2018

COCAINE CHOICE: A NOVEL PROCEDURE FOR INVESTIGATING NEURONAL ACTIVATION MEDIATING COCAINE PREFERENCE

Jonathan Jenn-Sheng Chow
University of Kentucky, jjchow3@g.uky.edu
Author ORCID Identifier:
https://orcid.org/0000-0001-5999-145X
Digital Object Identifier: https://doi.org/10.13023/etd.2018.497

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Jonathan Jenn-Sheng Chow, Student

Dr. Joshua S. Beckmann, Major Professor

Dr. Mark T. Fillmore, Director of Graduate Studies

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DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

Jonathan Jenn-Sheng Chow

Lexington, Kentucky

Director: Dr. Joshua S. Beckmann, Assistant Professor of Psychology

Lexington, Kentucky

2018

ABSTRACT OF DISSERTATION

Cocaine Choice: A Novel Procedure for Investigating Neuronal Activation Mediating Cocaine Preference

Cocaine use disorder is a significant health problem, negatively impacting individuals afflicted. While preclinical self-administration research has provided invaluable insight into the neurobehavioral mechanisms that underlie cocaine abuse, cocaine use outside of the laboratory occurs within an environment where other goods are also available ubiquitously. Although there is an ever-increasing literature investigating drug vs. non-drug choice in rodent models and how alternative goods can compete with the subjective value of cocaine, the neurobiological mechanisms that are associated with cocaine preference remains largely unknown. Additionally, current drug vs. non-drug choice studies use procedures that confound preference with intake, such that preference measures are directly reflective of individual experience with drug and non-drug reinforcers earned through the choices that are made; simply, preference and intake are the same. Moreover, differences in cocaine experience can result in differential neural adaptations, thus making it difficult to determine if the neurobiological mechanisms underlying choice are related to preference or drug intake. Herein a novel choice procedure, which controls for reinforcer intake (controlled reinforcer ratio; CRR), was used to explore how certain reinforcer dimensions (i.e., magnitude and frequency) influence cocaine preference. In addition, neuronal activity, measured via c-fos expression, in the orbitofrontal cortex and nucleus accumbens, areas associated with decision-making and valuation, for cocaine and food were independently targeted and labeled using fluorescent in situ hybridization and fluorescent immunohistochemistry. First, unlike prototypical choice procedures where preference and intake are confounded, the CRR choice procedure was able to dissociate the two. Under the CRR choice procedure, it was revealed that both magnitude and frequency, independent dimensions of reinforcement, greatly influence preference for cocaine. Furthermore, the CRR choice procedure was sensitive to manipulations known to influence cocaine preference while keeping reinforcer intake constant. When neuronal activity was examined after CRR training, the number of cocaine activated cells, relative to food activated cells, did not correlate with individual

preferences for cocaine despite overall reinforcer intake being held constant. Instead, results suggest neuronal activity for cocaine was related to overall cocaine intake. Overall, these results give impetus for utilizing the CRR choice procedure to better investigate how drug and non-drug reinforcers are afforded differential subjective value and compete for preference. Moreover, use of a CRR choice procedure may lead to identification of specific neurobehavioral mechanisms and lead toward future development of more effective pharmacological and behavioral treatments to ameliorate substance use disorders.

KEYWORDS: Choice, Cocaine, Decision-making, Matching Law, Orbitofrontal Cortex, Nucleus Accumbens

Jonathan Jenn-Sheng Chow
Student's Signature

November 12, 2018
Date

Cocaine Choice: A Novel Procedure for Investigating Neuronal Activation Mediating Cocaine Preference

By

Jonathan Jenn-Sheng Chow

Joshua S. Beckmann, Ph.D.
Director of Dissertation

Mark T. Fillmore, Ph.D.
Director of Graduate Studies

November 12, 2018

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. Joshua S. Beckmann, who has been an exemplary mentor and friend. Dr. Beckmann has continuously encouraged and provided me with the necessary resources to explore and pursue my research interests. I would also like to express my appreciation to Dr. Michael Bardo for serving as a co-mentor and facilitating me with opportunities to advance my professional goals throughout my graduate career. Moreover, I would like to thank my dissertation committee members, Dr. Kimberly Nixon and Dr. Thomas R. Zentall, for providing insights and challenges throughout this process; without their support and guidance, this dissertation would not have been as successful as it is. I would also like to thank my outside examiner, Dr. Michael J. Wesely, for being able to attend my defense. I would also like to thank Joshua N. Lavy, Emily Denehy, Linda A. Simmerman, Dr. K. Martin Chow, and Dr. Rebecca S. Hofford for their technical assistance. This research was funded by the National Institute of Health grants DA033373 and DA016176.

