 2006; Kufahl et al., 2012; Kufahl et al., 2013; Orio et al., 2010; Parsegian et al., 2011; Reichel et al., 2012; Reichel et al., 2011; Rocha and Kalivas, 2010; Rogers et al., 2008; Schwendt et al., 2012; Schwendt et al., 2009; Watterson et al., 2012; Wee et al., 2007; Yuan et al., 2011), MDPV (Watterson et al., 2012), and methylphenidate (Marusich et al., 2010). This phenomenon is not unique to psychostimulants as robust escalation has also been shown with heroin (Ahmed et al., 2000; Dalley et al., 2005; Edwards et al., 2012; Greenwell et al., 2009a; Greenwell et al., 2009b; Lenoir and Ahmed, 2007, 2008; Walker et al., 2003). In addition a non-human primate phencyclidine self-administration study showed moderate escalation (Carroll et al., 2005), but LgA nicotine self-administration did not lead to escalation of intake (Paterson and Markou, 2004).
It is well established that the LgA procedure produces escalation of intake, a change in consumptive behavior. Those data alone do not provide insight into the effects of the procedure on subsequent appetitive processes. However, a number of studies have run experiments examining the effects of a LgA history on measures of subsequent drug seeking. Most PR studies have demonstrated increases in breakpoint (Hao et al., 2010; Orio et al., 2009; Paterson and Markou, 2003; Wee et al., 2008; Wee et al., 2009), although three have shown no changes (Liu et al., 2005a; Morgan et al., 2005a; Quadros and Miczek, 2009). Extended access has also been shown to result in increases in reinstatement responding in response to cues (Kippin et al., 2006), stress (Mantsch et al., 2008a), and cocaine priming (Ahmed and Cador, 2006; Kippin et al., 2006; Knackstedt and Kalivas, 2007; Mantsch et al., 2008a; Mantsch et al., 2008b) but one study did not see increased reinstatement responding (Morgan et al., 2005a). Two studies have applied a behavioral economic analysis to LgA cocaine self-administration. These studies tested rats with a history of either LgA or ShA self-administration, and interestingly found that LgA rats had a decreased Pmax (maximal price paid for cocaine) when tested on a between-session threshold procedure (Oleson and Roberts, 2009) but an increased Pmax when tested on a within-session threshold procedure (Zimmer et al., 2012). The primary difference between within- vs between-session threshold procedures is that in a within-session procedure Pmax is assessed at the end of the procedure whereas between-session procedures assess Pmax at the beginning of the session. Therefore, this disparity in results could indicate that LgA rats show
increased motivation to continue a binge (i.e. at the end of the session they will work hard to prolong it) but decreased motivation to begin one. Taken together, these many studies make a compelling argument that LgA self-administration of cocaine results in increased drug-taking and drug-seeking relative to rats that remain on ShA.

Extensive analysis of the neuronal changes in response to LgA self-administration of cocaine have been examined. The following paragraphs detail these neurobiological changes associated with LgA self-administration of cocaine.

**Anatomy:** Several studies have demonstrated adaptations in functional anatomy in response to LgA cocaine self-administration. For example, 30 days of abstinence after LgA self-administration of either cocaine or sucrose resulted in blunted fMRI activation in response to a cocaine challenge in the medial prefrontal cortex, anterior cingulate cortex, medial dorsal thalamic nuclei, and hippocampus relative to naïve controls (Lu et al., 2012). While both sucrose and cocaine LgA self-administration both showed blunted activation in these regions, the magnitude of the response was highly correlated with the total amount of cocaine previously self-administered but no such relationship was seen in the sucrose group (Lu et al., 2012). Immunohistochemical staining for cFos (a marker for neuronal activation) showed much greater expression in the lateral habenula, PFC and NAc core in ShA rats vs naïve controls (Ben-Shahar et al., 2004). However, this increased expression was absent in LgA animals, suggesting tolerance to the stimulating effects of cocaine following a history of...
LgA self-administration (Ben-Shahar et al., 2004). LgA cocaine self-administration has also been shown to be associated with greater spine density in the NAc core in LgA rats relative to ShA (Ferrario et al., 2005).

**Glutamate system:** Functional activation of mGluR2/3 receptors in the NAc was found to be significantly elevated following LgA cocaine exposure, and the mGluR2/3 agonist LY379268 dose-dependently decreased breakpoints in LgA rats but only the highest dose tested reduced ShA breakpoints (Hao et al., 2010). In contrast, mGluR5 expression and functional activation was significantly decreased in LgA rats in the NAc, and the mGluR5 antagonist MTEP reduced breakpoints in ShA but not LgA rats (Hao et al., 2010).

**Cannabinoid system:** Microdialysis showed that anandamide (an endogenous cannabinoid) levels were significantly reduced in the NAc shell in ShA but not LgA rats, and CB1 receptor expression was upregulated in LgA vs ShA in both NAc and amygdala (Orio et al., 2009). Administration of the CB1 receptor antagonist rimonabant significantly decreased LgA breakpoints but only the highest dose tested affected ShA breakpoints (Orio et al., 2009).

**Dopamine system:** Changes in the dopamine system between ShA and LgA have been documented as well. D2 receptor mRNA and protein expression was significantly decreased in LgA rats in the medial PFC (Briand et al., 2008). Interestingly, a microdialysis study showed that self-administered cocaine produced higher levels of cocaine and dopamine in rats with a history of LgA relative to ShA, but no differences between groups were observed following passively administered cocaine (Ahmed et al., 2003). LgA cocaine self-
administration has also been shown to produce tolerance to the actions of cocaine at the dopamine transporter relative to ShA or naïve controls (Calipari et al., 2013).

**Hypothalamic-pituitary-adrenal (HPA) axis:** Many changes in the HPA axis system have also been demonstrated. LgA cocaine exposure produced a reduction in baseline plasma corticosterone (CORT) levels relative to saline controls, and administration of dexamethasone (an anti-inflammatory steroid) reduced CORT levels to a greater extent in the saline animals vs cocaine (Mantsch et al., 2007). The same study also showed reduced expression of glucocorticoid receptors in the dorsomedial hypothalamus (Mantsch et al., 2007). In addition, LgA rats showed higher levels of corticotropin releasing factor in the basolateral amygdala (at one time point only) and dorsal raphe (Zorrilla et al., 2012).

In conclusion, it has been well demonstrated that the LgA procedure produces robust changes in consumption levels, although the mechanism driving these changes is the subject of debate (for example see Beckmann et al., 2012). It is also established that appetitive responding as measured by PR breakpoints, reinstatement responding and behavioral economics is increased following LgA self-administration as well. Additionally, many neurobiological adaptations produced by LgA self-administration have been discovered, and it has been well demonstrated that this protocol produces wide ranging effects on many neurotransmitter systems and in many brain regions (see paragraph above). The question remains which neuroadaptations underlie which aspects of behavioral
change. That is, moving forward, it is difficult to predict whether treatments aimed at reversing one particular LgA-induced neurobiological change will produce changes in consumptive or appetitive behaviors. This has implications for the clinical validation of the model, and future preclinical studies examining the underlying neurobiological consequences of LgA self-administration in relation to the relevant behavioral changes will help elucidate the relevance of those consequences.

4. Escalation of drug seeking

This interesting procedure used a schedule of reinforcement designed to assess appetitive responding (PR), and found that a behavioral history of responding under this schedule produced an escalation in breakpoints over time. In this procedure, immediately after acquisition of cocaine self-administration rats self-administered cocaine under a PR schedule of reinforcement for 2 weeks. A sensitized response (i.e. increased breakpoints over time) was shown in these relatively naïve animals, and the increases in breakpoint were robust (Morgan et al., 2006). Interestingly, in rats with more extensive training (i.e. more training days or more injections per day) PR sensitization did not occur and instead their subsequent breakpoints remained much lower than those with minimal cocaine training (Morgan et al., 2006). Similarly, PR sensitization was shown to be dependent on the dose of cocaine with lower doses of cocaine during PR responding yielding greater sensitization of breakpoints, and speed of injection with faster infusions producing more robust sensitization (Liu et al., 2005b). This
sensitization was shown to affect the entire PR dose-response curve (Morgan et al., 2006). These data speak to the importance of initial drug-taking experiences on subsequent susceptibility to drug abuse (Roberts et al., 2007).

5. Intermittent access self-administration

Above we have largely discussed patterns of consumption that are modeled after binges and their consequences on appetitive and consumptive behavior. In this section, we will examine a unique pattern of consumption, intermittent access, that has produced profoundly different appetitive and neurobiological consequences.

As discussed above, the development of binge-abstinence patterns of psychostimulant use is considered a hallmark of the addiction process (Gawin, 1991), and it is thought that each binge experience progresses the addiction process further (Koob and Le Moal, 2001). One remaining question to this theory is what process leads subjects to begin taking psychostimulants in binges in the first place? The intermittent access (IntA) procedure has attempted to model these early sporadic exposures to cocaine, and has demonstrated that IntA can produce robust increases in motivational responding for cocaine despite relatively low intake (Zimmer et al., 2012).

A recent study applied a behavioral economic analysis on responding for cocaine following a variety of behavioral histories. Rats self-administered cocaine under classical ShA or LgA conditions, and a separate group self-administered cocaine
under IntA conditions which consisted of 5 minute access periods, during which no limits were placed on the number of earnable infusions, separated by 25 minute timeout periods (i.e. 2 access periods per hour) over the course of a six hour session. Under this contingency, rats had access to cocaine for 1 hr each day, but access was separated into 12 5-min bins (Zimmer et al., 2011; Zimmer et al., 2012). Therefore subjects self-administering under this contingency were exposed to repeated spiking cocaine levels as opposed to the sustained high levels resulting from most other self-administration procedures (Zimmer et al., 2012). After 2 weeks of exposure to cocaine under ShA, LgA or IntA conditions, all subjects self-administered cocaine on a within-session threshold procedure (Bentzley et al., 2013; Oleson et al., 2011) and a behavioral economic analysis was performed to assess changes in the maximal price (Pmax) rats would pay for cocaine. As expected exposure to LgA conditions produced a significant increase in Pmax values relative to ShA. However, the IntA exposure produced a robust increase in Pmax which was significantly higher than both ShA and LgA groups (Zimmer et al., 2012). These results are of special interest given that total cocaine intake in the IntA subjects was not statistically different than ShA subjects. These data suggest that exposure to intermittent spiking cocaine levels produces a robust increase in drug seeking.

This model of drug addiction could represent an important tool for understanding adaptations that progress subjects from initial, recreational use of psychostimulants to high-intake binges. For example, a recent study examined dopamine release and uptake and the potency of cocaine dopamine transporter
(DAT) inhibition in the NAc using in-vivo voltammetry in tissue from subjects with a history of ShA, LgA or IntA cocaine self-administration. Results showed that release and uptake of dopamine were unchanged following ShA and LgA histories, however IntA exposure produced increases in both release and uptake of dopamine in the NAc (Calipari et al., 2013). These histories produced profound differences in cocaine potency as well. LgA exposure to cocaine resulted in tolerance to cocaine’s DAT-inhibiting effects, whereas IntA exposure produced sensitization of cocaine’s effects at the DAT. Thus, IntA produced a sensitized response to cocaine whereas LgA produced tolerance to the same effects (Calipari et al., 2013).

6. Concluding remarks

Drug addiction involves the emergence of unique patterns of drug taking and drug seeking. The purpose of this review was to organize results from a variety of self-administration procedures in terms of their consequences on subsequent appetitive and consumptive behavior. Examining the self-administration literature through this unique lens is one method of applying translational relevance to the myriad self-administration results.


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CHAPTER TWO

The selected self-administered dose varies as a function of brain concentration

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Introduction

Cocaine self-administration procedures typically involve the infusion of a single unit dose of drug contingent upon the performance of an operant response (most commonly a lever press). These procedures have shown that the reinforcing effects of cocaine can be described by an ascending dose-response curve. The peak of the progressive ratio dose-response curve is typically in the range of 1.5 mg/kg (for review, see Arnold and Roberts, 1997; Stafford et al, 1998), and studies offering a choice between different doses of cocaine (Johanson and Schuster, 1975; Llewellyn et al, 1976; Lynch et al, 1998; Ward et al, 2005) have shown that larger doses on the ascending limb are generally preferred. Given that unit doses up to 1.5 mg/kg are preferred, the results from an experiment using a behaviorally dependent dosing (BDD) schedule were unexpected (Morgan et al, 2009). Under the BDD schedule, the syringe pump was activated for the length of time that a lever was held down. The procedure was devised in order to provide access to a range of doses rather than a fixed unit dose selected by the experimenter. Morgan et al. (2009) showed that rats quickly learned to self-administer cocaine on the BDD schedule. Total intake during a 3-h BDD session was found to be almost identical to drug intake during a standard 3 h fixed ratio (FR1) session. When the concentration of cocaine was manipulated over a 16-fold range, rats adjusted their responding such that the total intake was consistent across concentrations. Although the findings seemed to indicate that the rats could effectively regulate their cocaine intake, the size of the self-administered doses was surprisingly low. Analysis of the lever responses
revealed that rats generally held the lever down for very brief periods (~0.25 s) corresponding to very small doses of cocaine. These data show that rats appear to tightly regulate their cocaine intake on a BDD procedure; however, the doses self-administered were a full order of magnitude smaller than those predicted to be the most preferred from PR and choice studies (cited above). The goal of the present study was to understand why doses self-administered using the BDD schedule are far smaller than those thought to be the most reinforcing. In the first experiment, animals were given access to cocaine on the BDD schedule and two new methods of analysis were used to better understand the behavior. First, having observed that BDD self-administration occurs in bursts of responses followed by long post-infusion pauses, we examined whether accounting for these clusters might more accurately reflect the size of the doses self-administered. The majority of doses were found to be within a range commonly used in self-administration studies (~0.75 mg/kg; Caine and Koob, 1994; Ito et al, 2002; Liu et al, 2005; Pettit and Justice, 1991; Quadros and Miczek, 2009). Unexpectedly, however, a number of relatively large doses of cocaine (~4 mg/kg) were also found. Second, we applied a mathematical model to calculate brain-cocaine concentrations (as described by Pan et al, 1991) and examined whether the size of the self-administered dose was correlated with current drugs levels. Very large doses were found to be self-administered at the beginning of the session when brain levels were low; however, later in the session much smaller doses were self-administered. In the second experiment, we introduced a range of timeout periods (10–25 min) in order to produce variability in brain-cocaine
concentrations. The dose of cocaine was found to correlate with current brain-cocaine levels. One possible explanation of this relationship is that high levels of cocaine reduce the ability of the animal to hold the lever down. This hypothesis was tested in the third experiment in which animals self-administered on a BDD schedule with interspersed 5 min probe trials during which the lever was still present but responding did not activate the pump. Hold-down responding was shown to be increased during the probe trials, demonstrating that animals are fully capable of making the hold-down response. The data show that the dosage of cocaine self-administered changes within the session and is predicted by brain drug levels. These data have implications for the interpretation of results from more traditional self-administration procedures using experimenter-selected unit doses.
Methods

Animals, Surgery, and Housing

All experiments were conducted using male, Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing B350 g at the beginning of the experiment. Animals were given a week to acclimate to the laboratory environment. Animals were then anesthetized using a combination of ketamine (100 mg/kg; i.p.) and xylazine (8 mg/kg; i.p.), and a chronic, indwelling Silastic cannula was implanted into the right jugular vein that exited through the back of the animal in the region of the scapulae (Roberts and Goeders 1989). Ketoprofen (5 mg/kg; i.p.) was used as a postoperative analgesic, and animals were allowed to recover from surgery for a minimum of 3 days. Following surgery, animals were housed in 30_30_30 cm operant chambers under a reverse 12-h light/dark cycle with ad libitum access to food and water. All procedures were conducted in concordance with the Wake Forest University Animal Care and Use Committee guidelines.

Self-Administration Training

The beginning of the self-administration session was indicated by the extension of the lever into the self-administration chamber, which began 7 h into the dark cycle. A single response on the lever resulted in either a 0.75 or 1.5 mg/kg infusion (see below) of cocaine over 4 s period, followed by a 20 s timeout period during which a light cue above the lever was illuminated. The sessions occurred 7 days/week, and lasted 6 h. Two training procedures were used. Groups received access on an FR1 schedule to either (A) 0.75 mg/kg infusions until the maximum number of infusions (20) per session was self-administered for
2 consecutive days or (B) 1.5 mg/kg infusions until the maximum injections (40) per session was self-administered for 5 consecutive days. No differences between the two training protocols (A and B) were observed, and their data were combined. After completion of the FR1 training criteria, all rats were switched to a BDD schedule (see below for description of the BDD procedure) until responding was stable across three sessions. BDD Schedule of Reinforcement Using a Hold-Down Response The BDD schedule used the hold down as the operant response, in which depression of the lever resulted in the infusion pump turning on until the lever was released. In this way the volume of the injection was dependent on the length of time the animal held the lever down. The concentration of cocaine in the syringe and the pump speed were held constant at 5.0 mg/ml and 1.6 ml/min, respectively. The LED stimulus light above the lever was illuminated whenever the lever was depressed. Sessions lasted 3 h.

**BDD with Intermittent Timeout Periods**

Timeout periods of various lengths were introduced in order to force brain-cocaine levels to decline within a session. Under this condition, drug access was given for a 5-min period on the BDD schedule (as described above). Following the 5 min access period, the lever was retracted and a forced timeout period began that lasted 10, 15, 20, or 25 min depending on the experimental condition. All BDD timeout sessions lasted 6 h.

**BDD with Probe Trials**
For this experiment, six rats were given access to cocaine using a BDD schedule during daily 3 h sessions. Six equally spaced probe trials were inserted into each session. Every 25 min, a 5-min probe trial was introduced. During the probe trials, the lever remained extended and responding on it illuminated the stimulus light but did not activate the syringe pump. Three of the animals had extensive experience on the BDD schedule. To ensure that the results were not because of the behavioral history of these animals, an additional three animals were added to the experiment, having been trained to self-administer using training procedure A (see self-administration training above).

**Modeling Brain-Cocaine Levels**

Whole-brain levels of cocaine were mathematically modeled using a two-compartment model for rats receiving a chronic i.v. cocaine regimen derived by Pan et al. (1991). Briefly, we used the equation

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

where \(c\) is the concentration in the brain, \(d\) is the dose, \(k\) is the rate constant for transfer from the blood to the brain (0.233), \(v\) is volume of the brain (0.044), \(\alpha\) and \(\beta\) (0.642 and 0.097, respectively) are constants representing the flow of cocaine between the blood and brain compartments and the elimination of cocaine from the body, and \(t\) is the time in min since the last infusion. All constants were based on the chronic i.v. administration values (Pan et al, 1991). Each infusion of cocaine was calculated independently and then summed in 5 s intervals to obtain the total concentration of cocaine (Stuber et al, 2005b).
**Data Collection and Storage**

Two IBM-compatible computers were used to control 16 self-administration chambers. Programs for controlling the equipment, storing the data, and analyzing the results were written in Borland Developer Suite (Embarcadero Technologies). Every event related to the physical equipment and the experimental session (lever up, lever down, pump on/off, session start time, etc.) was entered into a data stream with a time stamp in ms. Responses on the BDD schedule were analyzed into clusters; responses occurring within a specific time period of each other (measured from the end and beginning of two adjacent responses) were summed together.