In addition to the expertise and technical assistance received above, I also received valuable support from my family. I am grateful to my parents for instilling me with a sense of curiosity and a love for science at an early age. I know my decision to pursue a Ph.D. was something that they strongly advised against due to their own experiences, but despite my decisions they have been very supportive, and for that, I am thankful. Finally, I am indebted to my brother, Christopher, for his help in fixing and refining some of the tools that I have used during this process.

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Introduction

The use of cocaine, a psychostimulant, can be traced back as early as the 6th century (Petersen, 1977). Cocaine, or more specifically coca leaves (Erythroxylon coca), was chewed by natives in western South America, present day Peru and Bolivia, for ceremonial purposes and, in some instances, chewed for its performance enhancement effects to aid in laborious tasks at high altitudes (Siegel, 1977; Karch, 2005). Due to its noted ability to stimulate activity, efforts were made to extract the psychoactive properties contained in the coca leaf. Soon after the isolation and purification of cocaine in the late 1800's, it was quickly marketed as a therapeutic (Musto, 1999); with Sigmund Freud as one of the most notable proponents for cocaine as a panacea (Byck, 1974). However, as cocaine use increased throughout the late 1800's and into the early 1900's it became clear cocaine use was associated with adverse-effects (e.g., hallucinations, paranoia, and psychosis) and that the pharmacological actions of the drug could result in death as well (i.e., overdosing; Petersen, 1977). Cocaine use was quickly viewed as a danger to the public causing legislators in 46 out 48 states, at the time, to pass state laws limiting the distribution and sale of cocaine (Ashley, 1975). Following state legislation, the federal government passed legislations (e.g., Pure Food and Drug Act, 1906; Harrison Narcotics Tax Act, 1914) limiting access of narcotics, including cocaine, to the public. Eventually, the Comprehensive Drug Abuse Prevention and Control Act (1970) was passed in attempts to protect the public from the dangers of drugs and other abuse-liable substances; cocaine was classified as a Schedule II drug making it a controlled

substance with an acceptable medical use (i.e., local anesthetic and vasoconstrictor), but a high potential for abuse. Despite cocaine's intended purpose as a therapeutic being quickly overshadowed by its adverse-effects and federal efforts to regulate drugs and abuse-liable substances, cocaine is still recreationally used and, in some cases, abused.

Cocaine use has been attributed to induce feelings of euphoria, invigoration, enhanced sexual stimulation, increased energy, enhanced self-confidence, and increased sociability (Ashley, 1975; Gawin, 1991). In short, cocaine's subjective-effects can be viewed as positive. Although cocaine use is also associated with some physiological side-effects (e.g., cardiovascular problems; Pilgrim et al. 2013; Bodmer et al. 2014; Qureshi et al. 2014), it does not produce any severe physiological withdrawal symptoms like other drugs of abuse (e.g., opioids, benzodiazepines, and alcohol). Cocaine's adverse-effects seem to be primarily psychological; symptoms include anxiety, anhedonia, agitation, insomnia, and intense cravings for cocaine (Gawin, 1991). However, there are instances where cocaine use, like other drugs of abuse, can be characterized by a pathological pattern of drug-seeking and drug-taking, where an individual spends an inordinate amount of time preoccupied with such behavior regardless of the detriments to one's well-being (Hasin et al. 2006, 2013). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; APA, 2013), an authoritative guide outlining the criteria and symptoms of mental disorders, some features of cocaine use disorder includes: increased usage; failure to abstain; spending a lot of time obtaining, using, and/or

recovering from use; cravings and urges; continued use despite negative consequences or interferences to personal and interpersonal events; and development of withdrawal. In a survey by the Substance Abuse and Mental Health Services Administration (SAMHSA; 2016) it was estimated 28.6 million individuals, aged 12 or older, are current users of illicit drugs, with about 2 million individuals having used cocaine within the past month and about 4.8 million individuals having used cocaine within the past year. In addition, reports have also suggested that around 968,000 individuals initiated cocaine use for the first time within the past year, the highest since 2007, and that cocaine related deaths are approaching 7000 annually with predictions that these numbers will continue to rise (National Drug Threat Assessment; NDTA, 2017). Furthermore, the estimated cost of substance use disorders exceeds $700 billion annually, of which illicit drugs (e.g., cocaine, methamphetamine, marijuana, and heroin) accounts for $193 billion (United States Department of Justice, 2011). In all, cocaine use is a significant public health problem.

A Human Issue

The effects of cocaine and cocaine use disorders have been documented and studied in humans since its premiere in the 1880's (Byck and Van Dyke, 1977). Of note, cocaine use disorders, like substance use disorders in general, are markedly exclusive to human nature; thus, it would seem reasonable to primarily focus scientific efforts in understanding these disorders from the human perspective. However, this is complicated by the heterogeneity of the human

experience and genetic predispositions. Controlling for these factors can prove both difficult and time consuming when collecting data from a willing population. One could argue that researchers could design experiments to specifically control for these factors in a laboratory setting to better understand substance use disorders in humans; however, ethical guidelines regarding human experimentation may limit, or even prohibit, certain research questions from being explored. However, one fact that is easily discerned from human behavior is that drugs of abuse, like cocaine, can serve as reinforcers. A reinforcer is operationally defined as a stimulus or event which follows behavior in a way that increases the likelihood an organism will behave in the same manner (Ferster and Skinner, 1957). Reinforcers often relate to some biological function (e.g., feeding) necessary for an organism's survival; for drugs of abuse this is not necessarily the case. Drugs of abuse function by eliciting positive feelings, such as "reward" and "pleasure" in the user. By eliciting feelings of "reward" or "pleasure," drugs of abuse, like cocaine, are hypothesized to subsequently cause individuals to repeatedly engage in behavior which leads to the procurement and use of a drug to obtain hedonic feelings (Schuster, 1975; Wise and Bozarth, 1987; Gawin, 1991).

Although humans are the predominant species that display substance use disorders, ethical guidelines protecting human participants limits what can be done. However, the use of animals (i.e., preclinical models) has been an invaluable substitute, allowing for scientific endeavors into psychological and biological research to rapidly advance (National Research Council, 2010; Hajar,

2011). Preclinical models for studying substance use disorders have relied on the "gold-standard" of intravenous self-administration, where a chronic indwelling-catheter is implanted into the animal's jugular vein which allows for a drug of interest to be delivered (Weeks, 1962; Thompson and Schuster, 1964). Intravenous self-administration is highly lauded for its almost instant and direct delivery of a drug into the central nervous system, via the blood stream, which bypasses first-pass metabolism and allows for precise dosing. Using preclinical self-administration, it was demonstrated that drugs of abuse, mirroring humans, function as reinforcers in animals. Moreover, animals do not need to be dependent on a drug of abuse before it is self-administered, suggesting, like humans, animals will engage in drug (e.g., cocaine) use for its rewarding properties (Pickens and Thompson, 1968; Deneau et al. 1969). Finally, animals are shown to self-administer drugs that are abused in humans; and drugs that are not abused in humans are not self-administered in animals (Schuster and Thompson, 1969). Collectively, these findings support the use of preclinical models in studying substance use disorders.

Drug Reinforcement in Preclinical Models

Operant behavior can be described as the selection of behavior by its consequences (Skinner, 1953, 1963, 1985). For example, if behavior is maintained by the presentation of a stimulus (e.g., environmental or biological event), the stimulus is referred to as a positive reinforcer. Similarly, if behavior is maintained by the termination of a stimulus, the stimulus is then referred to as a

negative reinforcer. In both instances, the stimulus acts as a reinforcer, but the presentation or termination of the stimulus, contingent on the emitted behavior, serves as reinforcement, increasing the likelihood that the behavior will be repeated. Research into operant behavior has spanned many decades and has provided insight into the determinants necessary for an event to function as a reinforcer, as well as the effects that the arrangement of scheduled consequences have on behavior (Ferster and Skinner, 1957; Honig, 1966; Morse and Kelleher, 1970). Furthermore, these principles of reinforcement have been applied to substance use disorders research and has served as a framework for how drugs of abuse function as reinforcers.