**Drugs**

Cocaine hydrochloride (National Institute on Drug Abuse Research, Research Triangle Park, NC) was dissolved in physiological saline. All drug concentrations are represented as the weight of the salt.

**Statistics**

Dependent measures were the size of clusters, the peak brain levels of cocaine within a 5-min BDD access period, and the trough brain levels following each timeout period. Peak levels of cocaine were analyzed by repeated measures analysis of variance. Trough levels failed the equal variance test, and therefore the w2 test was performed in place of ANOVA. Our post hoc analysis was the Tukey test, and our probability level was 0.05. Correlation coefficients were
calculated using Microsoft Excel, and all other statistical analyses were performed using SigmaPlot version 11.
Results

A total of 17 rats satisfied our acquisition criterion. All but one of the 17 showed stable responding on the BDD schedule for 3 consecutive days. Four rats displayed a loss of patency before finishing the study and were therefore excluded from all analyses. The 12 remaining animals were used in the analyses of this study.

Figure 1a shows the cumulative duration of the syringe pump throughout a 3-h BDD session in a representative animal. The beginning of the session shows a steep incline, indicating the animal self-administered a large amount of cocaine in a small period of time, whereas the rest of the session is marked by a slower, stable rate of intake. Figure 1b illustrates the histogram of doses self-administered during the same BDD session. Pump intervals were grouped into bins of 0.1 mg/kg, and the peak of the histogram demonstrates that the majority of responses were for a dose of <0.1 mg/kg. Closer examination of the data revealed a tendency for responses to be clustered together. Figure 1c represents the raw event record of the data. The height of each line represents the duration of the pump for each event (scale=1.0 mg/kg/inf). We accounted for clustering by grouping responses that occurred within 20 s of each other. Figure 1d shows a histogram of the same data after accounting for the clusters of responses. A consistent outlier was observed (arrow), and this outlier always occurred at the beginning of the session when blood levels were low. This observation prompted us to model whole-brain levels of cocaine using a two-compartment model described by Pan et al. (1991). Figure 1e shows the calculated brain-cocaine
concentration over the 3 h BDD session. A sharp increase in brain levels is observed at the beginning of the session, and for the remainder of the session the animal maintained a consistent blood level of cocaine. Figure 1f represents the brain-cocaine concentration at the start of each cluster of responses the animal self-administered. Closed circles represent doses that were self-administered within the first 5 min of the session. Note the one outlier (arrow) occurs within the first 5 min of the session when brain levels are low. This led us to hypothesize that large doses are self-administered when brain levels are low, whereas small doses are taken when brain levels are high. However, to test this hypothesis it was necessary to manipulate brain-cocaine levels and measure the corresponding doses.
Figure 1

A

B

C

D

E

F

[Graphs and plots demonstrating different data distributions and trends]
Analyses of behavior on a BDD schedule of reinforcement. Data from a representative animal self-administering on a 3-h BDD schedule were graphed using multiple methods of analysis. (a) A cumulative record of the syringe pump throughout the session. (b) A histogram of the 218 responses on the lever. All responses were grouped into bins of 0.1 mg/kg infusions. (c) An event record in which each line shows 1 h of the session. Bars represent a response on the lever, and the height of the bar (scale equals a 1.0 mg/kg infusion) represents the size of the dose injected. (d) A histogram of responses identical to (b) except that clusters of responses were accounted for such that any responses occurring within 20 s of each other were counted as a single behavior. Note the change in scale between (b) and (d). (e) The calculated brain-cocaine concentration throughout the 3 h session. (f) A scatter plot of each cluster of responses. The size of the injection is plotted on the ordinate, and the calculated brain-cocaine concentration is plotted on the abscissa. Closed circles represent responses occurring within the first 5 min of the session, whereas open circles occurred during the remainder of the session. The arrows in (d and f) highlight an extremely large dose (4.2 mg/kg) that the animal self-administered. The rate of consumption during this session was 9.80 mg/kg/h.
In order to manipulate the brain levels of cocaine, we used a discrete trial procedure that consisted of 5 min periods of BDD access to cocaine followed by a timeout period of 10, 15, 20, or 25 min (5–10, 5–15, 5–20, and 5–25, respectively). Figure 2a shows the event record of a representative animal on a 6-h 5–25 BDD schedule (top line). Note the large clusters occurring each time access is available. A representative 5 min access period shows that the majority of responses occur in one large cluster at the very beginning of the access period (Figure 2a, middle line). Also shown is the 20 s period in which this large cluster occurred (bottom line). Figure 2b shows the cumulative pump time during the same session. Note that the animal consistently took nearly 10 s of pump time (roughly 4.0 mg/kg) during each 5 min access period. Figure 2c shows the calculated brain-cocaine levels during this session. Note that the rat rapidly increased its brain-cocaine levels during each 5 min access period, and during the 25 min timeout period, brain levels decrease to a very consistent level (~1.5 µM).
Figure 2
The effects of a timeout period on BDD self-administration behavior. Data from a representative animal during a 6 h BDD schedule consisting of alternating 5 min access periods to the BDD lever and 25 min timeout periods. (a) The event record. Bars indicates a response on the lever, and the height of the bar (scale equals a 1.0 mg/kg infusion) represents the size of the injected dose. The top line shows responses during the full 6 h session. The middle line illustrates the event record of a single 5 min BDD access period. The bottom line is an expansion of a 20-s period in which the animal self-administered a cluster of responses consisting of a mixture of relatively long and short HD responses. (b) The cumulative pump duration throughout the session. The gray shading indicates the 5 min access period. (c) The modeled brain-cocaine concentration throughout the session.
Figure 3a shows the calculated cocaine levels in a representative animal following a 10 min (top left, green), 15 min (top right, blue), 20 min (bottom left, red), or 25 min (bottom right, black) timeout period. The dashed line on each graph shows the mean level that cocaine levels fell to following each timeout period. These data illustrate that the length of the timeout determined the extent of the decrease in calculated cocaine levels. Figure 3b shows the data from Figure 3a (color matched) as a correlation ($r = -0.77, \ P<0.001$) between the dose the animal self-administered and the brain-cocaine level just before that dose was taken. Closed circles represent the first dose in each 5 min period.

Correlation coefficients for all rats (n=12) and all conditions (n=4) revealed 33 out of 48 significant negative relationships (mean $r$ value = -0.55, range = -0.27 to -0.80). The 15 non-significant days were due to low responding (e.g., only self-administering during one of the available 5 min access period). Figure 4 shows the average highest and lowest points of modeled brain-cocaine concentrations for all rats during BDD sessions with varying timeouts. The length of the timeout had no significant effect on the peak cocaine concentration within each 5 min access period ($F(3, 33) = 2.149, \ p=0.113$); however, there was a significant effect on the degree to which cocaine levels decreased ($\chi^2 (3) = 24.500, \ p < 0.001$).

Post hoc analysis revealed that 5–10 trough levels were significantly higher than 5–25 or 5–20, and 5–15 levels were significantly higher than 5–25.
Figure 3

A

B

Brain-Cocaine Concentration (μM)

Time (min)

Figure 3

Brain-Cocaine Concentration (μM)

Time (min)

Dose Selected (mg/kg)

Brain-Cocaine Concentration (μM)
Figure 3

Current brain levels of cocaine predict subsequent dose. Data from a representative animal during four 6 h sessions consisting of alternating 5 min access periods to the BDD lever and timeout periods of varying length. (a) The calculated brain-cocaine concentration during a session with a 10 min (top left, green), 15 min (top right, blue), 20 min (bottom left, red), or 25 min (bottom right, black) timeout period. Dashed lines emphasize how far brain-cocaine levels declined following each timeout period. (b) The relationship between brain-cocaine concentration and subsequent doses animals self-administered. Data points are from the same sessions illustrated in (a). Colors represent a 10, 15, 20, or 25 min timeout period (green, blue, red, or black, respectively). Closed circles represent the first response of a 5-min BDD access period, and open circles represent a dose self-administered within the remainder of the period.
Figure 4

Animals maintain consistent brain-cocaine levels despite timeout length. Average peak and trough levels of brain-cocaine concentration for all animals during 6 h sessions of alternating access and timeout periods are shown. Data are sorted into sessions consisting of 10, 15, 20, or 25 min timeout periods. Closed circles represent the average (±SEM) peak level for all rats, and open circles represent the average trough levels following the timeout period. Data points significantly lower than the 5–10 or 5–15 trough levels are indicated by * and w symbols, respectively.
A third experiment was conducted to test the hypothesis that increasing cocaine levels decreased the ability of rats to respond on the lever. In this experiment, access to cocaine alternated 6 times during the 3 h session between a 25-min period of the BDD schedule and a 5-min probe trial during which responding on the lever did not activate the syringe pump. Responses on the lever were summed into 5 min bins. Figure 5a shows the total time the lever was depressed (seconds) within each 5 min bin averaged across animals. Results were converted to dose and are expressed on the right y axis as a reference. However, it should be noted that the syringe pump was not activated during probe trials. Responding during the 25 min BDD schedule periods (open circles) was consistent with our results above. However, during the 5 min probe trials (closed circles), responding on the lever increased significantly ($F(35, 126) = 1.662, p < 0.05$). Figure 5b shows the distribution of response durations for probe trials (closed circles) and the 5 min of BDD schedule immediately preceding each probe trial for all six animals. Figure 5c shows the same data converted to the amount of time the syringe pump was activated. Note that 49.8% of the total drug self-administered came from response durations < 0.5 s, 21.8% came from responses >0.5 s and <1.0 s, and 28.4% came from response durations >1 s.
Figure 5

A

Reinforced
Non-reinforced

Lever depression (sec)

Time (min)

Dose (mg/kg)

B

Reinforced
Non-reinforced

Number of responses

Response duration (sec)

C

Reinforced
Non-reinforced

Pump time (sec)

Response duration (sec)
Within a BDD session, animals are capable of making the HD response. Animals were given access to cocaine on a BDD schedule of reinforcement, and twice an hour, 5 min probe trials were presented in which the lever was present but responding on it did not activate the pump. Open circles represent normal BDD 5 min bins and closed circles represent the probe trials in which the syringe pump was inactivated. (a) The total time the lever was depressed within each 5 min bin averaged across animals (±SEM). Rats dramatically increased their responding during the 5 min probe trials, indicating that they are capable of making a large HD response within a session when brain-cocaine levels are high. (b) The distribution of response durations for all six animals combined during the 5 min probe trials (closed circles) or the 5 min bin preceding each probe trial (open circles). (c) The same distribution converted to the amount of total pump time each response accounted for.
Discussion

The primary objective of the present study was to explain the discrepancy between the small doses of cocaine self-administered under the BDD schedule using a holddown response (Morgan et al. 2009) and the larger doses predicted by choice and PR procedures to be the most reinforcing. After accounting for the clustering of BDD responses, we found that the majority of doses were well within a range commonly used in self-administration studies (~0.75 mg/kg; Caine and Koob, 1994; Ito et al, 2002; Liu et al, 2005; Pettit and Justice, 1991; Quadros and Miczek, 2009). However, a few surprisingly large doses (~4mg/kg) were also noted. These high doses occurred at the beginning of the session when brain levels would be very low; smaller doses were observed later in the session when steady rates of drug intake were being maintained. We undertook an analysis of the relationship between self-administered dose and calculated brain-cocaine levels. A range of timeout periods were introduced in order to force predictable declines in cocaine concentrations. The present data show that the dose is inversely related to the current brain levels of cocaine and can change predictably during the session. These results raise issues concerning the determination of 'preferred' doses and reinforcing efficacy. Depending on the method of analysis, the data from a BDD session can be characterized in a variety of ways. On the BDD schedule, a range of doses are available to the subject by holding the lever down continuously for several seconds or by clustering many responses together; presumably the spacing within a cluster offers control over the speed of injection. Morgan et al. (2009) showed that total
cocaine intake during a BDD session was almost exactly the same as the total intake during a FR1 session using a fixed unit dose (1.5 mg/kg/inj). Rats self-administering cocaine under a BDD schedule maintained a relatively constant rate of responding throughout the session, which was readily apparent by inspection of a cumulative record (see Figure 1a). Cumulative records, however, are not particularly useful in illustrating the fact that BDD responses are often grouped together; a point we failed to recognize in our initial report (Morgan et al, 2009). Plotting the data using histograms of the doses self-administered per session (see Figure 1b) and cumulative records (see Figure 1a) did not reveal clustering. However, event records (see Figure 1c) illustrated that multiple small injections were being self-administered in a short time span followed by what appeared to be a typical postinfusion pause. By combining responses that occurred within a short time frame (ie, 20 s; see below for further discussion on the selection of this criterion), we found that the majority of doses self-administered during the session were more in line with those commonly used in the literature (Caine and Koob, 1994; Ito et al, 2002; Liu et al, 2005; Pettit and Justice, 1991; Quadros and Miczek, 2009). The mechanisms controlling drug intake at various times during a self-administration session are a matter of some debate. It has long been recognized that the initial period in a self-administration session is characterized by a burst of responding (Ettenberg et al, 1982; Wilson et al, 1971). This has been termed the ‘loading’ phase to reflect the presumption that the animal is attempting to increase the brain-cocaine concentration above some satiety threshold (Tsibulsky and Norman, 1999) or trigger point (Ranaldi et
al, 1999; Wise et al, 1995). After these loading bursts, single infusions with consistent postinfusion pauses are typically observed (Wilson et al, 1971). This pattern of responding is often referred to as the ‘maintenance’ phase, during which animals presumably titrate their brain levels of cocaine within a preferred range (Tsibulsky and Norman, 1999; Wise et al, 1995). It would appear that BDD sessions also show characteristic loading and maintenance phases. Based on the present observations that during both the loading and maintenance phases the size of the self-administered cluster resulted in brain-cocaine concentration increasing into a relatively narrow range, we hypothesized that a single regulatory mechanism influences the self-administered dose throughout the session; specifically, this dose appears to reflect the amount of drug necessary to elevate brain levels into a preferred range. In order to test this, timeout periods were incorporated into the second experiment so as to manipulate brain levels of cocaine within a session. This consisted of repeated BDD access (5 min) periods followed by forced timeouts of varying length. The length of the timeout period predictably reduced brain-cocaine levels; the longer timeout periods (ie, 25 min) resulted in lower brain concentrations. We then compared the calculated brain-cocaine concentrations with the subsequent doses the animals self-administered. Results (illustrated in Figure 3) showed that introducing a timeout induced loading phase behavior, and that the size of the loading dose was highly correlated with the extent of the decline in brain levels of cocaine. These data illustrate that animals will self-administer a wide range of doses and that loading and maintenance doses simply represent the two extremes of a continuum. The
size of the dose appears to be determined by the current levels of cocaine in the brain, and this single principle seems to be at work during the entire self-administration session. Although the data clearly show that rats self-administer predictably different dosages during a session, it is presently unclear whether these varying doses should be considered to have been volitionally selected, are the most ‘preferred’, or are the most-reinforcing at that moment in the session. We suggest the most likely explanation of the fluctuations in doses is that termination of drug taking is controlled by interoceptive feedback or satiety mechanisms; the lower the brain levels are when responding begins, the longer it takes for these terminating processes to have an effect. This would be consistent with evidence and theoretical speculations concerning titration of blood levels with fixed unit doses (see above). If this is the case, it would not be necessary to invoke a volitional ‘selection’ of individual doses. Nonetheless, given the wide and predictable dose range that animals self-administer on the BDD schedule, it seems appropriate to consider whether the ‘preferred’ dose can in fact change throughout a session and whether the reinforcing effects might also be dynamically affected. As parameters such as discrete trial interval and timeout necessarily force a decline in brain levels of cocaine, we predict that these parameters could systematically influence the results from a variety of self-administration studies. For example, one method of studying reinforcing efficacy has been to determine the peak of the dose-response curve on a PR schedule using experimenter-selected unit doses (Bergman and Paronis, 2006). In typical PR studies, rats are able to ‘load-up’ while the response requirements are
minimal (ie, drug is cheap); however, as the time required to complete larger response ratios increases, greater declines in blood levels are observed (Nicola and Deadwyler, 2000). The breaking point is therefore presumably affected by the decline in blood levels and whether the experimenter-selected unit dose is sufficient to return brain concentrations to a preferred level. The present results suggest that a fixed unit dose may not be the most appropriate at any given point in time. Future work should consider whether allowing access to a range of doses using a BDD schedule at each stage of the PR session (rather than a single experimenter-selected unit dose) might yield a better estimate of the maximal response output and the optimal dose. Choice procedures that use a discrete trial in order to avoid the ‘direct effects’ of a drug might be similarly affected. The inter-trial interval would produce a dramatic decrease in brain-cocaine levels. Our data show that insertion of these timeout periods can increase the dose an animal self-administers, and could explain why choice studies in both rats (Lynch et al, 1998; Ward et al, 2005) and non-human primates (Johanson and Schuster, 1975; Llewellyn et al, 1976) typically report that animals prefer a higher dose of cocaine over the lower one. A competing hypothesis that might account for the correlation between blood levels and dose during a BDD session is that higher drug concentrations interfere with the ability of the subject to hold the lever down appropriately. The idea that high drug levels somehow interfere with the ability of an animal to respond dates back to the earliest self-administration papers (Pickens and Thomspn 1968). In order to test this hypothesis, a third experiment investigated the effect of a non-reinforced
probe trial at various intervals during a BDD session. Rats were given access to cocaine on a BDD schedule of reinforcement during 3 h sessions; twice an hour, 5 min probe trials occurred during which holding the lever down did not activate the syringe pump. Hold-down responding during these probe trials was greatly increased (Figure 5a), even though brain-cocaine levels were at high, mid-session levels. We interpret the increase in responding to reflect an attempt at titration and frustrative nonreward (Amsel, 1958). Regardless of the mechanism for the increase, the data clearly demonstrate that the animal is quite capable of clustering hold-down responses during periods when drug levels are relatively high. We therefore conclude that the observed relationship between brain levels of cocaine and self-administered dose is not because of an inhibition of the animal’s ability to hold the lever down. In this study, responses during a BDD procedure were grouped together into ‘clusters’ (see Materials and methods) in order to better characterize the size and duration of each drug bolus. One question that remains is why animals exhibit this clustered response pattern at all, when holding the lever down for the same total duration would presumably require less effort. One possible explanation is that holding down a lever for a significant duration is simply not in the rat’s natural behavioral repertoire. Perhaps rats cannot or will not perform this type of response without behavioral shaping. This possibility can be ruled out because long hold-down responses (44 s) have been frequently observed when rats were given BDD access to heroin (Morgan et al. unpublished observations). Another possibility is that this behavior reflects the animal’s preferred rate of injection. That is, by taking multiple
microinfusions of drug within a short period of time, the animal is able to
determine the speed of the infusion.