Early preclinical models utilizing intravenous self-administration demonstrated that rats (Weeks, 1962) and monkeys (Thompson and Schuster, 1964) would emit responses (e.g., lever pressing) to receive injections of morphine. However, these subjects were first made physically-dependent, via experimenter-administered drug exposure, prior to self-administration. Hence, these findings established the principles of negative reinforcement applied to drug use, such that experimental subjects were emitting responses for an infusion of morphine which would subsequently alleviate the symptoms of opioid withdrawal. Following the demonstration of negative drug reinforcement, researchers later examined if positive drug reinforcement could be shown in naïve preclinical subjects. As it turns out, rats (Pickens and Thompson, 1968) and monkeys (Deneau et al. 1969) would self-administer drugs of abuse (e.g., cocaine) without having to be physically-dependent. Moreover, experimental

subjects readily self-administered drugs in a similar manner as food and water, where the drug of interest maintained consistent behavior across training sessions. Collectively, these studies provided evidence that known drugs of abuse in humans also functioned as reinforcers in animals.

While early studies utilizing intravenous self-administration serve as evidence that drugs of abuse function as reinforcers, Pickens and Thompson (1968) also noted a few interesting features regarding cocaine self-administration. First, cocaine-reinforced behavior functioned similarly to food-maintained behavior, where the dose of cocaine and the response-ratio required to earn said drug were directly related such that high doses, relative to low doses, were needed to maintain self-administration at large ratio requirements. Second, cocaine reinforcement occurred within a certain range of doses, if the dose was too low "ragged performance" was observed and if the dose was too high responding would stop entirely. Finally, cocaine-reinforced behavior was regularly spaced with long pauses after each reinforcer delivery, similar to food-maintained behavior when non-contingent cocaine infusions were intermittently-administered, suggesting that the pharmacological properties of cocaine can have disruptive effects on performance. Importantly, these observations would generalize to other drugs of abuse.

The procedures used by Pickens and Thompson (1968) for cocaine self-administration, based off Weeks and Collins (1964), would serve as the prototypical intravenous drug self-administration procedure, where two levers are presented such that responding, under a fixed-ratio schedule of reinforcement,

on one lever resulted in drug delivery, while responding on the other lever resulted in no scheduled consequences. A common feature observed under a fixed-ratio schedule of reinforcement for drug self-administration is the dose-response curve that it produces. The dose-response curve can be described as an “inverted U-shape” where low and high doses maintain low rates of responding, while intermediate doses maintain the highest rates of responding (Kelleher and Morse, 1968; McMillan and Leander, 1976; Spealman and Goldberg, 1978; Katz, 1989). Although fixed-ratio schedules are the most commonly used schedule of reinforcement applied to substance use disorders research (Spealman and Goldberg, 1978; Banks and Negus, 2012), other well-known schedules of reinforcement such as variable-ratio, variable-interval, and fixed-interval (Ferster and Skinner, 1957) have also been applied to drug self-administration research. However, the use of these schedules by themselves is seldom seen, in-part, due to observed effects of drugs on the rate of response, a fundamental measure for behavioral analysis (Honig, 1966; Kelleher and Goldberg, 1975; Katz, 1989). For example, drugs tend to have dose-dependent effects on rate of responding, where somewhat high-doses or cumulated low-doses can affect emitted behavior, thus under variable-responding, which promotes sustained responding, the response rates observed could be influenced by how much drug is in the subject’s system. Under interval schedules, the first response after a specified interval of time results in drug delivery; since these intervals are preset, the rate of responding for drug is relatively independent of inter-reinforcement intervals due to the long post-

reinforcement pauses associated with drug intake (Spealman and Goldberg, 1978).

A variation of the fixed-ratio schedule, known as progressive ratio schedule (Hodos, 1961; Richardson and Roberts, 1996) has also been heavily utilized in substance use disorders research, where the required response ratio for each successive reinforcer is systematically increased until the subject stops responding. The response requirement that results in incompletion is known as the breakpoint, which serves as a measure for reinforcer strength. Interestingly, studies using progressive ratio demonstrated dose-dependent effects where low doses produce low breakpoints and high doses produce higher breakpoints up to a point; after a high-enough dose, the breakpoint plateaus or begins to drop off (Griffiths et al. 1978, 1979; Richardson and Roberts, 1996). It has been argued that unlike the fixed-ratio schedule, progressive ratio schedules allow for quantitative measurements of the reinforcing properties of a drug due to the breakpoint measure since the “inverted U-shape” seen under fixed-ratio schedules is suspect to interpretation (Richardson and Roberts, 1996; Arnold and Roberts, 1997). Under fixed-ratio schedules, the inverse relationship seen between dose and drug intake has been interpreted as a type of compensatory mechanism. For low doses, higher rates of drug intake are necessary to compensate for the decrease in reinforcing efficacy of the drug, and for high doses, lower rates of drug intake are due to an increase in reinforcing efficacy of the drug (Yokel and Wise, 1975). In a series of studies, it was demonstrated that after injecting 6-hydroxydopamine into the brain (e.g., nucleus accumbens),

which results in the blunting of the reinforcing effects of drugs, rats did not increase their drug (e.g., cocaine) intake; suggesting the interpretation that the inverse relationship seen between rate of drug intake and the reinforcing efficacy of the dose is unlikely and that decreases in drug intake at high doses should instead represent a decrease in reinforcing efficacy, possibly some adverse effect of being too high a dose (Roberts and Koob, 1982; Zito et al. 1985). Although there have been arguments made regarding the interpretation of the dose-response curve seen under a fixed-ratio schedule, fixed-ratio schedules and variations of the fixed-ratio schedule (e.g., progressive-ratio) are still utilized today in substance use disorders research.

Theories Regarding Substance Use Disorders and Preclinical Models

During the last few decades, many different theories attempting to elucidate and capture the behavioral and biological processes that underlie substance use disorders have emerged. Many of the theories that investigate the advent of substance use disorders have relied on preclinical models to explore the neurobehavioral underpinnings involved. The following are a few examples of contemporary theories and the preclinical models associated with them.

One of the most contemporary explanations for substance use disorders is the allostatic hypothesis of drug addiction which views substance use disorders as a transition from impulsive (i.e., voluntary) drug use into compulsive drug use (Koob and Le Moal, 1997, 2001, 2005). This theory functions as a combination of two supposed processes: allostasis and the opponent-process theory for

motivation (Koob et al. 2004; Wise and Koob, 2014). Allostasis can be described as the process of regulating to stability in an ever-changing environment, where efficient regulation requires anticipation and preparation for future events (Sterling and Eyer, 1988; Sterling, 2004, 2012). The opponent-process theory of motivation states that when an affect is experienced, the opposite affect follows creating a contrast which gives relevance to what was experienced. However, through repeated experiences, onset of the opposite affect eventually occurs simultaneously with the primary affect, resulting in a net-decrease in the affect experienced (Solomon and Corbit, 1974; Solomon, 1980). Combining these two processes, the allostatic hypothesis of drug addiction functions under the notion that initial drug use results in feelings of drug "reward" that occurs in some "normal" state. Meanwhile, the biological systems involved simultaneously undergo allostasis adapting for the presence of the drug taken which results in preserving the initial opponent-process for drug "reward". However, as drug use continues, the allostatic processes that regulates the biological systems shifts away from a "normal" drug-free state. Through repeated drug exposures, drug presence is now part of the "normal" state, such that for feelings of "reward" to be achieved, greater amounts of drug must be taken. Eventually, through continued use, the allostatic processes involved in regulating drug presence ends up in some dysregulated state, where instead of an ongoing opponent-process for drug "reward," it becomes an opponent-process for withdrawal "relief." In short, with repeated and sustained use, drug use shifts the biological systems involved in regulating the opponent-process that results in positive drug reinforcement

towards a state of negative reinforcement, such that drug use is necessary to alleviate the negative physiological- or psychological-consequences of drug-withdrawal. Although the allostatic hypothesis of drug addiction posits the development of substance use disorder as a transition from positive to negative reinforcement, an important aspect of this theory is that individuals will increase drug use over time. To study how increased drug use can affect the transition from positive to negative reinforcement, escalation has been utilized (Ahmed and Koob, 1998, 1999, 2005). Escalation is a preclinical model designed in such a manner that subjects (e.g., rats) are assigned to a condition where they have long-access (6 hours) or short-access (1 hour) to self-administer a drug of abuse on a fixed-ratio schedule of reinforcement. Animals within the long-access group, over training sessions, will increase or "escalate" drug intake relative to animals in the short-access group. In addition, animals that undergo escalation for drug (e.g., cocaine) show markedly changed neuroadaptations relative to short-access animals (Wolf, 2010, 2016). Moreover, animals that escalated cocaine intake under long-access also showed a decrease in response to intracranial self-stimulation relative to animals that were assigned to short-access; suggesting that escalated intake compensates for the brain's shift in reward processing, where escalated intake is a compensatory mechanism for the decrease in drug-reward over time (Ahmed et al. 2002).