An unresolved issue is how best to define a cluster. Upon examination of a BDD
event record, grouping responses into meaningful clusters is often easy and
obvious. For example, subjectively counting the number of clusters during the
last hour in Figure 1c (bottom line) would almost certainly yield a result of 15
clusters despite the fact that 40 responses actually occurred during this time.
However, the distinction between one cluster and the next can often be much
more difficult to discern (eg, the first hour of Figure 1c, top line). We spent a
considerable amount of effort comparing results using different criteria, and
concluded that a wide range of criteria could be used without affecting the
statistical significance or conclusions of the study. For example, the BDD session
illustrated in Figure 1 consisted of 218 total responses. A cluster criterion of 10,
20, or 30 s reduced these responses to 76, 66, and 59 clusters, respectively. We
chose a 20 s cluster criterion because it represented a relatively conservative
criterion and corresponded with the post-infusion forced timeout period that is
normally imposed following each injection during more traditional self-
administration procedures. In addition, we have increased confidence that this
criterion is appropriate as its use reveals a high correlation between cluster size
and post-infusion pause (Zimmer et al, 2011; in preparation). The accuracy of the
modeled brain-cocaine concentrations deserves comment. The equations have
been extensively used in behavioral self-administration (Ahmed and Koob, 2005;
Samaha et al, 2002; Zernig et al, 2007), electrophysiological (Nicola and
Deadwyler, 2000; Peoples and Cavanaugh, 2003; Peoples et al, 2004, 2007), microdialysis (Wise et al, 1995), and voltammetric studies (Hermans et al, 2008; Stuber et al, 2005a, b), and a remarkable temporal relationship between modeled brain-cocaine concentration, NAc dopamine levels (Hermans et al, 2008; Shou et al, 2006; Wise et al, 1995), and cocaine-induced locomotor behavior (Shou et al, 2006) have been demonstrated. It is acknowledged that the relationship demonstrated here used modeled and not actual brain levels; nonetheless, the conclusions are based on relative changes in brain-cocaine concentrations and not the absolute magnitude. It should be noted that the modeling is determined for each infusion regardless of size, and is completely independent of the clustering analysis and criterion.

In summary, we have shown here that on the BDD schedule of reinforcement, hold-down responses are often clustered together, resulting in a bolus injection of various sizes - some up to 4 mg/kg/inj. Rats typically self-administered large doses during the loading phase, followed by small, maintenance doses for the remainder of the session. The data are consistent with the idea that brain concentrations of cocaine are titrated within a restricted range and the size of the dose self-administered at any point during the session depends largely on the amount of cocaine already on board. The present results suggest that the 'most preferred' or 'most reinforcing' dose of cocaine might fluctuate according to the brain levels at any particular moment.
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CHAPTER THREE

Examination of behavioral strategies regulating cocaine intake in rats

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Introduction

The pattern of cocaine self-administration has long been studied to understand the mechanisms controlling drug intake. It has been recognized that the rate animals self-administer psychostimulants such as cocaine and amphetamine is inversely related to the size of the unit dose (Gerber and Wise 1989; Pettit and Justice 1991; Pickens and Thompson 1968; Wilson et al. 1971) which is the preselected dose per injection usually held constant throughout the session. The fact that the total amount of drug per session is relatively stable regardless of the size of the unit dose has been offered as evidence that drug intake is somehow titrated. Further experimental evidence has added great weight behind this titration hypothesis by showing that amphetamine levels (Yokel and Pickens. 1974), estimated cocaine levels (Tsibulsky and Norman 1999), and nucleus accumbens dopamine levels (Ranaldi et al. 1999; Wise et al. 1995)—typically thought to play a critical role in the reinforcing effects of psychostimulants—are maintained above a theoretical threshold during self-administration sessions. The titration hypothesis is consistent with behavioral data showing that animals typically self-administer several unit doses quickly at the beginning of the session, which has been called the “loading” phase. A maintenance phase follows during which injections are regularly spaced (Ettenberg et al. 1982; Pickens and Thompson. 1968;Wilson et al. 1971;Wise et al. 1995). Previous studies have typically held the unit dose constant (but see for example Gerber and Wise 1989; Pettit and Justice 1991); however the development of a behaviorally dependent dosing (BDD) schedule provides a method to address
whether dose is a factor in the regulation of intake. When the unit dose is held constant, it is not available as a dependent variable. Thus the only titration strategy available to the animal is to adjust the inter-infusion intervals and spacing of injections. A BDD schedule described by Morgan et al. (2009) allows the behavior of the animal to determine the injection size. On this schedule, the duration that the syringe-pump is activated is contingent upon a holddown response rather than a simple lever press. That is, the syringe pump is directly controlled by depression of the lever, such that the duration of each infusion is determined by the length of time the lever is held down. Morgan et al. (2009) demonstrated that manipulation of the concentration of cocaine in the syringe led to proportional changes in responding; and, similar to findings from fixed unit dose studies, the total intake per session remained relatively constant (Morgan et al. 2009). It is important to note that both dose size and inter-infusion interval can be used as dependent variables on the BDD schedule. This provides a unique opportunity of examining the regulation of drug intake in a situation where both variables can vary and interact with each other.

We recently used a BDD procedure to examine the relationship between estimated brain levels (EBLs) of cocaine and the size of the self-administered dose (Zimmer et al. 2011). EBLs were calculated using equations adapted from standard pharmacokinetic principles (Pan et al. 1991) that have been used in many behavioral self-administration studies (Ahmed and Koob 2005; Samaha et al. 2002; Zernig et al. 2007; Zimmer et al. 2011, 2012). The accuracy of these equations have been demonstrated using electrophysiological (Nicola and
Deadwyler 2000; Peoples and Cavanaugh 2003; Peoples et al. 2004, 2007), voltammetric (Hermans et al. 2008; Stuber et al. 2005a, b), and microdialysis (Wise et al. 1995) techniques. Forced timeout periods were introduced into the session to allow EBLs to decline to predictable levels. It was shown that the self-administered dose at the beginning of each trial was highly correlated with EBLs. That is, larger doses—sometimes as large as 4 mg/kg—were self-administered when EBLs were very low. It appears that the size of the loading dose closely matched the amount necessary to elevate drug levels into a theoretically preferred range. This single large dose phenomenon parallels the loading phase observation when animals group multiple fixed unit doses together (Ettenberg et al. 1982; Pickens and Thompson 1968; Wilson et al. 1971; Wise et al. 1995).

The present study extended these findings to an analysis of the maintenance phase. If animals can indeed titrate their intake on a BDD procedure we would expect to see a relationship between the size of each selected dose and subsequent inter-infusion interval. On a BDD procedure dose size is adjusted by either holding the lever down for a long duration or by emitting a large number of very small responses in a short period of time. We have observed previously that rats self-administering cocaine appear to use the latter strategy, responding in clusters rather than a single, long response (Zimmer et al. 2011). In this study, we undertook a rigorous analysis of these infusion-clusters to determine the most appropriate criterion to classify which infusions constitute a cluster. Animals were given unrestrained access on a BDD schedule with various concentrations of cocaine, and the subsequent infusion-clusters and inter-cluster intervals (ICIs)
were measured. Results show that both clusters and ICIs changed in response to concentration manipulations. Additionally, the magnitude of the cluster self-administered was found to correlate with the size of the subsequent (but not previous) ICI. These results offer support to the hypothesis that EBLs of cocaine are maintained within an elevated zone, and show that both dose and ICI are modified in response to concentration changes.
Methods

Animals, surgery, and housing

Male, Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing approximately 350 g were used for all experiments. Following a 7-day acclimation period, a chronic, indwelling Silastic cannula was implanted into the right jugular vein. The catheter exited through the back of the animal in the region of the scapulae. Animals were anesthetized during surgery by a combination of ketamine (100 mg/kg; i.p.) and xylazine (8 mg/kg; i.p.), and ketoprofen (5 mg/kg; s.c.) was used as a postoperative analgesic. After surgery, animals were housed in a stainless steel operant chamber (30×30×30 cm), and were allowed to recover from surgery for at least 3 days. Room lights were on a 12-h reverse light/dark cycle (lights on at 3 PM), and food and water were provided ad libitum. All procedures were approved by the Wake Forest University Animal Care and Use Committee and followed all NIH guidelines outlined in the Guide for the Care and Use of Laboratory Animals.

Self-administration training

During the training period, a single press on the operant response lever (fixed ratio—FR1) resulted in a 0.75-mg/kg infusion of cocaine delivered over ~4 s depending on the weight of the animal. A 20-s timeout period followed each injection, during which the lever was retracted and a stimulus light above the lever was illuminated. Six-hour training sessions began 6 h into the dark cycle and took place 7 days/week. Training was complete when the animal self-
administered the maximum (20) number of infusions on two consecutive days and displayed a stable pattern of responding. After training acquisition, all animals were trained to self-administer cocaine on a BDD schedule of reinforcement (5 mg/ml concentration). All animals continued on this schedule until responding was stable, which was defined as self-administering ~1.0 ml or more per day (~40 s of pump-time) for three consecutive days.

Behaviorally dependent dosing schedule of reinforcement

Under the BDD schedule of reinforcement, the syringepump was activated for the duration that the lever was depressed. Releasing the lever caused the syringe-pump to immediately switch off. Therefore the magnitude of the infusion was directly related to the length of time that the lever was held down. The concentration of cocaine was 10, 5, 2.5, or 1.25 mg/ml depending on the experimental condition, and the pump speed was held constant at 1.6 ml/min. The stimulus light was only illuminated when the lever was in a down state.

Estimated brain level calculation

A two-compartment model was used to calculate whole-brain levels of cocaine as previously described (Pan et al. 1991). The equation \[ c = \frac{d}{v(\alpha - \beta)} \left( e^{-\beta t} - e^{-\alpha t} \right) \] was used where \( d \) represents the dose self-administered, \( k \) is the rate constant representing the flow between the two compartments [0.233 min\(^{-1}\)], \( v \) represents
the apparent volume of the brain \([0.15 \text{ Lkg}^{-1}]\), and \(\alpha\) and \(\beta\) [0.642 and 0.097 \text{ min}^{-1}, \text{respectively}]\) are constants that represent the redistribution and elimination of cocaine, respectively, and \(t\) (minutes) is the time since the previous infusion. The time course of brain levels for each infusion was calculated independently with a 5-s resolution and then summed.

Drug

Cocaine hydrochloride (National Institute on Drug Abuse Research, Research Triangle Park, NC, USA) was dissolved in physiological saline and passed through a microfilter (0.45 \(\mu\text{m})\). All drug concentrations are represented as the weight of the salt.

Statistics and data analyses

The primary dependent measures were total session intake (milligrams), estimated brain levels (EBLs—as calculated above), inter-cluster intervals, and infusion-clusters. Infusion-clusters were defined as the total milligrams of a sequence of infusions that occurred within a short period of time as previously described. This is a necessary calculation when using the BDD schedule of reinforcement as it has been shown that animals self-administer large clusters of infusions rather than holding the lever down for a single period of time (Zimmer et al. 2011). The criteria used to determine which infusions should be grouped together were varied systematically in this study (see Figs. 2 and 4). A repeated measure one-way ANOVA was used on datasets that passed the Shapiro–Wilk
test for normality. A Friedman repeated measures ANOVA on ranks was performed on datasets that failed the normality test. Post hoc evaluations were made using the Holm–Sidak or Neumann–Keuls method. Correlation coefficients and their corresponding t values were determined using Microsoft Excel (2007), and all ANOVA tests were performed using SigmaPlot (version 11). The probability (p) level was set to 0.05.
Results

All animals (n = 10) were offered each concentration (1.25, 2.5, 5, and 10 mg/ml) of cocaine for 3 days under a BDD schedule of reinforcement. Figure 1a shows cumulative records for an example animal. Decreasing the cocaine concentration in the syringe produced a relatively proportional increase in responding illustrated in Fig. 1a by a steeper slope of the cumulative record. This apparent compensation is quantified in Fig. 1b which shows the mean total pump-time for all rats across each concentration. Data failed the Shapiro–Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations ($\chi^2(3) = 28.92$, $p<0.001$), and a Newman–Keuls post hoc analysis demonstrated a significant difference between all comparisons. This response pattern led to a relative equalization of intake within the session. Figure 1c shows the mean total intake per session for all animals at each of the four concentrations. Data failed the Shapiro–Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations ($\chi^2(3) = 16.44$, $p<0.001$), and a Newman–Keuls post hoc analysis demonstrated a significant difference between every concentration (all six pairwise comparisons).
Fig. 1 Self-administration behavior on the behaviorally dependent dosing schedule with four cocaine concentrations. a Cumulative responses for a representative animal at each of the concentrations during 3-h sessions. b Average pump time for all animals at each concentration. c Average intake for all animals at each concentration. Statistically different (p<0.05) values are indicated by asterisks different than 1.25 mg/ml, number sign different than 2.5 mg/ml, and dagger different than 5.0 mg/ml.
Figure 2 illustrates the clustered pattern of responding on a BDD schedule of reinforcement. The top line shows the self-administration pattern of an animal within an 80-s period during the middle of the session. Forty responses occurred during this period. In our previous study (Zimmer et al. 2011), a relatively arbitrary criterion of 20 s was chosen to define a cluster. That is, any responses occurring within 20 s of each other were combined. In the present study, an analysis of several criteria ranging from 1 to 30 s was performed in order to characterize the most appropriate cluster criterion. Combining events that occurred within 1 s of each other produced four clusters and the intervals in between these clusters are shown (Fig. 2a—top). Note that increasing the criterion to 20 or 30 would reduce the number of clusters to 2 and 1, respectively. The bottom line in Fig. 2a shows a higher resolution of the responses that occurred during a 4-s period. Within this period the pump was activated 12 times for a total duration of 2.25 s. Figure 2b shows the average number of clusters calculated for all rats at all concentrations using a 0-, 1-, 10-, 20-, or 30-s cluster criterion. Note that applying a 1-s criterion combines many responses together and increasing the cluster criteria beyond 1 s has less dramatic effects. Figure 3 shows the EBLs of cocaine during representative sessions of one rat self-administering cocaine at each of the four concentrations (Fig. 3a–d). Horizontal lines represent the mean EBL for the session. Note that at all concentrations, EBLs fluctuated around the mean with relatively little variability, and there was little difference between mean EBLs despite an eightfold change in concentration.
Fig. 2 Visual representation of the clustered pattern of responses on the BDD schedule. a An event record of an 80-s period within a 3-h session for a representative animal responding for cocaine is shown on the top line. The 40 responses the animal made in this period were clustered into four bouts. An expansion of the 4 s during which one of these bouts occurred is shown on the bottom line. Within this period the animal activated the pump 12 times for a total 2.25 s. b The average number of clusters for all rats at all concentrations using a 0-, 1-, 10-, 20-, or 30-s cluster criterion.
Figure 3f shows the relationship between infusion-clusters (1 s cluster criteria—see Methods) and EBLs. The same data represented in Fig. 3d are plotted at a higher resolution (minutes 60–90) and the corresponding infusion-clusters that occurred during that period are on the lower panel of Fig. 3f. The average maintained EBLs for all rats at all concentrations are shown in Fig. 3e. Data failed the Shapiro–Wilk normality test. A Friedman repeated measures ANOVA on ranks revealed a significant difference between concentrations ($\chi^2(3) = 15.60$, $p<0.001$), and a Neumann–Keuls post hoc analysis demonstrated a significant difference between five of six comparisons (no statistically significant difference was observed between 2.5 vs 5.0 mg/ml).
Fig. 3 Estimation of brain levels of cocaine during BDD self-administration sessions with four different concentrations of cocaine. Brain levels within a session are shown for a representative animal at each concentration (a–d). Horizontal lines represent the average brain level maintained during each session. e The average of these means (±SEM) for all animals is shown at each concentration. The data from (d) are shown at higher resolution (f) with the corresponding infusion-clusters plotted in the lower panel for comparison. Statistically different (p = 0.05) values are indicated by asterisks different than 1.25 mg/ml, number sign different than 2.5 mg/ml, and dagger different than 5.0 mg/ml.
Figure 4 shows the relationship between cluster size and subsequent inter-cluster interval for an example session using all five cluster criteria (Fig. 4a–e). Note the low correlation value observed between the two variables when no cluster criterion was applied (panel a). A stronger relationship between cluster size and ICI emerged when any of the five cluster criteria were used (panels b–e). Each animal (n = 10) self-administered cocaine at each of the four tested concentrations for 3 days, yielding a total of 120 self-administration sessions (10 rats×4 concentrations×3 days). Sessions were excluded if daily intake was below 0.2 ml. A correlation coefficient was determined for each day, and the statistical significance of each point was determined by calculating the t statistic and corresponding probability (p, one-tailed). The results from our correlation analysis are displayed in Fig. 4f. Cluster criteria had relatively little effect on the number of sessions that showed a significant relationship (88 % were significant with no cluster and 84–96 % were significant using a criteria of 1–30 s). However, applying a cluster criterion had an effect on the magnitude of the average r value as shown in Fig. 4f. Non-significant sessions were often due to an outlying ICI (i.e., a 20-min period of non-responding).
Figure 4

A

No cluster

B

1 sec cluster

r=0.90

C

10 sec cluster

r=0.88

D

20 sec cluster

r=0.86

E

30 sec cluster

r=0.84

F

Correlation coefficient (r)

Dose (mg/kg)

Cluster criterion

- 1.25 mg/ml
- 2.50 mg/ml
- 5.00 mg/ml
- 10.0 mg/ml
Analysis of selected infusion-clusters compared to the previous ICI revealed a weaker relationship. The average magnitude of the significant correlations was much lower than those calculated for infusion-cluster vs subsequent ICI. Specifically, with a cluster criterion of 0, 1, 10, 20, or 30 s the mean r value for cluster vs previous ICI was 0.20, 0.29, 0.29, 0.31, and 0.31, respectively. For cluster vs subsequent ICI the mean was 0.30, 0.57, 0.56, 0.58, and 0.55, respectively.

Because titration of EBLs across concentrations could be accomplished by either adjusting the size of the selected infusion-cluster or the length of the ICI, an analysis of both of these variables was performed. The mean (±SEM) pump duration and corresponding dose at each concentration with either a 0 or 1 s cluster criterion is shown in Fig. 5a and b, respectively. When clusters were not accounted for, a one-way repeated measures ANOVA revealed no statistically significant difference in the average pump durations across concentration (Fig. 5a, closed circles, F[9, 27]=0.13), which explains why the average dose increased significantly and proportionally to the change in concentration (Fig. 5b, closed circles, F[9, 27]=72.49, p<0.001). A one-way repeated measures ANOVA on the average pump duration with a 1-s cluster criterion revealed a significant difference between concentrations (Fig. 5a, open circles, F[9, 27] = 14.02, p<0.001). A post hoc analysis (Holm–Sidak) revealed all comparisons were
significantly different except 2.5 vs 5.0 mg/ml, indicating that the average pump duration decreased as concentration of cocaine increased. The corresponding average dose was also significantly different across concentrations, as revealed by a one-way repeated measures ANOVA (Fig. 5b, open circles, $F[9, 27] = 33.36, p< 0.001$). Post hoc analysis (Holm–Sidak) revealed a significant difference for all comparisons except 1.25 vs 2.5 mg/ml. The mean ICI also increased as a function of concentration, such that the largest concentrations led to the largest ICIs (Fig. 5c). A Friedman repeated measures ANOVA on ranks (data failed normality test) revealed a statistically significant difference between concentrations with a 1-s cluster ($\chi^2(3) = 22.68, p< 0.001$) and without clustering ($\chi^2(3) = 28.92, p<0.001$). A Student–Neumann–Keuls post hoc analysis without the clustering criteria demonstrated a significant difference between all comparisons and with a 1-s cluster all comparisons were significant except 10.0 vs 5.0 and 2.5 vs 1.25 mg/ml.
Fig. 5 Analysis of dose size and inter-infusion interval. a The mean pump duration for all animals at each cocaine concentration without clustering responses (closed circles) or with a 1-s cluster criterion (open circles). b The mean cluster self-administered at each concentration with no cluster criteria (closed circles) or a 1-s cluster criteria (open circles). c The mean inter-cluster interval at each concentration with no cluster criterion (closed circles) or a 1-s cluster criterion (open circles). Statistically different (p < 0.05) values are indicated by asterisks different than 1.25 mg/ml, number sign different than 2.5 mg/ml, and dagger different than 5.0 mg/ml.
Discussion

The present study examined the regulation of cocaine self-administration using a novel BDD schedule of reinforcement. This schedule allows for a broad range of doses that depend on the behavior of the animal. One of the unique attributes of this schedule is that the size of dose and inter-infusion interval can both be used as dependent variables. Similar to other schedules of reinforcement (Gerber and Wise 1989; Ranaldi et al. 1999; Tsibulsky and Norman 1999; Wilson et al. 1971; Wise et al. 1995), relatively constant estimated brain levels were maintained within each session on the BDD schedule (Fig. 3). Following manipulation of cocaine concentration, compensatory adjustments were seen in both infusion-cluster size and inter-cluster interval (Fig. 5b and c). Finally, a significant relationship between the size of each cluster and the subsequent (but not previous) ICI was found (Fig. 4).

Several mechanisms have been proposed to explain the regulation of psychostimulant intake. One possibility is that cocaine has a direct effect on the motor behavior of animals such that they become incapable of responding for some period of time (Pickens and Thompson 1968). This explanation is unlikely given the results from experiments examining concurrent intracranial self-stimulation (ICSS) and i.v. drug self-administration. Wise et al. (1977) reported an augmentation of ICSS rates during post-infusion pauses, demonstrating that rats are fully capable of responding during this interval. An alternate hypothesis is that cocaine levels are maintained within a range—either above a threshold and/or below a ceiling (Tsibulsky and Norman 1999; Wise et al. 1995; Wilson et
al. 1971; Pickens and Thompson 1968). It has been demonstrated that cocaine has anxiogenic properties and can produce avoidance behavior (DeVries and Pert 1998; Ettenberg and Geist 1991, 1993) and conditioned taste aversions (Booth et al. 1977; Goudie et al. 1978). However, microdialysis studies of psychostimulant self-administration do not appear to support the idea that dopamine levels fluctuate below a theoretical ceiling (Wise et al. 1995; Ranaldi et al. 1999). These authors argued that if titration was due to the animal avoiding upper aversive effects, it would be expected that a frequent low dose would be preferred over larger ones. However, animals consistently choose larger doses (Johanson and Schuster 1975; Llewellyn et al. 1976; Lynch et al. 1998; Ward et al. 2005), suggesting that the aversive properties of cocaine cannot fully account for the titration of brain levels. Additionally, a microdialysis study demonstrated that amphetamine levels at the time of each response were relatively constant despite a wide range of available doses suggesting that rats titrate drug levels above a threshold value (Yokel and Pickens 1974).

The titration hypothesis—that rats self-administer to maintain brain levels of drug above a theoretical threshold—makes several predictions. First, when current drug levels fall below the threshold, the subsequent cluster selected should correlate with the length of the previous ICI. That is, the further below the threshold drug levels fall the greater the dose required to return levels to the threshold. Second, when animals are maintaining drug levels above the threshold it would be expected that the size of the dose selected would correlate with the subsequent ICI. That is, a large dose would push brain levels
significantly above the threshold and animals would wait the appropriate length of
time for brain levels to return to the satiety threshold before resuming
responding. Our previous report demonstrated that large loading clusters could
be induced by introducing forced timeout periods and that the size of these
clusters correlated with the length of the previous ICI as predicted (Zimmer et al.
2011). The results of the current study lend support to the interpretation that
animals titrate brain levels above a threshold by showing that cluster size
correlated more strongly with the subsequent ICI than the previous one when
animals were maintaining drug intake with no forced timeouts.

Panlilio et al. (2003) have argued that the titration hypothesis predicts an
autocorrelation between sequential latencies. That is, an error in one inter-
infusion interval should produce a compensatory adjustment to the next one.
However, they were unable to demonstrate such a relationship and concluded
that the temporal regularity of responding was not due to compensatory
adjustments of inter-infusion intervals. Their failure to observe a significant
correlation may be due to the insufficient variability in Inter-infusion intervals
produced by using a fixed unit dose. We would argue that the correlations
observed in the present experiment using the BDD schedule are due to the much
larger variance in dose and ICIs.

There remains one issue that is hard to reconcile with the titration hypothesis.
Studies consistently report that EBLs and nucleus accumbens dopamine levels
are not constant across a range of doses (Pettit and Justice. 1991; Pickens and
Thompson 1968). The present study replicated these observations by showing an increase in mean maintained EBLs and intake with larger concentrations (Figs. 1c and 3e). These findings appear to be in contrast to the titration hypothesis, which posits that changes in dose are corrected by a proportional change in responding. One possible explanation of this phenomenon is that the elimination kinetics of cocaine produces a non-linear function of dose and subsequent inter-infusion intervals (Tsibulsky and Norman 1999). This explanation works well with the behavioral data, but is hard to reconcile with dose-dependent nucleus accumbens dopamine levels and estimated brain-cocaine concentrations. Other possible explanations include differences in work output with changing doses or the sensitivity of interoceptive cues.

To the extent that animal studies can provide an understanding of human drug taking, previous research using fixed, unit doses would suggest that spacing of inter-injection intervals is the primary regulatory strategy. The BDD schedule offers a different dimension (manipulation of dose) and points to the fact that animals will self-administer quite different doses throughout the session and use a variety of strategies to regulate their intake. This work would predict that human regulation of cocaine levels would be accomplished by a combination of adjustments in both the size and spacing of injections. This prediction appears to match human regulation of other drugs such as nicotine. The study of nicotine consumption has confirmed that dose (puff size) and inter-puff interval interact in the regulation of smoking behavior and that smokers appear to titrate nicotine levels (Ashton et al. 1979; McMorrow and Foxx 1983; DeGrandpre et al. 1992).
To our knowledge clinical studies of cocaine intake have not yet reached this detailed level of analysis.

The present study confirmed previous results that the selected dose on the BDD schedule can be described by clusters of multiple short responses. The first study characterizing the BDD schedule of reinforcement showed animals selecting surprisingly small doses of cocaine (Morgan et al. 2009). Further analysis suggested that multiple responses are typically clustered within a very short period of time (Zimmer et al. 2011). The present study confirmed these findings by showing that the average pump duration for a single response does not change across concentration but the number of these responses occurring within a cluster does (Fig. 5a). Further evidence that a single hold down response is not a predictive unit of measure but needs to be grouped together comes from the observation that single responses do not show expected relationships while clusters of responses do. For example, clustering responses that occurred within even 1 s of each other resulted in a dramatic increase in the predictive ability of clusters on the subsequent ICI (Fig. 4). Analyzing the data with multiple cluster criteria demonstrated similar significant results for a range of criteria (1–30 s). A 1-s criterion was used for all subsequent analyses as it represented the most conservative option and it appeared that using a larger criterion provided no added advantage and could potentially group unrelated clusters together.

Finally, the use of specific terms in this study deserves comment. A number of proponents of the titration hypothesis have offered theoretical explanations using
terms such as “hedonic set point”, “satiety threshold”, “trigger point”, “compulsion zone”, etc. (Ahmed and Koob 1998; Wise et al. 1995; Tsibulsky and Norman 1999). These terms have been helpful in focusing attention on core concepts of the titration hypothesis. However, in some cases these terms imply a level of conscious control. We wish to emphasize that the present support for the titration hypothesis should not, by itself, be interpreted as evidence for a specific conscious or emotional state. Homeostatic mechanisms do not necessarily require conscious awareness (e.g., blood pressure), and it remains unclear the degree to which awareness contributes to the regulation of cocaine concentrations in the brain. For example, levels of nicotine appear to be titrated within a narrow range by human smokers without them having an explicit awareness of the phenomenon (Ashton et al. 1979; Gritz et al. 1976; Herning et al. 1985). Some stimuli that control drug seeking and taking are conscious while others are not (Childress et al. 2008). Therefore, it would be inappropriate to presume the processes described here involve conscious awareness, volition, or any particular emotional state of the animal. Such conclusions would require additional data.

The present study manipulated cocaine concentrations in order to examine the self-administration behavior on a BDD schedule of reinforcement during the maintenance phase. In agreement with previous studies, animals appear to titrate EBLs around a relatively constant level by adjusting both cluster size and ICI. In addition, a positive correlation between cluster size and the subsequent
ICIs was found, which suggests that animals maintain EBLs above some presumed threshold.
References


CHAPTER FOUR

The Acute Effects Of High Intake “Binges” On Motivation To Self-Administer Cocaine In Rats

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Introduction

One of the hallmarks of psychostimulant addiction is the appearance of a binge-abstinence pattern of drug use. Addicts progress from occasional and recreational drug consumption to periods when use occurs in binges of prolonged durations (6 – 36 hrs and occasionally more) and high intake (Gawin, 1989, 1991; Gawin and Ellinwood, 1989). This binge-abstinence cycle contrasts with the abuse patterns of many other drugs (e.g. alcohol, tobacco, opiates) in which the addiction process is usually marked by a daily use pattern (Gawin, 1991). Clinical studies and case reports demonstrate that the psychostimulant binge-abstinence pattern of self-administration is predictive of the progression of the addiction process in individuals (Baker, 1989; Foltin and Fischman, 1998; Gawin, 1989, 1991; Gawin et al, 1989).

Binge-abstinence patterns are also seen in preclinical models. The earliest IV self-administration studies demonstrated that unlimited access to psychostimulants produce erratic swings between high intake followed by periods of abstinence in both rats (Bozarth and Wise, 1985), and non-human primates (Balster and Schuster, 1973; Johanson et al, 1976). A number of toxic events were noted within these binges, such as extreme exhaustion, sleep-deprivation, grooming deficits, weight loss, and seizures. Typically, the severity of toxic effects increased over the course of 4-5 binge-abstinence cycles ultimately resulting in lethality (Balster et al, 1976; Bozarth et al, 1985; Johanson et al, 1976). The length of the binge can be extremely variable, lasting from a few hours to more than several days (Baker, 1989; Gawin, 1991). The mechanisms
that serve to terminate a binge are unclear, although suggested possibilities include physical exhaustion, sleep and food deprivation, or in some cases seizure. The high mortality rate in experiments with no limits on access makes studying this naturally occurring binge-abstinence pattern problematic. For this reason subsequent psychostimulant self-administration studies have imposed constraints on intake. These constraints have included limits on dose (Carroll and Lac, 1987; Carroll et al., 1989), the number of infusions available per hour (Fitch and Roberts, 1993; Roberts et al., 2002), or session length per day (Ahmed and Koob, 1998, 1999). This last option has become increasingly popular as a method that allows animals to self-administer a relatively short binge but has no substantial health problems associated with it. In general the type of constraint used depends on the focus of the study, and consequently many different protocols have all been described as “binges”.

The finding that animals self-administer psychostimulants in binge-abstinence patterns similar to humans has led to a series of studies focusing on two important questions regarding binge intake; the consequences of binge intake and the mechanisms that regulate it. In studies examining the consequences of binge intake, the binge sessions are independent variables (e.g. Ahmed et al., 1998; Zimmer et al., 2012). Whereas studies examining the regulation of binge intake typically place few or no constraints and treat measurements of the binge session itself (e.g. number of infusions/binge, length of continuous self-administration within a binge, etc.) as a dependent measure (e.g. Covington and Miczek, 2001, 2005b; Quadros and Miczek, 2009).
Studies examining the effects of cocaine binges on subsequent behavior and physiology have shown a variety of behavioral and physiological consequences. These studies have used a variety of binge lengths, from 6-48 hours. As referenced above, daily 6-hour binges (frequently termed long access, LgA) have become a popular procedure to examine the consequences of binge intake (Ahmed et al, 1998, 1999). The effects of these binges has been examined thoroughly, and many behavioral and neurochemical consequences have been described including increases in responding for cocaine and increased cocaine intake over time (Kenny et al, 2005; Orio et al, 2009; Wee et al, 2008; Wee et al, 2009; Zimmer et al, 2012; Zorrilla et al, 2012). Studies examining access periods longer than 6 hours typically need to be separated by experimenter-imposed abstinence days to ensure the survival of the subjects. These studies have demonstrated disruptions of intake regulation and autonomic homeostatic mechanisms (Tornatzky and Miczek, 1999, 2000). Twenty-four hour binge sessions have also shown decreased glucose metabolism in a large number of brain regions (Hammer et al, 1993), and increases in measures of stress and anxiety are also observed following 12 and 48 hour binges (Mutschler and Miczek, 1998).

Studies examining how binge intake is regulated have shown that factors such as stress and sensitization can play prominent roles. This literature has described controlling factors that influence total intake and regularity within a binge. It has been demonstrated that the highly regular pattern of intake normally seen during self-administration of psychostimulants (Gerber and Wise, 1989; Pettit and

Procedures frequently used to engender sensitization to the locomotor effects of cocaine have been shown to increase intake during a 24 hour binge (Covington et al, 2001). Stressors such as social defeat or confrontation also increases intake, intensity and duration of cocaine binges (Covington et al, 2001, 2005b; Quadros et al, 2009), and these stress-induced changes have been shown to persist up to 2 months following the stressful events (Covington et al, 2005a).

Because cocaine binges can last 24 hours or more, circadian cycles become an important factor that must be considered. Circadian rhythms have been shown to influence psychostimulant self-administration under some circumstances, but the extent to which they affect a cocaine binge is unknown. The circadian influences on self-administration behavior are typically more observable when behavior is constrained, such as restricting hourly intake by limiting the number of discrete trials per hour available or by reducing the unit dose available (Fitch et al, 1993; Roberts et al, 2002). In these situations self-administration is seen almost exclusively in the dark/active phase, and the observed diurnal pattern persists in the absence of external light-dark entraining cues suggesting a strong endogenous regulator of cocaine self-administration (Bass et al, 2010).

As highlighted above, studies have increased our understanding of the mechanisms that regulate a binge by demonstrating factors that influence intake and regularity (Covington et al, 2005a; Covington et al, 2001, 2005b; Quadros et al, 2009; Tornatzky et al, 2000). The present study sought to examine the time-
course of motivational changes within a binge. It can be presumed that at the termination of a binge the motivation to self-administer is too low to sustain responding. However, the time-course of motivational change within a binge is unknown. The primary goal of the present study was to examine the motivation to sustain cocaine self-administration throughout a binge. In experiment 1, rats self-administered cocaine under an FR1 schedule of reinforcement with no limits for sessions of 0, 12 or 24 hrs immediately followed by a PR schedule. In addition, the start times were staggered to evaluate if circadian cycles played an observable role in changes in PR responding during cocaine binges. In experiment 2, the influence of cocaine dose during a binge session (of length 6, 12, or 24 hours) was assessed. Finally, in experiment 3, we examined shifts in the PR dose-response curve following cocaine binges of 12 or 24 hours.
Materials and methods

Animals

Male, Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) weighing approximately 350 g at the start of procedures were used in all experiments. Lights in the animal room were maintained on a reverse 12 h light/dark cycle (lights on at 5 p.m), and each rat was habituated to this schedule for a minimum of 7 days before surgery. Throughout all experiments, food and water were made available ad libitum. Subjects were individually housed in custom-made, stainless steel self-administration chambers (30 x 30 x 30 cm) 24 hrs per day.

Catheter implantation

Rats were anesthetized with the combination of ketamine (100 mg/kg) and xylazine (8 mg/kg) i.p., and a chonically indwelling Silastic catheter (CamCaths, Cambridgeshire, UK) was implanted into the jugular vein. The catheter was attached to a subcutaneous plastic anchor in the region of the scapulae. The plastic anchor was attached to Tygon tubing, enclosed by a stainless steel tether for protection, which connected the anchor to a counterbalanced fluid swivel (Instech Laboratores, Inc., Plymouth Meeting, PA., USA). Tygon tubing also connected the fluid swivel to an infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) located outside of the self-administration chamber. All catheters were flushed daily with heparinized saline, and rats were given 3-5 days to recover following surgery before self-administration sessions were initiated.

Cocaine self-administration training
All self-administration sessions were signaled by the extension of the lever into the chamber. Training sessions began in the middle of the dark phase, lasted six hours, and were run 7 days per week. During training a single response on the lever (FR1) resulted in an infusion of cocaine (1.5 mg/kg/inf) that lasted 4-5 seconds depending on the body weight of the animal. A 20 sec timeout period followed each infusion, during which a cue-light above the lever was illuminated and the lever was retracted. Training was considered complete when the maximum infusions allowed per session (40) had been self-administered in five consecutive sessions and a consistent pattern of post-infusion pauses was observed.

**Progressive ratio schedule of reinforcement**

Throughout this study progressive ratio (PR) schedules were used to determine the acute reinforcing efficacy of cocaine following various manipulations. Several doses (0.3, 0.56, and 1.5 mg/kg/inf) of cocaine were tested as described in each experiment. Under a PR schedule, the response requirement was increased for each consecutive infusion. The response requirements increased according to the following sequence: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc (Richardson and Roberts, 1996). The breakpoint was defined as the total number of infusions earned within a single 6 hour PR session.