Although theories of substance use disorders have primarily attributed this problem to either negative- or positive- reinforcement (Wise and Bozarth, 1987), or in some cases, a transition from positive into negative reinforcement (e.g.,

Koob et al. 2004), these theories are not without criticism. A major critique of the allostatic hypothesis of drug addiction is that withdrawal is the driving mechanism (i.e., negative reinforcement) behind compulsive drug use. For example, there are studies demonstrating that reinstatement (e.g., relapse-like behavior) of drug-taking, after a period of extinction, is markedly more intense following a priming injection of heroin than an injection of an opioid antagonist, which can induce withdrawal in previously drug-exposed animals (Stewart and Wise, 1992; Shaham et al. 1996). Moreover, in humans, some individuals will relapse into drug use despite being past the window where withdrawal symptoms are present; challenging the concept that compulsive drug use is driven by negative reinforcement (O'Brien, 1997). Instead, a large number of studies have attributed substance use disorders to positive reinforcement (Wise and Bozarth, 1987). Positive reinforcement is without its issues since by definition, positive reinforcement only describes the relationship between a drug as a reinforcer and the behavior emitted for said drug but says nothing about how drugs are addicting (Robinson and Berridge, 1993). Moreover, positive reinforcement does not fully describe instances where environmental stimuli that are associated with drug use are repeatedly shown to elicit drug-craving or relapse (Stewart et al. 1984; Wise and Bozarth, 1987); going against the notion that positive reinforcement is the driving mechanism behind substance use disorders. One theory that has emerged is the incentive sensitization theory of drug addiction (Robinson and Berridge, 1993, 2000, 2001, 2008). Like other contemporary explanations, incentive sensitization functions under the notion that drugs of

abuse produce long-lasting neuroadaptations, especially in areas that are responsible for motivation and reward. Due to the changes, via drug use, the brain's reward system becomes hypersensitize or "sensitized" to drugs and drug-related stimuli; importantly, sensitization only mediates incentive salience (i.e., "wanting") and not the "rewarding" effects of the drugs. Thus, explaining how drug-related cues can motivate individuals to relapse after periods of abstinence (Shalev et al. 2002; Shaham et al. 2003; Lee et al. 2006). Drug sensitization is seen through behavioral sensitization, a procedure where subjects (e.g., rats) are repeatedly exposed to a drug, via experimenter-administration, and subsequently placed into an open-field. Over repeated drug exposures, drugs that result in sensitization will typically increase an animal's locomotor activity. Consequently, animals that show increased locomotor activity also acquire drug (e.g., cocaine and amphetamine) self-administration on fixed-ratio schedules of reinforcement relatively faster than controls (Horger et al. 1990; Piazza et al. 1989; 1990); suggesting that drug sensitization changes the motivational properties for drugs. However, recent research into incentive sensitization has focused primarily on incentive salience (i.e., how reward-predictive cues can elicit wanting) via autoshaping procedures (e.g., Hearst and Jenkins, 1974; Flagel et al. 2011; Meyer et al. 2012). There is evidence suggesting individuals that have a propensity to attribute incentive salience to reward-predictive stimuli have a propensity for drug self-administration and have higher breakpoints for drugs on a progressive-ratio schedule of reinforcement, all of which indicate that individuals, who attribute value to reward-predictive cues, are more liable for

substance use disorders (Saunders and Robinson, 2010; 2011; Anderson and Spear, 2011; Beckmann et al. 2011; Peters and DeVries, 2014). Altogether, the incentive sensitization theory of drug addiction posits that through repeated drug use and neuroadaptations, the stimuli associated with drugs of abuse become responsible for motivating drug-seeking and drug-taking behavior (Robinson and Berridge, 1993, 2001).