**Binge self-administration protocol**

Experiment 1 examined the acute effects of cocaine binge self-administration on progressive ratio (PR) responding. The experiment consisted of a within-subject
design in which each subject received a baseline PR schedule of reinforcement, and a 12 or 24 hr binge (FR1, unlimited intake) followed immediately by a PR schedule. However, one concern with this design was that animals were kept on a 12-12 light/dark cycle. Therefore, baseline and 24 hr binge sessions would lead to the PR session occurring during the dark/active phase, whereas the 12 hr binge condition would end during the light/inactive phase. The experimental design was expanded to account for possible circadian effects on responding by adjusting the start time for each binge condition (baseline, 12 hr, and 24 hr binge) to occur 0, 6, 12 or 18 hours after lights on (hereon referred to as ZT 0, 6, 12, and 18 respectively). The order of binge length and start time was randomized. To ensure the health of the animals, a 24 hr period of abstinence was enforced following a 12 or 24 hr binge-PR pairing and animals continued responding daily on PR schedules until breakpoints returned to baseline before another binge condition was implemented. All PR sessions in experiment 1 used a dose of 0.56 mg/kg/inf cocaine, which was chosen because it is on the ascending limb of the dose-effect curve (Ward et al. 2005). All subjects experienced each binge length condition (0, 12 or 24 hrs) and each starting time (ZT 0, 6, 12, or 18) unless catheter patency failed before completion of the experimental design.

The goal of experiment 2 was to assess whether the dose of cocaine self-administered during a binge significantly altered the consequent breakpoints. Similar to experiment 1, animals self-administered cocaine under a PR schedule (0.56 mg/kg/inf) of reinforcement to establish a baseline, followed by cocaine binges (FR1 – no limits on intake) lasting 6, 12 or 24 hrs with a dose of cocaine
of either 0.56 or 1.5 mg/kg/inf. The order of binge length and dose was randomized and each rat experienced each combination unless catheter patency failed before completion.

The purpose of the third experiment was to determine if the breakpoint-reducing effects of binge cocaine self-administration were dose dependent. All animals received initial PR training using 0.56 mg/kg/inf as the unit dose. In this experiment all cocaine binges consisted of 1.5 mg/kg/inf unit doses, and as before no limits were placed on intake within the binge. Each rat self-administered cocaine on a PR schedule of reinforcement using one dose of either 0.3, 0.56, or 1.5 mg/kg/inf immediately following a 0 (baseline), 12 hr, or 24 hr binge of cocaine. Each subject experienced each binge condition (0, 12 and 24) at each PR dose (0.3, 0.56 and 1.5) unless catheter patency failed.

**Drugs**

Cocaine HCl (National Institute on Drug Abuse, Rockville, MD) was dissolved into sterile saline (0.9%) at concentrations of 1.0, 1.87, 3.73 or 5.0 mg/ml. All concentrations were passed through a micro filter (0.45 µm, Fisherbrand) before use and are expressed as the weight of the salt. Changes in the self-administered dose were accomplished by changing the concentration (see above) of cocaine while maintaining the same infusion time with one exception: for the binges using a lower dose (0.56 mg/kg/inf), the pump durations were halved (~ 2 sec/inf) and the appropriate concentration (3.73 mg/ml) was calculated to achieve the desired dose.

**Data analysis**
The dependent variables examined were the final ratio achieved under a PR schedule of reinforcement and total intake during binges. As previously discussed (Richardson et al, 1996), the total number of reinforcers delivered (i.e. breakpoint) were used for all statistical analyses. This is necessary for statistical analysis because the exponential nature of PR data regularly violates the assumption of homogeneity of variance. All statistics and graphs were calculated using sigmaPlot (version 11), and the Holm-Sidak method was the post-hoc evaluation used for all statistically significant ANOVA results. The probability \((p)\) level was set to 0.05.
Results

Experiment 1

Fig 1 illustrates the experimental protocol by showing example event and cumulative records for an animal responding during the three binge conditions (baseline, 12 hr binge, and 24 hr binge). The cumulative record labeled “Baseline” represents responding on a PR session before any binge protocol had been implemented. Example event records for 12 hr and 24 hr binge sessions can be seen (fig 1, bottom lines) followed immediately by PR session cumulative records. This pattern of baseline, 12 hr and 24 hr binges was repeated four times with a different start time (ZT 0, 6, 12 and 18) for each rat in a randomized order.
**Fig. 1** PR breakpoints (0.56 mg/kg/inf) following self-administration binges (FR1) for 0, 12, or 24 hours. Data from a representative animal are shown to illustrate the experimental procedure. Event records of a 12 and 24 hour binge session are shown on the abscissa. Progressive ratio sessions were run immediately following each binge session to assess motivational responding, and are shown as cumulative records. Diagonal tick marks on the cumulative records represent completion of the response requirement and consequent infusion of cocaine. For event records, each tick mark represents an infusion of cocaine (FR1).
Figure 2A shows resulting breakpoints collapsed across starting times for the baseline (no preceding cocaine binge) and 12 or 24 hr binge (FR1) conditions. Figure 2B shows the same data separated into the staggered start times. Circles represent the baseline breakpoints at each time, squares represent breakpoints following a 12 hr binge and triangles represent breakpoints following a 24 hr binge. Note that the data points represent the time that the PR session began rather than the binge (e.g the 12 hr binge, ZT 12 data point represents binges that started at ZT 0). A repeated measures two-way ANOVA with binge length and time of day as factors revealed a significant effect of binge length (F [2, 28] = 50.65; p < 0.001), but no significant effect of time of day (F [3, 28] = 2.1; p = 0.11) or interactions (F [6, 28] = 1.75; p = 0.15) were found. A Holm-Sidak post-hoc analysis revealed that breakpoints following 12 and 24 hr binges were significantly lower than baseline, and that breakpoints following a 24 hr binge were significantly lower than those following a 12 hr binge.
Fig. 2 Effects of binge length and time of day on PR breakpoints. (A) Data from all staggered starting times are combined to show the overall effect of binge length on PR breakpoints. Bars represent the average (±SEM) breakpoints (corresponding final ratios are plotted on the right ordinate) for animals at baseline (no preceding binge), or immediately following a 12 or 24 hour binge. Statistical significance (p < 0.05) is indicated by * different than baseline and † different than 12 hour. (B) Same data from panel A, separated across staggered start times. The abscissa denotes the time that the PR session began (ZT0 = lights on; ZT12 = lights off). Each point represents the mean (±SEM) breakpoints (corresponding final ratios are plotted on the right ordinate) at baseline (no preceding binge – circles), or immediately following a 12 (squares) or 24 (triangles) hour binge session. Starting times were staggered to assess the effects of circadian influences on PR responding. No statistically significant effect of time of day on breakpoints was found.
Following each 24 hr binge session animals were given daily PR sessions until breakpoints returned to baseline. Each rat performed multiple 24 hr binges and recoveries with staggered start times. The mean breakpoint was calculated for each rat across start times, and the averages of these values are shown in figure 3. A repeated measures one-way ANOVA revealed a significant difference across days ($F[5, 44] = 18.16; p < 0.001$). A Holm-Sidak post-hoc analysis revealed that breakpoints following the 24 binge and the subsequent day were significantly lower than those from baseline. These data suggest that breakpoints recover within 48 hours.
**Fig. 3** Time-course of binge-induced deficits in PR (0.56 mg/kg/inf) responding. Circles represent the average (±SEM) breakpoints at baseline (no preceding binge), immediately following a 24 hour binge, and four consecutive days following the binge. Data points significantly different from baseline are indicated by * (p < 0.05).
Experiment 2

Total intake per session for each condition is shown in figure 4A. Cocaine binges with a unit dose of 0.56 mg/kg/inf are represented as circles and 1.5 mg/kg/inf are represented as squares. A two-way repeated measures ANOVA revealed a significant effect of dose (F[1, 4] = 42.30; p < 0.001), binge length (F[2, 4] = 108.50; p < 0.001), and there was a trend towards a dose x binge length interaction (F[2, 4] = 6.57; p = 0.054). The breakpoints following each binge condition are shown in figure 4B. A two-way repeated measures ANOVA revealed a significant effect of binge length (F[3, 8] = 8.61; p = 0.001), but no effect of cocaine dose (F[1, 8] = 3.34; p = 0.10) and no significant interaction (F[3, 8] = 2.37; p = 0.15). A Holm-Sidak post-hoc analysis revealed that breakpoints following a 24 hr cocaine binge were significantly lower compared to baseline.
Fig. 4 Effect of dose on cocaine binge intake and subsequent PR breakpoints. Animals responded for cocaine in binge sessions (FR1) for 6, 12 or 24 hours with a dose of 1.5 mg/kg/inf (squares) or 0.56 mg/kg/inf (circles). (A) Intake within cocaine binge sessions. Data points represent the average (±SEM) total intake for the entire session. A significant effect of dose (p < 0.05) and a significant effect of binge length (p < 0.05) were found, and there was a trend for a significant dose X length interaction (p = 0.054). (B) Breakpoints following cocaine binge sessions. Data points represent the average (±SEM) breakpoints at baseline (no preceding binge), or immediately following a binge (FR1) of 6, 12, or 24 hours using a dose of 1.5 mg/kg/inf (squares) or 0.56 mg/kg/inf (circles). A significant effect of binge length was found (p < 0.05), but no binge dose effect or dose X length interactions were found.
Experiment 3

The breakpoints from the PR dose effect curve are shown for each binge condition in figure 5. Breakpoints that were not preceded immediately by a cocaine binge (baseline) are represented as circles. Breakpoints immediately following a 12 hr binge are shown as squares, and after a 24 hr binge as triangles. A repeated measures two-way ANOVA revealed a significant effect of binge length ($F[2, 28] = 32.89; p < 0.001$), but there was no effect of PR dose ($F[2, 28] = 2.72; p = 0.1$) and no significant binge length by PR dose interaction ($F[4, 28] < 1$).
Fig. 5 PR breakpoint dose-effect curve following cocaine binges of 12 or 24 hours. Data points represent average breakpoints (±SEM) following baseline (no preceding binges - circles) or immediately following a binge (FR1) of 12 (squares) or 24 (triangles) hours. A significant effect of binge length was found (p < 0.05), but no effects of PR dose and no dose X length interactions were found.
Discussion

In this study, rats self-administered cocaine in “binge” sessions of 6-24 hrs followed immediately by progressive ratio (PR) sessions. A number of parameters were manipulated including start times, binge length, binge dose, and PR dose to ascertain the important factors driving motivation to self-administer cocaine within a binge. Results showed that breakpoints decreased steadily throughout a binge, and recovered to baseline levels within 48 hours. Circadian influences did not change motivation within a binge in a significant way, although in a few circumstances subtle trends appeared to contribute. Dose manipulation during the binge had subtle effects on intake, but not on breakpoints. Finally, binge administration of cocaine was shown to shift the PR dose-response curve downward.

The purpose of studying cocaine binge self-administration is to better model and understand important characteristics of human drug taking. Clinical reports suggest a high degree of variability and individual differences in drug taking. Addicts have been reported to binge for durations from 3 to 72 hours (Baker, 1989; Gawin, 1989, 1991). Abstinence periods often occur in between high-intake binges that can last several days, such that these prolonged binges typically occur 1 to 3 times per week (Gawin, 1989, 1991). Precise measurements of amounts and pattern of intake within a binge are difficult to attain in a naturalistic setting. However, case reports and interviews in clinical studies suggest that users take between 3 to 8 grams over the entire binge session and 40 to 50 grams per week, and users report administering the drug
once every 10 to 60 minutes (Baker, 1989; Gawin, 1989, 1991; Gawin et al., 1989). Regardless of the pattern of use within a binge, it is clear that experiencing high-intake binge administration of cocaine leads to long-lasting and harmful changes. For example, it has been demonstrated that users who had experienced a period of time when limits to psychostimulant access were removed (e.g. involvement in production or sale) self-administered significantly more cocaine even after the normal limiting factors such as price and availability were reinforced (Culbertson et al., 2009). Taken together, the clinical data demonstrate that binge self-administration of cocaine in humans is a crucial parameter that is indicative of the progression of the addiction process, but empirically studying psychostimulant binges in humans is ethically and pragmatically difficult.

Animal models of bingeing behavior have a long history in both non-human primates and rodents. Studies have demonstrated that non-human primates will readily self-administer large quantities of cocaine in a binge-like pattern to the point of lethality (Johanson et al., 1976). Another study gave monkeys a choice between food and cocaine 24 hours per day, and found that subjects chose cocaine exclusively and the experiment had to be terminated early because of deteriorating health (Aigner and Balster, 1978). More parametric work has been done using rodent models. These studies have shown that rodents will also self-administer cocaine to the point of lethality when given unlimited access (Bozarth et al., 1985; Johanson et al., 1976). In addition observable disruptions of intake regulation and autonomic homeostatic mechanisms have been demonstrated
(Tornatzky et al, 1999, 2000). Specifically, intake becomes dysregulated after roughly 18-24 hours and in some cases self-administration behavior ceases entirely (Tornatzky et al, 2000). Results from the present set of experiments fits well with this work. Breakpoints, a measure of work output, decreased significantly as the binge progressed (Fig 2). It has been suggested that highly stereotyped behavior induced during binge administration of psychostimulants may in some cases compete with the intake-disrupting effects, prolonging a self-administration binge (Fowler et al, 2007). In addition to the health-deteriorating effects, imaging studies have shown decreased glucose metabolism in a number of brain regions including the striatum, olfactory tubercle, and somatosensory and motor cortices following a 12 hour cocaine binge compared to shorter sessions of cocaine access (Hammer et al, 1993). Binge administration has also been shown to cause an increase in measures of stress including ultrasonic vocalizations and startle reflex responses (Mutschler et al, 1998). This combined with the finding that stress increases likelihood and intensity of subsequent binge administration (Covington et al, 2005a; Covington et al, 2001, 2005b) suggests a possible stress/high-intake cycle could be at play.

Designing a 24 hr binge experiment creates a natural circadian confound. Circadian influences on psychostimulant self-administration behavior has been demonstrated repeatedly, and it is well known that these changes are seen when constraints (e.g. limits on length of session, dose, number of discrete trials, timeouts, etc) on behavior are imposed (Bass et al, 2010; Brebner et al, 1999; Espana et al, 2010; Fitch et al, 1993; Lynch and Roberts, 2004; Roberts and
Andrews, 1997; Roberts et al, 2002; Roberts et al, 2003). In the present experiment, intake was not constrained during the binge episodes and therefore no differences in self-administration behavior were expected during these sessions. However, whether circadian influences could be detected in motivated behavior following a binge remained an unanswered question. Results show that although motivation to continue self-administration decreased throughout the binge session, no significant circadian effects were apparent (Fig 2B). The stimulating effects of high levels of cocaine are likely able to overcome the normal regulatory mechanisms that would reduce intake in other circumstances.

In conclusion, the binge-abstinent pattern seen in psychostimulant addicts is an important phenomenon that is difficult to study in naturalistic settings. Rodents given access to psychostimulants such as cocaine engage in a binge-abstinent pattern of intake. The face validity between human and animal binge-abstinence patterns of self-administration, combined with growing evidence that binge self-administration leads to important change in subsequent self-administration behavior, and that binge administration occurs despite severe consequences all suggest that unlimited-access cocaine binges are important features of the addiction process.

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CHAPTER FIVE

The Motivation to Self-Administer is Increased After a History of Spiking Brain Levels of Cocaine

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Drug addiction is a multifaceted disorder that presents with a variety of symptoms. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), a diagnosis of drug dependence requires the presentation of any three of seven distinct symptoms (American Psychiatric Association, 1994). These range from purely consumptive measures (eg, increased intake) to motivational criteria such as an inability to abstain. The diversity of these criteria suggests that drug dependence involves multiple neurobiological processes that may manifest differently in different individuals (Koob and Volkow, 2010). A comprehensive study of these underlying neurobiological mechanisms requires animal models that reflect the multiple addiction processes.

A number of cocaine self-administration procedures have been developed in rodents to assess specific DSM-IV symptoms associated with cocaine addiction (Vanderschuren and Everitt, 2004). The schedules and access conditions used, and the resulting patterns of cocaine intake, greatly affect the expression of the symptoms. It is important to note that many access conditions produce remarkably stable patterns of drug intake with no apparent change in motivational measures (Roberts et al, 2007). This demonstrates that simply allowing an animal to self-administer cocaine is not sufficient to produce an addicted phenotype. Successful rodent models of the progression of cocaine addiction have identified a number of critical features including increased daily access (Ahmed and Koob, 1998, 1999), intermittency (Morgan et al, 2002;
Morgan and Roberts, 2004), abstinence (Grimm et al, 2001; Pickens et al, 2011), and speed of drug injection (Liu et al, 2005b; Wakabayashi et al, 2010).

A long access (LgA) procedure in which rats self-administer cocaine during daily 6-h sessions on a fixed ratio 1 (FR1) schedule is perhaps the most widely used model for examining changes in self-administration behavior over time (Koob and Kreek, 2007; Koob and Volkow, 2010; Zernig et al, 2007). This procedure has been shown to produce a robust escalation in drug intake (~30–40%) over a 2-week period, which is not observed in short access (ShA) controls given access to only 1 h/day (Ahmed and Koob, 1998). These sessions, and all FR1 sessions, typically show a loading and maintenance phase (Ettenberg et al, 1982; Wilson et al, 1971). That is, animals load up at the beginning by self-administering several injections in a short period of time, and for the remainder of the session, infusions become more evenly spaced resulting in cocaine levels being maintained within relatively narrow limits (Ahmed and Koob, 2005). As both LgA and ShA sessions provide animals with the opportunity to engage in loading behavior, the critical difference between these procedures is the length of the maintenance phase. Therefore, it would appear that the escalation effect is due to brain levels of cocaine being maintained for an extended period of time.

Although the LgA model in rodents, in many respects, mimics a 6-h cocaine ‘binge’ (Ahmed, 2005), clinical data suggest that humans take cocaine in a somewhat different pattern. Recent survey data from cocaine users indicate that experienced individuals consume the same amount of cocaine in a similar period of time as less experienced ones. However, more experienced subjects reported
getting significantly fewer ‘uses’ (<4) from each purchase indicating that,
compared with less experienced users, they were self-administering larger doses
separated by longer intervals between intoxicating events (Beveridge et al,
2012). The inter-use-interval was often much greater than an hour. Given that the
half-life of cocaine in humans is B40 min (Javaid et al, 1983), blood
concentrations are not maintained at a high level; instead, substantial reductions
in blood levels occur which then ‘spike’ with each intense intoxicating event. It is
likely, therefore, that there are qualitative differences in the dynamics of cocaine
levels between human drug users and subjects in typical FR cocaine self-
administration experiments.

The clinical data suggesting that addicts exhibit spiking rather than relatively
stable blood levels during a binge prompted this study. Here, we investigated the
effects of 2 weeks of exposure to a cocaine self-administration procedure that
engendered a spiking pattern of cocaine intake. Rats were given intermittent
access (IntA) to cocaine during 5-min trials that alternated with 25-min timeout
periods. This IntA procedure, which was developed while investigating the
relationship between the preferred dose of cocaine and the amount of drug on
board, results in substantial fluctuations in cocaine brain levels (Zimmer et al,
2011). The IntA procedure offers the opportunity to test whether maintained
levels are necessary for a change in addiction phenotype and whether rapid
spikes in brain levels are sufficient. Two IntA groups were included in the present
experiment; during each 5-min trial one group was given access to cocaine using
a hold-down (HD) procedure (IntAHD) while the other group was given access to
cocaine on an FR1 schedule (IntA-FR). These two groups were compared with LgA, ShA, and two other control groups. After 2 weeks of daily access to cocaine, the performance of the six groups was compared using a within-session threshold (TH) procedure (Oleson et al, 2011). Pmax, defined as the unit-price at which maximal responding occurs (Hursh, 1991), was assessed using a behavioral economic analysis of data. Here, we report that cocaine self-administration procedures resulting in repeatedly spiking drug levels produce more robust increases in Pmax than procedures resulting in maintained high levels of cocaine.
MATERIALS AND METHODS

Animals, Surgery, and Housing

The Wake Forest University Institutional Animal Care and Use Committee approved all experiments before the study commenced. Male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing approximately 350 g at the time of surgery were used as subjects. Before entering the study, rats were anesthetized with ketamine (100 mg/kg) and xylazine (8 mg/kg) and implanted with chronically indwelling Silastic cannulae (CamCaths, Cambridgshire, UK) as previously described (Liu et al, 2007). On recovery, animals were individually housed in 30_30_30 cm experimental chambers located in a temperature-controlled room (20–21 °C) maintained on a 12-h light–dark cycle (lights on at 1500 hours). A counterbalanced fluid swivel (Instech Laboratories, Plymouth Meeting, PA) mounted above the experimental chamber was used to connect an infusion pump (Razel Scientific Instruments, Stamford, CT) to the cannula using Tygon tubing enclosed within a stainless steel tether. Cannulae were flushed daily with heparinized saline to help maintain patency. Food and water were available ad libitum.

Self-Administration Training

After a 3- to 5-day recovery period, all animals were given access (FR1) to a cocaine-paired lever which, when depressed, initiated an intravenous injection of
cocaine (0.75 mg/kg, infused over 4 s). When each infusion was initiated, the lever was retracted and a stimulus light above the lever was illuminated, signaling a 20-s timeout period. Sessions occurred 7 days per week and began in the middle of the dark cycle. Sessions were terminated after a maximum of 20 infusions or after 6 h, whichever occurred first. An animal was considered to have acquired if 20 injections were self-administered for 2 consecutive days and a stable pattern of post-infusion pauses was apparent. Following training, separate groups of animals were assigned to one of six daily access procedures (ShA, LgA, HD, TH, IntA-HD, or IntA-FR - see below) for a 2-week access period after which Pmax was evaluated using a within-session TH procedure.

**IntA-HD Group**

Following acquisition, rats (N=6) were given access to cocaine using a HD procedure as previously described (Morgan et al, 2009). Briefly, the subjects were provided access to a lever which, when depressed, activated an infusion pump until the lever was released. An LED stimulus light above the lever was illuminated when the lever was depressed; that is, the light and pump were activated–inactivated concurrently. The dose self-administered was determined by the length of time the animal held the lever down (infusion rate was 0.375mg/kg/s) and by the pattern of responding. There were no timeout periods during these HD training sessions. The concentration of cocaine was 5 mg/ml. Rats were given daily 3-h sessions until the response pattern stabilized (which
generally took 2–3 days). After they had acquired the HD response, the schedule was switched to an IntA schedule in which subjects were given access to the retractable HD lever for twelve 5-min access periods on the HD schedule as previously described (Zimmer et al, 2011). In between each 5-min trial, a 25-min timeout period was enforced during which the lever was retracted. The lever presence–absence served as the only signal for drug availability. Animals received daily IntA-HD sessions for 14 consecutive days.

IntA-FR Group A separate group (N=5) was tested using the same IntA procedure described above (twelve 5-min trials separated by 25-min timeout periods) except that, instead of using a HD procedure, responding was reinforced on an FR1 schedule. The concentration of cocaine was 5 mg/ml. Each response produced a 1-s infusion of drug (0.375 mg/kg/inf) signaled by the illumination of a 1-s presentation of an LED stimulus light. Apart from the 1-s interval during which the pump was active, no timeouts were imposed to limit the number or timing of infusions within the 5-min access periods. This procedure lies midway in the continuum of control of drug dose and injection speed with the HD procedure on the one extreme and a fixed unit dose on the other. That is, with a small unit dose and no timeout it is possible for an animal to self-administer, for example, 4–5 small injections in an 8- to 10-s interval. Pilot studies have shown that the patterns of intake with this procedure are similar to those observed on the HD schedule.

LgA Group
Subjects in the LgA group (N=9) were given access to cocaine (0.75 mg/kg; infused over 4 s) on an FR1 schedule during daily 6-h sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

**ShA Group**

One group (N=6) was given access to cocaine (0.75 mg/kg; infused over 4 s) on an FR1 schedule during 2-h daily sessions for 14 consecutive days. At the start of each infusion, a stimulus light signaled a 20-s timeout period during which the lever was retracted.

**HD Group**

Subjects (N=7) in this group self-administered for 14 daily, 2-h sessions on the HD schedule of reinforcement as described by Morgan et al (2009). Briefly, the syringe-pump became active when the lever was depressed and became inactive when the lever was released. The size and speed of each dose was determined by the duration and spacing of HD responses (infusion rate was 0.375 mg/kg/s). An LED stimulus light above the lever was illuminated when the lever was depressed; that is, the light and pump were activated–inactivated concurrently. No timeout periods were imposed to limit the size or number of doses self-administered.
Within-Session TH Group

Subjects in the TH group (N=5) were given access to cocaine for 14 consecutive days on the within-session TH procedure (Oleson et al, 2011). Rats were given access to a descending series of 12 unit doses of cocaine (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4, 1.3, and 0 mg/injection) on an FR1 schedule during consecutive 10-min bins within a 2-h daily session. An LED stimulus light signaled the duration of the infusion and the corresponding time-out period (ie, equal to the pump duration). The lever was not retracted at any time during the session. Doses were manipulated by holding the concentration constant and adjusting the pump duration (see supplementary material of Oleson and Roberts (2009) for a full characterization of this approach and validation that the appropriate quantity of drug is delivered across all pump durations). Each TH session lasted 2 h. The TH group was exposed to these conditions during the 14-day test period. All groups, including the TH group, self-administered on this schedule following their 14-day test period for 3 consecutive days.

Data Analysis

A behavioral economic analysis was used to quantify results from this procedure. Behavioral economic theory has been successfully applied to drug self-administration in general (Bickel et al, 1993; Hursh, 1991) and TH procedures in particular (Espana et al, 2010; Oleson et al, 2011; Oleson and Roberts, 2009). In the current TH procedure, the descending series of doses (listed above) resulted in rats receiving access to cocaine across the following 11 ascending unit-prices:
2.4, 4.2, 7.5, 13.3, 23.7, 39.9, 75, 134, 242, 417, and 750 responses/mg. The primary dependent measure analyzed was the maximal price paid for cocaine (Pmax), which was determined to be the unit-price corresponding to the apex of the price-response function as previously described (Espana et al, 2010; Oleson et al, 2011). An example of the calculation of Pmax is shown in Figure 4a. The dose that maintains the highest rate of responding is defined here as the TH dose. This can be converted to Pmax by calculating the responses required at the observed TH dose to obtain 1mg cocaine. For example, if the TH dose were found to be 7.5 mg/inj, then the Pmax would be 133.3 responses/1 mg cocaine. In the vocabulary of behavioral economics, Pmax coincides with the point at which cocaine consumption changes from being maintained (inelastic demand) to not being maintained (elastic demand). Pmax was calculated from data averaged across 3 consecutive days of TH test sessions.

The TH procedure affords the opportunity to assess both appetitive and consummatory responding (as discussed in Oleson et al, 2011). Drug intake measured during early phases (10–40 min) of the TH procedure yields a measure of consummatory responding relatively unconstrained by price. Mean intake during 10–40 min were calculated for each animal. Intake was calculated in the early stage of the TH procedure because this is a period when the price of cocaine is relatively inexpensive and therefore less likely to constrain the animal's intake. The first 10 min were excluded to avoid the loading phase of the session.
Brain-Cocaine Concentration Model

Brain-cocaine concentrations were calculated as previously described (Zimmer et al, 2011) using equations employed by Pan et al (1991). Briefly, the equation

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

estimates the amount of cocaine in the brain compartment at time \( t \). This equation accounts for the dose of cocaine \( (d) \), the transfer of cocaine between the blood and the brain \( (k=0.233\text{min}^{-1}) \), the apparent brain volume \( (v=0.15\text{ l kg}^{-1}) \), and the removal of cocaine from the blood through redistribution \( (a=0.642\text{min}^{-1}) \) and elimination \( (b=0.097\text{min}^{-1}) \).

Drugs

Cocaine HCl, obtained from the National Institute on Drug Abuse (Research Triangle Institute, NC), was dissolved in a solution of sterilized saline 0.9% and passed through a microfilter \((0.45\text{ mm pore size})\). A 5mg/ml cocaine solution was used for HD, within-session TH, and IntA experiments; a 2.5 mg/ml solution was used for acquisition, ShA and LgA.
RESULTS

Figure 1 illustrates representative response patterns generated by each of the six procedures and the corresponding modeled brain levels. Each panel consists of the mathematically modeled brain-cocaine concentration (left axis) and the cumulative dose (right axis) self-administered throughout the session. Animals self-administering on an FR1 schedule (ShA and LgA) titrated their brain levels of cocaine by regularly spacing their responses (Figures 1a and b). The HD group displayed a steady rate of responding as previously described (Morgan et al, 2009). This led to a relatively stable level of cocaine throughout the session (Figure 1c). The within-session TH group also showed a stable level of cocaine only during the first part of the session with brain levels of cocaine falling as the price of cocaine increased beyond the animal’s Pmax (Figure 1e). The IntA animals had access to cocaine on either an FR1 (0.375 mg/kg/inf) or HD procedure during 5-min trials followed by a 25-min timeout period. This pattern of access led to large fluctuations of brain-cocaine concentrations throughout the 6-h session (Figures 1d and f). The pattern of intake was virtually identical between the IntA-HD and the IntA-FR groups. Subjects were observed to self-administer large doses (43.0 mg/kg) mostly in the first minute of the trial as previously described (Zimmer et al, 2011). It is important to note that the IntA-FR animals had no timeouts within their 5-min access period allowing them to self-administer clusters of injections at the beginning of the access period in a similar manner as the IntA-HD group.
Figure 1 Intake and modeled brain levels of cocaine for representative animals tested using six distinct self-administration procedures. Each panel shows the modeled brain levels of cocaine (left axis) and cumulative intake (right axis) throughout a session for an individual rat self-administering for cocaine on a ShA (a), LgA (b), HD (c), IntA-HD (d), TH (e), or IntA-FR (f) procedure.
Figure 2a illustrates the mean daily intake for each group during the 2-week test period. The data failed a test for homogeneity of variance and normality and were therefore transformed to rank orders. A two-way repeated-measures analysis of variance (ANOVA) revealed a significant DAYS effect ($F(13, 406)=4.19, p<0.001$), and measures of the LgA group intake showed a 21.8% increase. No significant GROUP _ DAYS interaction was observed. The average intake per session for each group is shown in Figure 2b. A significant GROUP effect was observed ($F(5, 32)=98.19, p<0.001$) and a Holm–Sidak post-hoc test revealed a significant difference of the LgA group compared with each of the other groups. No other comparisons were statistically significant.

The average number of responses per session is shown in Figure 2c. The data set failed a test of homogeneity of variance, therefore a Kruskal–Wallis H-test was conducted instead of an ANOVA. A statistically significant GROUP effect was observed ($H (5)=31.73, p<0.001$). A Dunns multiple comparison procedure revealed that the animals in the TH, HD, and IntA-HD groups responded more than ShA animals. The TH and HD groups also differed from the IntA-FR group.
Figure 2 Average intake and responses during the 2-week test period. Panel (a) shows the daily intake self-administered for each day over the 14-day test period for each group. Each symbol represents the mean intake self-administered for that group (±SEM). Panel (b) shows the average (±SEM) intake per session for each group. LgA animals self-administered significantly more cocaine than all other groups (p=0.001). Panel (c) shows the responses per session for each group. Bar represents the mean (±SEM) number of responses per session. The TH, HD, and IntA-HD groups responded significantly more than ShA animals (*p=0.05), and both TH and HD groups responded more than the IntA-FR group (p=0.05).
Following the 2-week test period, during which each group self-administered on their respective schedules, all animals were tested using a within-session TH procedure. Figure 3 shows representative event records for a ShA (top) and an IntA-HD subject (bottom). This comparison illustrates how animals might have similar rates of responding in the early part of the session but cease responding at very different times (corresponding to different doses). The IntA-HD animal increased its response rate throughout the session compensating for the decrease in unit dose and continued responding into the eleventh bin, whereas the ShA animal ceased responding after the sixth bin. Mean (±SEM) responses during each 10-min bin for every group are shown (Figure 3, bottom) illustrating the differences between groups in response rate during the TH schedule. These differences in intake were subjected to a behavioral economic analysis.
Figure 3 Response patterns and intake levels during the within-session TH procedure. Top: representative event records of animals responding on the TH schedule are shown for a representative ShA and IntA-HD animal. Note that the IntA-HD animal continued responding much longer into the session despite the increase response rate required to maintain preferred brain levels of cocaine. Bottom: average (±SEM) number of responses during each 10-min bin for each group is plotted. The apparent differences in these groups prompted a behavioral economic analysis (see Figure 4).
Figure 4 shows the behavioral economic analysis of data derived from the TH procedure. An example session is shown in Figure 4a to illustrate the method used to determine $P_{\text{max}}$. Responses (closed circles) and intake (open circles) are graphed during the 120-min session. Note that the animal in this example maintained a stable intake during the first 80 min of the session by increasing the response output in each consecutive bin. In bin 8, the highest number of responses is observed, and all subsequent bins were marked by a failure to maintain stable cocaine intake. This inflection point ($P_{\text{max}}$, dotted line) was determined for each animal. Figure 4b shows the average (±SEM) $P_{\text{max}}$ for each group. As the $P_{\text{max}}$ values were derived from an exponential series of doses, a log transform was performed on all data before ANOVA. This transform was necessary in order to meet the requirement of homogeneity of variance. A two part analysis was performed. The first addressed the various control conditions. The TH and HD control groups were compared with the ShA group in order to test whether the HD response pattern and/or the high rate of responding engendered by the TH and HD procedures had an effect. No significant difference was revealed by a one-way ANOVA ($F(2, 17)=1.37$, $p=0.28$). No statistically significant difference was observed between the IntA-HD and IntA-FR groups (mean $P_{\text{max}}=480.6±114.7$ and $480.1±96.2$, respectively) and data from these two groups were combined in the subsequent analysis. The central hypothesis that spiking blood levels would have a greater impact on $P_{\text{max}}$ relative to LgA and ShA was tested using a one-way ANOVA, which revealed a significant difference ($F(2, 25)=13.07$, $p<0.001$) between groups.
Holm–Sidak post-hoc analysis demonstrated that LgA and IntA animals had higher Pmax values than ShA, and IntA animals had higher Pmax values than LgA animals.
Figure 4 Behavioral economic analysis of data obtained from the within-session TH procedure. Panel (a) shows a representative animal responding during the TH schedule. Closed circles represent the number of responses emitted during each 10-min bin. Note that the unit dose of cocaine decreased during each bin leading to an increase in responses. Open circles show the intake of cocaine during each bin, demonstrating that total intake of cocaine remained relatively stable through the first 80 min of the schedule despite the rising price of cocaine. The dotted line represents the inflection point (Pmax) at which the animal failed to increase responding to maintain a stable level of intake. The Pmax value was calculated for all animals as the price (responses/mg of cocaine) animals reached before responding dropped off. Average (±SEM) Pmax values are plotted for each group in panel (b). Values statistically higher (p<0.05) than the ShA are denoted by an (*), and values higher than the LgA group are denoted by a pound sign (#).
Drug intake measured during early phases (10–40 min) of the TH procedure yields a measure of consummatory responding relatively unconstrained by price. A two part analysis was performed on the groups as described above. A one-way ANOVA (F(2, 17) < 1) on the 3 control groups (ShA, TH, and HD) revealed no significant differences in consumption during the early phase of the TH procedure. Similarly, a one-way ANOVA (F(2, 25) < 1) revealed no significant differences between the main test groups (ShA, LgA, and IntA).
DISCUSSION

The present experiment was designed to test the hypothesis that IntA is sufficient to increase Pmax values, a behavioral economic measure of the motivation to self-administer drug (Bickel et al., 1993; Hursh and Winger, 1995; Oleson et al., 2011; Oleson and Roberts, 2009). Six groups with histories of different self-administration procedures, which produced very different patterns of drug intake and drug dynamics, were compared. Combined data from the two IntA groups (IntA-HD and IntA-FR) showed robust increases in Pmax relative to both ShA and LgA animals. These groups received 12 opportunities to self-administer each day during 5-min trials separated by 25-min timeouts. Rats in these groups often self-administered large doses of cocaine (e.g., 43.0 mg/kg) usually in the beginning of the trial and in a relatively short period of time (~60 s). This pattern is consistent with our previous report (Zimmer et al., 2011) showing that rats rapidly self-administer large doses of drug when blood levels have been forced to low levels. These results suggest that rapid spiking of brain-cocaine levels is sufficient to increase Pmax.

The application of a well-established mathematical model for estimating cocaine levels in brain (Ahmed and Koob, 2005; Nicola and Deadwyler, 2000; Pan et al., 1991; Wise et al., 1995) helps illustrate the important differences between the groups during daily self-administration sessions. Brain concentrations of cocaine rose relatively rapidly in all groups at the beginning of the session. This well-characterized phenomenon has been referred to as a loading phase (Wilson et al., 1971), and is typically followed by a maintenance phase during which drug
levels are maintained within relatively narrow limits. In this study, four groups were allowed to progress into the maintenance phase for varying lengths of time. The TH group maintained blood levels for only 60–90 min (depending on individual Pmax), the ShA and HD groups maintained blood levels for 2 h, and the LgA group for 6 h. By contrast, the two IntA groups were exposed to a very different drug-level dynamic—that is, 12 distinct cocaine spikes within each 6-h session. This spiking pattern of intake was associated with the highest Pmax values. Note that as the IntA procedures do not allow blood levels to be maintained, it would appear that sustained blood levels are not necessary and that spiking blood levels are sufficient to increase the maximum price paid for cocaine.