While the above are some examples of theories that are at the forefront in substance use disorder research, one common and vital theme is that drug use causes long-lasting neurobiological adaptations (Hyman and Nestler, 1996; Nestler, 2001; Hyman et al. 2006; Kalivas and O'Brien, 2008). Likewise, other theories have also emerged that emphasize the importance of neuroadaptations via drug use. Robbins and Everitt (1996,1999, 2002; Everitt et al. 2001; Everitt and Robbins, 2005) have conceptualized that the transition from voluntary to compulsive drug use as a byproduct of the neurobiological processes that underlie learning and memory, specifically habit-learning, for drugs of abuse. The mesocorticolimbic pathway (i.e., "reward-circuit"; c.f., Everitt and Robbins, 2005) is composed of multiple brain regions (e.g., prefrontal and orbitofrontal cortex, dorsal and ventral striatum, hippocampus, and amygdala) and within these regions reward-learning and processes related to reward-learning occur. An example of how neuroadaptive shifts within a brain region can influence drug-seeking behavior is hypothesized to occur within the striatum. It is theorized that initial acquisition of drug-seeking behavior is dependent on nucleus accumbens function such that individuals are seeking and taking drug purposefully. Through

prolonged drug-seeking and drug-taking behavior, the dorsal striatum becomes recruited and responsible for such actions. However, the dorsal striatum has also been implicated in processing drug-related stimuli as well, such that the through reward-learning the presence of drug-related stimuli can, in a sense, engender habitual-like drug-seeking behavior (Everitt and Robbins, 2005; Vanderschuren et al. 2005; Belin and Everitt, 2008; Murray et al. 2012). To explore habitual drug use (i.e., behavior insensitive to consequences), Pavlovian-instrumental transfer has been the go-to model. Pavlovian-instrumental transfer procedures were initially developed to determine the effects that appetitive- or aversive- cues have on operant behavior; especially, in relation to outcome devaluation (Vanderschuren and Everitt, 2004; Everitt and Robbins, 2005; LeBlanc et al. 2012). The procedures used in Pavlovian-instrumental transfer function a bit differently than a fixed-ratio schedule of reinforcement which is often designed to simply consist of an active and inactive operandum. Generally, rats are first trained on a Pavlovian component, where subjects are trained to associate a stimulus with some event (e.g., light predicts shock). Next, rats are then trained to complete a response-chain where completion of a random-ratio on a “seeking-operandum” produces the “taking-operandum” which results in reinforcer delivery upon completion of a fixed-ratio schedule of reinforcement. Finally, on test days, the two components, Pavlovian and operant, are presented within the same session. It is theorized that any changes in performance, via presentation of the previously trained stimulus associated with some event (i.e., Pavlovian component), demonstrates the excitatory or inhibitory properties of said stimulus,

allowing for direct investigation into how predictive-cues can influence seeking and taking behavior (Balleine, 1992; Balleine et al. 1995; Corbit and Balleine, 2003). For example, Vanderschuren and Everitt (2004), using Pavlovian-instrumental transfer, showed that the presentation of a stimulus that was previously associated with a shock could suppress cocaine self-administration. However, via long-term cocaine use, rats did not suppress cocaine self-administration during the presentation of the previously trained stimulus, but instead continued to self-administer. Likewise, Deroche-Gamonet et al. (2004) demonstrated that rats that exhibit “cocaine addiction”, via long-term self-administration, will continue to self-administer cocaine regardless of consequentially getting shocked when responding on an operandum that results in drug delivery. Altogether, demonstrating that long-term cocaine use results in compulsive behavior where subjects exhibit habit-like behavior and continue to take drug despite the possibility of adverse consequences.

In all, the emergence of theories pertaining to the occurrence of substance use disorders and the application of preclinical models have provided insight into the behavioral and biological mechanisms that underlie this problem. Moreover, these theories and preclinical models have greatly shaped the direction that behavioral neuroscience research has taken in resolving substance use disorders.

Advancing Preclinical Models

Preclinical intravenous self-administration research has provided invaluable translational insight into the neurobehavioral mechanisms associated with substance use disorders in humans. However, it should be noted that most preclinical models utilized (e.g., escalation, Ahmed and Koob, 1998; Pavlovian instrumental transfer, Vanderschuren and Everitt, 2004) are considered single-schedules; meaning subjects are only given access to one reinforcer. Furthermore, all data regarding the acquisition, maintenance, extinction, and reinstatement of drugs of abuse, including the effects that environmental (e.g., Schenk et al. 1987; Haney et al. 1995; Piazza and Le Moal, 1999; Kosten et al. 2000; Stairs and Bardo, 2009) and biological (e.g., Lynch and Carroll, 2000; Jackson et al. 2006; Belin et al. 2011) factors have on drug use, have been collected using single-schedules.