The hypothesis tested here that spiking cocaine levels might be important to the addiction process was prompted by both preclinical and clinical data. On the clinical side, textbooks and the research literature emphasize the importance of the ‘rush’ or intense subjective effects, which are mediated by larger doses and faster routes of administration (Seecof and Tennant, 1986; Kumor et al, 1989; Gorelick, 2009; Volkow and Li, 2009). Presumably, these intense intoxicating events increase the probability of future use. Over time, individual or grouped intoxicating events can progress to a cyclical binge-abstinent style of intake (Gawin, 1991). The idea that ‘binge-like’ intake is an important part of the addiction process has prompted the development of rodent models that allow for daily ‘binges’ to occur, such as in the LgA procedure (Ahmed and Koob, 1998).

What has been missing from the clinical literature is information on the pattern of
drug use within a binge. A recent survey of experienced cocaine users shows the interval between uses within a cocaine binge might be, on average, well over an hour (Beveridge et al, 2012). Given the short half-life of cocaine (~40 min; Javaid et al, 1983) in humans, these survey data suggest that blood levels are not maintained throughout the binge period but may decrease substantially between each intoxicating event. Although more clinical data on dose size and inter-use interval within a binge would be helpful, it appears that fast-rising and subsequent decline in blood levels is a pattern that can lead individuals down the addiction cycle.

The spiking cocaine-level hypothesis was also suggested by recent preclinical data. A self-administration procedure that produces spiking brain levels in rats was developed in our lab while we were investigating the relationship between the preferred dose of cocaine and drug levels in the brain (Zimmer et al, 2011). Animals were given access to drug during 5-min trials, and, in order to force a decline in blood levels, the trials were separated by timeout periods ranging from 10 to 25 min. The primary finding of the experiment was that the selected dose of cocaine was inversely related to blood levels. That is, rats selected relatively low doses of drug when blood levels were high and selected very large doses (~3 mg/kg/inj) of cocaine when blood levels were low. After completion of the experiment, animals were given access to cocaine on a progressive ratio (PR) schedule and were found to have markedly elevated breakpoints (unpublished). Although we had no control group to compare results with, it appeared that animals that had experienced a spiking pattern of cocaine intake showed much
higher breakpoints than was typically seen in our lab. This study represents an attempt to quantify the increases in motivation we observed relative to groups that self-administered cocaine with varying drug dynamics.

A within-session TH procedure was chosen to compare the effects of the different cocaine self-administration histories. This procedure was adapted from a between-session TH procedure in which a series of doses were tested on consecutive days (Oleson and Roberts, 2009; Zittel-Lazarini et al, 2007). The procedure involves giving access to cocaine on an FR1 schedule at a fixed unit dose, which is reduced through a series of 11 doses every 10 min (see Materials and methods section). A behavioral economic analysis of the response rate and drug intake at each interval yields measures of both consumption and maximum price paid and thus affords the opportunity to investigate appetitive and consummatory aspects of self-administration within the same procedure.

Consummatory behavior is reflected as cocaine intake early in the session (at high unit doses) when the response cost is low. Appetitive behavior is assessed later in the session by determining the lowest unit dose (ie, highest unit price) that maintains consumption. Typically in self-administration studies, price is manipulated by holding a drug dose constant and increasing the response requirement (Cosgrove and Carroll, 2002; Wade-Galuska et al, 2007); however, fixing the response requirement and decreasing the available unit dose accomplishes the same thing (Bickel et al, 1990). Here, the decreasing series of unit doses resulted in an ascending series of unit prices (2.4, 4.2, 7.5, 13, 24, 40, 77, 133, 244, 416, and 750 responses/mg). Pmax values are theoretically related
to breakpoints derived from a PR schedule. Although the PR and TH procedures measure different aspects of appetitive behavior – work output to obtain a large bolus injection (PR) vs work output to maintain a constant blood level despite diminishing returns (TH) – there seems to be a high correlation between the two dependent measures. Indeed, several pharmacological manipulations that have been shown to have an effect on PR breakpoints such as haloperidol (Depoortere et al, 1993; Roberts et al, 1989) or baclofen (Roberts et al, 1996; Brebner et al, 2000) had a similar effect on TH Pmax (Oleson et al, 2011). The PR breakpoint and the TH Pmax thus permit detailed assessment of the relationship between work output and obtained reinforcement and together may be particularly helpful in characterizing changes in drug intake produced by prolonged drug exposure (Vezina, 2004).

In the present experiments, spiking brain levels were produced using an IntA procedure with two different response requirements. The HD response was first described by Morgan et al (2009) as an attempt to bypass unit dose and give the animal more control over the size and speed of injections. Animals appeared to titrate their blood levels within a narrow range and adjusted their response pattern to compensate changes across a 16-fold range of cocaine concentrations (Morgan et al, 2009). For comparison, we also included an IntA group reinforced under an FR1 schedule during the 5-min access periods. It is important to note that no timeout was used with the FR1. Thus, animals were permitted to self-administer multiple injections (0.375 mg/kg/inf) within a few seconds. The IntA-FR and IntA-HD groups showed similar intakes during the 5-min trials and across
sessions. We found no observable differences in intake or Pmax values between these two groups. Comparing the two IntA groups with controls also allowed us to rule out the possibility that the increase in Pmax was produced by overall cocaine intake (Figure 2b) or by high rates of responding (Figure 2c). Therefore, the increases in Pmax observed in IntA animals are likely due to the spiking brain levels of cocaine throughout the session. Although the IntA-HD group offers some unique advantages in terms of data analysis (eg, selected dose size, speed), the IntA-FR schedule represents an easier method of generating the phenotype reported in this study.

The LgA procedure represents the best current model of escalation of intake. Animals in this paradigm reliably show increases in intake of 30–40% over the course of 2 weeks (Ahmed and Koob, 1998). In most cases, this procedure has resulted in an increase in motivational measures such as PR breakpoints (Paterson and Markou, 2003; Wee et al, 2008; but see Liu et al, 2005a; Quadros and Miczek, 2009) and drug-induced reinstatement responding (Mantsch et al, 2008). The effect of LgA on Pmax appears to depend on procedural variables. We previously observed a decrease in Pmax in LgA animals using a between-session TH procedure (Oleson and Roberts, 2009). In that procedure, the dose of cocaine decreased each day over the course of 11 days. In this study, the entire dose-effect curve was evaluated within a single session, and the LgA group showed an increase in Pmax. These two TH procedures address different aspects of the motivation to self-administer cocaine. The within-session TH procedure allows animals to take large loading doses of cocaine when the price
is relatively inexpensive; the price then increases through the session. Pmax in this case appears to measure the maximal price a rat will pay to maintain brain levels of cocaine. By contrast, the between-session TH procedure effectively measures the maximal price an animal will pay to load cocaine brain levels. It appears that LgA can, under some circumstances, produce an increase in some motivational measures.

Our conclusion, that spiking brain levels of cocaine produce an increase in the motivation to self-administer drug, depends on the accuracy of the mathematical model used to estimate brain-cocaine concentrations. The equation used by Pan et al (1991) is based on standard pharmacokinetic principles that have been validated for many different drugs and in many different systems (Karan et al, 2009). However, the specific variables used to represent rat blood volume, cocaine redistribution and degradation must be verified in order to have confidence in applying the equation to this study. Fortunately, use of this model has been widespread. Many self-administration studies have applied the equation to estimate brain concentrations of cocaine in live animals (Ahmed and Koob, 2005; Samaha et al, 2002; Zernig et al, 2007; Zimmer et al, 2011), and the equations have also been applied in studies using electrophysiology (Nicola and Deadwyler, 2000; Peoples and Cavanaugh, 2003; Peoples et al, 2004, 2007), microdialysis (Wise et al, 1995) and voltammetry (Hermans et al, 2008; Stuber et al, 2005a, b). These studies have demonstrated that the modeled brain-cocaine concentrations
are highly correlated with NAc dopamine levels (Hermans et al, 2008; Shou et al, 2006; Wise et al, 1995) as well as cocaine-induced locomotor behavior (Shou et al, 2006).

An extensive literature has demonstrated that continuous vs intermittent administration of psychostimulant drugs produces very different consequences on behavior and neurochemistry (for review, see Robinson and Berridge, 2008). For example, it has been shown that daily injections of cocaine induce long-lasting behavioral sensitization whereas continuous (minipump) administration produces behavioral tolerance (Reith et al, 1987; King et al, 1992). Differences in neurochemistry have also been shown, such as subsensitivity or supersensitivity of the D2 autoreceptor following intermittent or continuous administration of cocaine respectively (Jones et al, 1996). Intermittency typically refers to daily or every-other-day administration of drug; however, the present data suggest that the theoretical intermittency/continuous distinction might reasonably apply to 6-h cocaine self-administration sessions. Given that a 25-min timeout period allows for a ~90% clearance of cocaine, the twelve 5-min access periods used here would constitute an intermittent dosing regimen; a 6-h LgA session would more closely represent continuous drug delivery. It has been suggested that continuous administration is the better model of a human binge (eg, King et al, 1994) based on the assumption that addicts self-administer at frequencies that result in sustained cocaine levels. However, recent clinical studies have challenged this assumption (Beveridge et al, 2012; see Introduction section). In
fact, the pattern of human intake may more closely resemble intermittent administration. Future studies will be necessary to fully characterize the role of intermittency on the transition to drug addiction as well as the underlying neurobiological mechanisms involved. Here, we report that the schedules that induce both fast rise-times and large fluctuations in brain-cocaine concentrations produce the most robust increases in motivation to self-administer cocaine. The observed increases in \( P_{\text{max}} \) could not be accounted for by intake, contingency or rates of responding. Measures of \( P_{\text{max}} \) were higher in IntA animals than LgA animals, indicating that when it comes to producing an increase in motivation the pattern of intake is likely more important than the total amount consumed.
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SUMMARY AND CONCLUSIONS
The purpose of creating animal models of any disorder is to establish the underlying physiology and make predictions about the potential success of various treatments. Substance use disorder is associated with multiple, complex symptoms that can prove especially difficult to translate to rodent models (5th ed.; American Psychiatric Association, 2013). Since drug addiction involves the emergence of unique patterns of drug taking and drug seeking, one strategy to assist with the interpretation of results from basic science research is to simplify the numerous symptoms into changes in consumptive or appetitive behavior (Roberts et al. 2013). Applying an emphasis on how results from the myriad animal models of drug addiction can be interpreted as changes in drug-seeking vs drug-taking provides a useful tool to organize the literature and is a major theme underlying the work of this dissertation.

As discussed in chapter 1, behaviors directed towards interacting with and consuming the drug are fundamentally different from those focused on seeking out and attaining the drug, with distinct underlying neurobiological mechanisms. However, it was also discussed that making this distinction in IV self-administration procedures can be difficult since the consumptive and appetitive-directed behaviors are both directed towards the same operant response. Therefore, careful analysis is needed when interpreting results from self-administration studies to identify the resulting changes on appetitive or consumptive behaviors. It is hoped that a better understanding of the underlying neurophysiology associated with these behavioral changes will provide a focus
for targeted pharmacological interventions to treat or reverse symptoms of this problematic disease.

One of the major focuses of this work has been on improving our understanding of how drug taking is regulated in rodent self-administration paradigms. In chapter 2 it was shown that when dose was made a dependent measure, a wide discrepancy in selected doses was observed within the same session. Specifically, large loading doses were selected at the beginning of the session and smaller maintenance doses were selected for the remainder of the session. Additionally, it was shown that loading doses could be induced mid-session by introducing forced timeout periods, and there was a significant inverse relationship between the size of these induced loading doses and the extent of the decline of estimated brain-cocaine levels. In chapter 3, the regulation of the maintenance-sized doses was investigated by changing the concentrations of cocaine under HD schedule conditions and measuring the resulting self-administration behavior. It was shown that the total time the pump was activated changed in a near proportional manner with the manipulations of cocaine concentration resulting in similar cocaine intake levels regardless of the concentration. In addition, the size of each selected dose was correlated significantly with the subsequent – but not previous – inter-dose interval. This finding indicated that the selected dose determined the length of the subsequent post-infusion pause. These studies have greatly increased the depth of our understanding of cocaine intake regulation by demonstrating separate classes of loading and maintaining behavior and showing how each of these behaviors are
regulated within a session. This work has provided strong evidence in support of
the titration hypothesis of cocaine intake. That is, it appears that cocaine
consumption behavior is driven by titration principles with brain-cocaine levels
being tightly regulated around an elevated satiety threshold (Tsibulsky and
Norman, 1999; Wise et al. 1995; Zimmer et al. 2011, 2013). When brain levels
are at or near the satiety threshold, small maintenance-sized doses are selected
(and presumably preferred) to elevate brain levels above the threshold, and post-
infusion pauses are initiated until brain levels return to this threshold (Zimmer et
al. 2013). When brain levels are markedly low, large loading-sized doses are
selected to rapidly increase brain levels to the satiety threshold (Zimmer et al.
2011).

One important concept that has emerged from the series of experiments
examining psychostimulant intake regulation is the interpretation of unit doses of
cocaine in self-administration paradigms. The self-administration literature has
traditionally placed much emphasis on unit doses, with focused interpretations of
shifts in dose-effect functions. However, the results presented here have shown
that greater emphasis should be placed on the titration of brain levels of cocaine
around a constant level. That is, consumption of cocaine in a highly regulated
manner can in some circumstances provide misleading results that if interpreted
using traditional concepts of unit dose lead to incorrect conclusions. The clearest
example of this is in interpreting FR dose-effect curves. Traditional operant
experiments have a long history of interpreting FR response-rates as signifying
the reinforcing value of the reward substance. However, with IV cocaine self-
administration behavior the FR response rate follows rules of cocaine
consumption rather than reinforcing value at each dose. Thus, the lowest doses
of cocaine (above a threshold) result in the greatest responding and increasing
doses results in decreased response rates on FR schedules. Whereas with
schedules of reinforcement geared towards examining motivational aspects of
responding the exact opposite relationship between dose and responding is
observed. Additionally, this work has demonstrated that the self-selected dose of
cocaine changes within a single session and can be accounted for by concepts
of drug titration. These findings suggest concepts such as the “most reinforcing”
dose of cocaine change dynamically from moment to moment.

Another primary focus of this body of work was examining the effects of various
self-administration histories on subsequent drug-seeking behavior. Results from
chapter 4 demonstrated that PR breakpoints decreased steadily throughout a
cocaine binge (FR1 for 12-24 hrs). These decreases recovered to baseline within
2-3 days, and it was also shown that the breakpoint-decreasing effects applied to
the entire PR dose-response curve. Additionally, manipulating the dose of
cocaine within the binge sessions resulted in subtle changes on intake but led to
no measurable changes in breakpoint. Results from chapter 5 demonstrated
robust increases in the behavioral price rats would pay for cocaine following a
history of repeated spiking of estimated brain-cocaine levels. This study
demonstrated that sustained blood levels are not necessary to produce
increased motivational responding for cocaine and spiking blood levels appear to
be sufficient. Therefore, it appears that pattern of intake can be an important
factor in the progression of the addiction process. Taken together, this work has demonstrated that heavy use of psychostimulants such as cocaine is not necessarily more effective at progressing the addiction process, and it appears that pattern of intake can have profound effects on subsequent drug-seeking behavior.

In conclusion, substance use disorder is a chronic disease effecting a very large population, and to date no treatments have been approved for the treatment of psychostimulant addiction (Preti 2007; Vocci et al. 2005). This is true despite the fact that the underlying neurophysiological changes associated with cocaine use have been extensively examined for nearly half a century. Perhaps one contributing factor to this disturbing failure to establish successful treatments is the weakness of the behavioral models used to study drug-induced changes in physiology. The bulk of the studies presented and discussed in this dissertation were focused on dissecting and improving our understanding of the basic self-administration models used to study drug addiction. Interpreting results as changes in drug-seeking or drug-taking was emphasized although distinguishing these two classes of behavior in IV drug paradigms can be difficult. The regulation of cocaine consumption mechanisms was discussed, and the effects of many different procedural protocols on subsequent drug-seeking or drug-taking behavior was reviewed and presented. Much future work is still needed to further elucidate the effects of various behavioral histories on subsequent self-administration behavior. For example, while it was shown in Chapter 5 that pattern of intake is a powerful factor in the development of an addictive
phenotype, this procedure has not been extensively characterized. Many parameters can be manipulated in this paradigm such as the number of access periods provided per hour, the number of hours in each session, the number of days the animal receives IntA, etc. Additionally, future work is needed to specifically characterize the underlying neurobiology associated with changes in drug-seeking or drug-taking. The use of sophisticated behavioral models will help separate neurobiological changes that are relevant to the progression of the addiction process from those that are associated with acute drug use.
References


Appendix I

Reduction of the reinforcing efficacy of cocaine by continuous d-amphetamine treatment in rats: importance of active self-administration during treatment period

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Introduction

While many dozens of compounds have been tested in clinical trials for the treatment of psychostimulant abuse, only a few have shown promising outcomes (Mendelson and Mello 1996; Preti 2007; Voci and Elkashef 2005; Voci and Ling 2005; Voci et al. 2005). One of these treatment drugs is orally administered d-amphetamine, which has been shown to safely and effectively reduce amphetamine intake in amphetamine abusers (Charnaud and Griffiths 1998; Fleming and Roberts 1994; White 2000). More recently, studies examining the effects of oral d-amphetamine on methamphetamine abuse have shown that it can reduce craving, withdrawal symptoms and intake (Galloway et al. 2010; Longo et al. 2009). With the agonist therapy treatment model in mind (Shearer, 2008), these findings have been extended to another addictive psychostimulant, cocaine. In three randomized, double-blind, placebo-controlled clinical trials, cocaine-dependent individuals showed modest but significant decreases in cocaine use relative to placebo after treatment with d-amphetamine (Grabowski et al. 2001, 2004; Shearer et al. 2003). Additional experiments have demonstrated that d-amphetamine treatment reduces the subjective effects and decreases the dose of cocaine selected in a double-blind choice procedure of two cocaine doses (Rush et al. 2009, 2010).

These promising clinical outcomes are consistent with data from non-human primate studies on the therapeutic efficacy of sustained d-amphetamine treatment. While initial investigations failed to show a specific effect of either oral (Foltin and Evans 1999) or acute pre-injections (Mansbach and Balster 1993) of
d-amphetamine on cocaine- versus food-maintained responding, subsequent studies showed more promising results. *Continuous* d-amphetamine administered by a constant iv drip produced dose-dependent effects in a variety of self-administration paradigms including second-order (Negus and Mello 2003b) and progressive ratio (PR) schedules of reinforcement (Czoty et al. 2010, 2011; Negus and Mello 2003a), and a choice procedure in which animals selected between cocaine (0–0.1 mg/kg/inj) and food pellets (Negus 2003). In each of these studies, specific d-amphetamine doses were identified that produced a decrease in cocaine self-administration with little or no effect on food-maintained responding. Notably, Czoty et al. (2011) reported such a result in every animal tested when the dose of d-amphetamine was adjusted each week on an individual basis, in an effort to mimic clinical treatment conditions. Breakpoints, the primary dependent measure of progressive ratio schedules, remained low for the duration of the treatment period (in some cases, many weeks), but returned to baseline shortly after d-amphetamine treatment was suspended.

These decreases in cocaine-reinforced breakpoints shown in non-human primate studies have been replicated with rodents, and the dose-dependent interactions between d-amphetamine and cocaine have been extensively characterized. The effect depends on the length of treatment and the self-administered dose of cocaine. Testing across a range of self-administered cocaine doses (0.19-1.5 mg/kg/inf) showed that the lowest doses were the first to be effected. That is, one week of d-amphetamine delivered at a constant rate (5 mg/kg/day via osmotic
minipump) resulted in a decrease in breakpoints only at the lowest unit dose. When d-amphetamine treatment was extended to two weeks, significant decreases in breakpoints were observed at cocaine doses up to 0.75 mg/kg/inf (Chiodo et al. 2008). Interestingly, a follow-up study showed that d-amphetamine treatment during low-dose (0.19 mg/kg/inf) cocaine self-administration resulted in a sustained downward shift of the entire PR dose-effect curve including the highest dose tested (1.5 mg/kg/inf; Chiodo and Roberts 2009).

Another aspect revealed by rodent studies is that animals receiving two weeks of d-amphetamine treatment (5 mg/kg/day) without the opportunity to self-administer cocaine during this time showed no reductions in post-treatment breakpoints (Chiodo et al. 2008). Taken together, these studies suggest that d-amphetamine can produce a significant reduction in the entire PR dose-effect curve; however, this reduction appears to depend on an interaction between cocaine and d-amphetamine.

The current study tested two separate hypotheses of why co-administration of d-amphetamine and cocaine is necessary for the putative therapeutic effect. The first hypothesis is that the mechanism driving the reduction in breakpoints is a purely pharmacological interaction between the two drugs. That is, co-administration of cocaine and d-amphetamine could be producing cross-tolerance or other receptor-mediated changes that decrease the reinforcing efficacy of cocaine. If this mechanism were responsible, it would be predicted that sustained levels of d-amphetamine in combination with passively administered cocaine would be sufficient to reduce breakpoints when tested after
the treatment period. An alternative hypothesis is that the reduced breakpoints reflect an associative process taking place during the d-amphetamine treatment period, in which the animals learn that the rewarding effects of cocaine are decreased and/or that the aversive effects of the drug have become more potent. This hypothesis would predict that animals must self-administer cocaine during treatment for the reductions in breakpoint to occur. The present study was designed to discriminate between these two potential mechanisms by providing continuous d-amphetamine while rats simultaneously received cocaine, either by active self-administration or non-contingent cocaine infusions. Here we show that active responding is an important factor for the d-amphetamine-induced decreases in breakpoint to be observed, suggesting that associative learning processes are involved.
Materials and methods

Animals

Subjects were male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) weighing approximately 350 g (~12 weeks old) at the start of the experiment. All animals were maintained on a reverse 12 h light/dark cycle (lights on at 1500 h), and were habituated to this schedule for a minimum of 7 days before surgical implantation of a catheter. Food and water were made available ad libitum. Animals were housed individually in their stainless steel custom-made self-administration chambers (30 x 30 x 30 cm) throughout the experiment.

Surgery

Catheter implantation

Subjects were anesthetized with an intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (8 mg/kg), and implanted with a chronically indwelling Silastic jugular catheter (CamCaths, Cambridgeshire, UK). The catheter was attached to a subcutaneous (SC) plastic anchor that exited through the skin on the dorsal surface in the region of the scapulae. Tygon tubing, enclosed by a stainless steel protective tether, connected the plastic anchor to a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA., USA; Part # 375/22PS) mounted above the experimental chamber. An infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) located outside of the self-administration chamber was connected to the swivel by Tygon tubing. Rats
were given a 3-5 day recovery period before starting self-administration. Catheters were flushed daily with heparinized saline to maintain patency.

**Mini-pump implantation**

Rats were anesthetized with gas containing oxygen, nitrogen and halothane (3% to induce anesthesia and 1.5% for the remainder of the surgery). An osmotic mini-pump (Alzet Model 2001, Durect, Cupertino, CA, USA) containing saline or d-amphetamine (5 mg/kg/day) was implanted (SC), rostral to the plastic catheter anchor (see above) with the flow moderator pointing rostrally. After 7 days all mini-pumps were removed immediately following that day’s self-administration session so that subjects had 18 hours of recovery before self-administering the following day.

**Cocaine self-administration**

The start of a self-administration session was signaled by the extension of the lever into the experimental chamber. Sessions were run 7 days/week starting at 1000 h (during the dark phase) and lasted 6 hours. Session length was chosen to conform to previous experimental parameters (Chiodo et al 2008; Chiodo and Roberts 2009). Successful completion of the response requirement led to an infusion which consisted of ~0.1 ml of cocaine infused over 4-5 second (depending on body weight). Self-administration training consisted of 1.5 mg/kg injections under an FR1 schedule of reinforcement followed by a 20 second timeout period that was signaled by the retraction of the lever and illumination of
a cue light located immediately above the lever. Animals continued the training protocol until they self-administered the maximum infusions available (40) within the 6 hour session for five consecutive days and displayed consistent post-infusion pauses.

**Progressive ratio**

Upon completion of the training protocol, rats in the contingent-cocaine group were switched to a progressive ratio schedule of reinforcement (0.19 mg/kg/inj). This dose was chosen to conform with previous reports that d-amphetamine reduced breakpoints when rats self-administer low-dose but not at higher doses of cocaine (Chiodo et al 2008; Chiodo and Roberts 2009). On this schedule the response requirement was increased for each consecutive infusion in the following sequence: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. The breakpoint was defined as the number of infusions earned before a one hour period elapsed without completion of the next ratio. Breakpoints are traditionally defined as a period of non-responding (Richardson and Roberts 1996); 60 minutes was selected for consistency with previous publications and because this criteria represents a very conservative time parameter. Most rats reached breakpoint criteria within ~90 minutes, therefore all sessions were run for 6 hrs to ensure no sessions ended before a breakpoint had been reached.

**Non-contingent cocaine administration**

Upon completion of the training protocol, rats in the non-contingent-cocaine group were given passive infusions of cocaine during the 7 day treatment period.
The dose size was identical to the contingent group (0.19 mg/kg/inj). The number and timing of infusions was determined by taking the mean time to each injection and total number of injections from the contingent animals. That is, the rats in the d-amphetamine-contingent-cocaine group took an average of 2 minutes between the first and second infusions and 4 minutes between the second and third, etc. These group means were calculated for each of the 7 days the animals self-administered to adjust for potential changes in the spacing of infusions over the course of the treatment period.

**Drugs**

Cocaine HCl (National Institute on Drug Abuse, Rockville, MD) was dissolved in sterile saline (0.9% NaCl) in concentrations of 0.625, 1.25, 2.5 and 5 mg/ml and passed through a micro filter. All concentrations are expressed as the weight of the salt. d-Amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline (0.9%).

**Data analysis**

The primary dependent variable of interest was the final ratio achieved under a PR schedule of reinforcement. Because the exponential data produced from PR schedules violates the assumption of homogeneity of variance, the total number of reinforcers delivered (breakpoint) was used in all statistical analyses (Richardson and Roberts 1996). A two-way, repeated measures ANOVA was
used for statistical comparison between groups and across days, and the Holm-Sidak method was used for all post hoc evaluations. The probability (p) level was set to 0.05. All statistics and graphs were conducted using SigmaPlot (version 11).
Results

Figure 1 shows the average breakpoint completed for each group for the duration of the experiment. The gray shading indicates the treatment period during which animals received a constant SC infusion of either saline (0.9%; squares; N = 7) or d-amphetamine (5 mg/kg/day; circles; N = 8). Note that the non-contingent cocaine group (closed circles; N = 8) also received d-amphetamine during the treatment period. However, no data appear on the graph during this period because animals in this group were not self-administering cocaine but were instead receiving passive infusions in a pattern similar to that of animals in the contingent-cocaine groups. Because the non-contingent group did not reach breakpoints during the treatment period, separate statistical tests were run on the baseline (days 1-3), treatment (days 4-10), and post-treatment (days 11-17) days.

A two-way, repeated measures ANOVA revealed no significant differences between groups [F(2, 20) < 1], days [F(2, 40) < 1], and no interaction [F(4, 40) < 1] during the baseline period.

During the treatment period, breakpoints in the d-amphetamine treatment group were reduced by 35% (treatment day 7 vs last day of baseline). A two-way, repeated measures ANOVA revealed a significant effect of group [F(1, 13)=5.45; p<0.05], and a group X day interaction [F(6, 78) = 4.67; p < 0.001]. No effect of day was found [F(6, 78) < 1]. Post hoc analyses showed significant differences between saline and d-amphetamine treatment on days 8-10 (p < 0.05 – indicated by asterisks in Fig 1). These results indicate that the elevated levels of d-
amphetamine (5 mg/kg/day) via osmotic minipump significantly reduced PR breakpoints relative to the saline controls.

During the post-treatment period (days 11-17), breakpoints in animals that self-administered cocaine while receiving d-amphetamine treatment remained depressed initially for 4 days before gradually recovering. Animals that received d-amphetamine combined with passive infusions of cocaine during the treatment period showed no differences in breakpoints relative to the saline controls. A two-way, repeated measures ANOVA revealed a trend towards a significant effect of group [F(2, 20) = 2.90; p = 0.08] in the post-treatment period. No significant effect of day was found [F(6, 120) < 1], but a significant group X day interaction was present [F(12, 120) = 2.57; p < 0.01]. A Holm-Sidak post hoc test revealed significant differences between the two contingent-cocaine groups (i.e., d-amphetamine and saline mini-pump groups) on days 12-14 (p < .05 – indicated by asterisks in Fig 1) and also between the two d-amphetamine treatment groups (i.e., contingent-cocaine and non-contingent-cocaine) on days 11-14 (p < .05 – indicated by crosses in Fig 1).
**Fig. 1** Effects of d-amphetamine treatment on self-administration of low-dose cocaine (0.19 mg/kg/inf) under a PR schedule. Points represent the mean (±SEM) breakpoints (corresponding final ratios are shown on the right y axis). Shaded portions represent the d-amphetamine treatment period (7 days of 5 mg/kg/day). Circles represent amphetamine treatment and squares represent saline control. The non-contingent cocaine group (closed circles) received d-amphetamine during the treatment period combined with passive cocaine, and therefore no breakpoints were achieved during this time. Breakpoints that were statistically lower (p < 0.05) than the contingent-cocaine-saline group (open squares) are marked with asterisks, and those lower than the non-contingent-cocaine (filled circles) group are marked by daggers.
Discussion

The present study adds to a growing collection of preclinical studies in both rodents (Chiodo et al. 2008; Chiodo and Roberts 2009; Peltier et al. 1996) and non-human primates (Czoty et al. 2010, 2011; Negus 2003; Negus and Mello 2003a, 2003b) that demonstrate robust decreases in cocaine-maintained responding following continuous d-amphetamine treatment. Specifically, responding for cocaine was reduced under a PR schedule of reinforcement while d-amphetamine was continuously administered to rats via SC osmotic mini-pumps and responding remained low for four days following cessation of treatment. However, rats that received the same d-amphetamine treatment in conjunction with passive cocaine infusions showed no change in breakpoints following the treatment period (Figure 1). Thus, this study demonstrates that active responding for cocaine during the treatment period is an important factor in the putative therapeutic value of d-amphetamine.

The current study taken together with previous findings provide insight into the mechanisms responsible for the effect of continuous d-amphetamine treatment on cocaine self-administration. Chiodo et al. (2008) demonstrated that when animals were deprived of the opportunity to self-administer cocaine during the treatment period d-amphetamine had no effect on subsequent breakpoints. This finding suggested that having cocaine present during the treatment period increased d-amphetamine efficacy. A simple explanation of this effect is that a pharmacological interaction between these two drugs was occurring such as cross-tolerance that decreased subsequent responding for the drug (Peltier et al. 200...
1996). However, the results of the present experiment, that d-amphetamine has no effect on breakpoints when administered simultaneously with passive cocaine infusions, appear to rule this explanation out. Instead, the mechanism driving d-amphetamine efficacy appears to be an associative process that requires active drug seeking during the treatment period.

The question remains as to the importance of the self-administration schedule in creating these observed changes in self-administration behavior. The efficacy of continuous d-amphetamine on reducing rates of responding for psychostimulants has been demonstrated using PR (Chiodo et al. 2008; Chiodo and Roberts 2009; Czoty et al. 2010, 2011; Negus and Mello 2003b; Peltier et al. 1996) and second order (Negus and Mello 2003a) schedules of reinforcement. The PR schedule of reinforcement is generally used as a method to systematically increase cocaine price until responding is abolished (Richardson and Roberts 1996; Roberts et al. 2007). It is possible that the associative changes observed in the present study are facilitated by the rat seeking cocaine under a schedule of reinforcement that requires a high work output. This hypothesis would explain why d-amphetamine treatment in combination with low doses of cocaine appears to be more effective at reducing breakpoints than high doses, since the price of cocaine (i.e., responses per mg) would be much higher with a low dose of cocaine.

Finally, the present findings are consistent with a growing clinical literature showing that some pharmacological treatments for drug dependence show greater efficacy when patients simultaneously use the abused drug. For example, naltrexone reduced the subjective effects when patients engaged in their normal
drinking patterns (Ray et al. 2007; Rosenthal 2006), and individuals who drank more regularly during the naltrexone treatment benefited the most (Ray et al. 2010). One possible explanation of these findings is that the combination of alcohol and naltrexone engaged extinction learning processes. Other drugs such as disulfiram have been shown to have clinical success in reducing use of alcohol by triggering intense aversive reactions (e.g., nausea, headache, etc.) when alcohol is concurrently consumed (Wright and Moore 1990). These treatments that produce greater effects when patients use the abused drug could in some ways be ideal, given that they would have even greater efficacy when patients relapse.

In conclusion, the present experiment along with previous studies demonstrates that continuous d-amphetamine can decrease cocaine-maintained responding on a PR schedule and can dramatically shift the cocaine dose-response curve downward (Chiodo et al. 2008; Chiodo and Roberts 2009; present study). Additionally, there appears to be residual effects since breakpoints remained low in the post-treatment period. It appears that drug seeking during the treatment period is an important factor for the development of the putative therapeutic effect suggesting that learning mechanisms play an important role. This could be an important point for future clinical studies examining the effects of d-amphetamine on psychostimulant abuse. Treatment with d-amphetamine might be more efficacious in settings where patients have access to drugs such as an outpatient facility as opposed to ones that include forced abstinence from the drug.
References


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CURRICULUM VITAE

Education

2008-Present Wake Forest University School of Medicine
Winston-Salem, NC
• Earning a PhD in Neuroscience
• Advanced to PhD candidate 8/29/2011

2004-2006 University of Tennessee at Chattanooga
Chattanooga, TN
• Bachelor of Science in Psychology with a minor in Biology
• Overall GPA: 3.975
• Magna Cum Laude

2001-2003 Chattanooga State Technical Community College
Chattanooga, TN
□ General Education
□ Overall GPA: 4.0

Professional experience

2006-2008 Emory University – Dr. Jay Weiss Atlanta, GA
Research Specialist
□ Maintained and screened animal models of depression and bipolar disorder using selective breeding after screening in either the forced swim test or ambulation following a foot-shock paradigm respectively.
□ computer skills required

2005-2006 UTC Neuroscience Laboratory Chattanooga, TN
Research Technician
• Research investigator: responsible for mixing drugs, overseeing experimental design, coordinating student research hours, observing animals, perfusion, and other surgical procedures.
• Animal caretaker: responsible for the cleanliness and well-being of animals, and for keeping in strict compliance with all NIH animal care rules and procedures.

2003 (6 months) Hello English School Oyama, Japan
English Teacher
Taught English as a second language to native students from age 2 - 80 years old.

Technical Proficiency
Since beginning research in behavioral neuroscience in 2005, I have acquired proficiency in a number of techniques from training received in the laboratories of Deborah Kreiss (University of TN, Chattanooga), Jay Weiss (Emory University), and Dave Roberts (Wake Forest University). These techniques include many behavioral assays (e.g. forced swim test, locomotor assessment, open field test), rodent self-administration of psychostimulants, intra-cranial self-stimulation, brain perfusion, neuroanatomical dissection, jugular catheterization, and stereotaxic surgery. Professional skills learned include high proficiency in Microsoft Office programs, SigmaPlot, GraphPad Prism, and programming proficiency using pascal and python languages.

Awards

Ulrey K. Wilson Psychology Award presented by the University of Tennessee for academic excellence in Psychology; 2006.

Outstanding Senior Award, presented by the Student Body Government of the University of Tennessee for overall academic excellence; 2006.

Second place in the Persuasive Speech Competition, offered by the Department of Theatre and Speech at the University of Tennessee; 2006.

Neuroscience Tutorial Speaker Award presented by Wake Forest University Neuroscience Program; 2009

Presentations


Zimmer BA, Dobrin CV, and Roberts DCS (2009). The time course of the motivation to self-administer cocaine within a binge. Society for Neuroscience Annual Conference. Chicago, IL

Zimmer BA, and Roberts DCS (2010). A hold-down procedure demonstrates that the preferred dose of cocaine
is related to drug levels. Society for Neuroscience Annual Conference. San Diego, CA


**Publications**


**Zimmer BA**, Oleson EB, and Roberts DCS. The Motivation to Self-Administer is Increased After a History of Spiking Brain Levels of Cocaine. *Neuropsychopharmacology*. 2012 Jul; 37(8):1901-10

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Zimmer BA, Roberts DCS. Examination of the reinforcing efficacy of cocaine within a 24-hour binge. *In submission*

Gabriele A, Zimmer BA, Roberts DCS. Cocaine self-administration on a two-lever progressive ratio schedule of reinforcement: separation of appetitive and consummatory responses. *In preparation*