While single-schedule preclinical models have served as a framework for behavioral studies within the field of substance use disorders research, one often overlooked issue is that human behavior for drugs of abuse is nested in an environment where many other reinforcers (e.g., food, monetary goods, and interpersonal relationships) are, for the most part, also simultaneously available. In brief, humans interact with an environment where choices exist. There is evidence that suggests the presence of other reinforcers (e.g., work and interpersonal relationships) within an individual's environment can promote an individual's ability to abstain from drug use and in some instances permanently quit (Robins, 1993; Klingemann et al. 2010). Moreover, clinical studies have

demonstrated that the availability of alternative reinforcers, such as money and vouchers for goods, can shift use away from cocaine, and other drugs of abuse, and promote abstinence in individuals with substance use disorders (Silverman et al. 1999; Hart et al. 2000; Higgins et al. 2004, 2008; Prendergast et al. 2006; Stoops et al. 2010, 2012; Vosburg et al. 2010; Festinger et al. 2014; Greenwald et al. 2014; Foltin et al. 2015; Moeller and Stoops, 2015; Holtyn et al. 2017). Thus, the question becomes whether complex human behavior can be modeled in preclinical subjects.

Within the past decade, there has been an increase in the number of studies examining the effects of alternative reinforcers on abuse-like behavior in preclinical models, especially rodent-models, in attempts to better understand the neurobehavioral mechanisms that underlie the decision-making processes involved in choice for drugs of abuse (Ahmed, 2010; Banks and Negus, 2012; Ahmed et al. 2013). Interestingly, the use of choice procedures has complicated the interpretation of some of the more contemporary behavioral models for studying substance use disorders such as escalation of drug intake (Lenoir et al. 2007; Cantin et al. 2010; Caprioli et al. 2015) and habit-like behavior for drug (Kosaki and Dickinson, 2010; Halbout et al. 2016; Singer et al. 2018). Specifically, the addition of a non-drug alternative (e.g., saccharin or food pellet) has repeatedly been shown to shift behavior away from drug (e.g., cocaine) towards said non-drug alternative, going against the notion that animals, that show escalated drug intake or display habit-like behavior, may not be compulsively using drugs. Moreover, there is evidence that escalation and habit-

like behavior for drugs of abuse are a byproduct of the single-schedules used (Kosaki and Dickinson, 2010; Beckmann et al. 2012; Hogarth, 2018). In all, these results mirror findings seen in human clinical studies, giving impetus for studying substance use disorders within the context of choice.

Choice Theory

Although many different theories regarding substance use disorders have emerged over the past few decades, one word that has often appeared to describe individuals with this problem is “compulsive.” For example, the word compulsive is associated with “loss of control” and “habitual drug use”; all of which would imply that the individual is insensitive to consequences. However, research has demonstrated that individuals diagnosed with substance use disorders have the ability to control their behavior (e.g., Higgins et al. 2008). Furthermore, data has suggested that most individuals diagnosed with substance use disorders are sensitive to consequences concerning financial and familial matters and will modify their behavior (i.e., reduce drug intake or quit), despite having an extensive history of drug use that results in physical alterations in the brain which supposedly causes problematic use (Warner et al. 1995; Waldorf et al. 1991; Klingemann et al. 2010). In all, these findings are contrary to the contemporary models for substance use disorders (e.g., the allostatic hypothesis of drug addiction and incentive sensitization theory of drug addiction).

Choice theory, different from normative theories such as rational choice theory (Scott, 2000) and optimal foraging theory (Stephen and Krebs, 1986)

which ascribe to maximization, views substance use disorders as an issue in value-based decision-making (Herrnstein and Prelec, 1991, 1992; Heyman, 1996, 2009, 2013; Ainslie, 2000). Specifically, choice for drugs of abuse is dependent under the context in which all reinforcers (i.e., drug and non-drug) are presented, and that substance use disorders appears under conditions where drugs of abuse has greater value relative to all other obtainable reinforcers (Heyman, 2013). Thus, understanding choice behavior can provide insight into substance use disorders.

Choice behavior has been studied through concurrent schedules of reinforcement for more than a half-century. Concurrent schedules function such that two or more distinct operandum are presented, each with its own scheduled consequences, which the organism can freely allocate behavior across the given options (Ferster and Skinner, 1957; Findley, 1958; Herrnstein, 1958, 1961; Catania, 1963, 1966). Through concurrent scheduling, choice theory developed. The basis of is rooted in matching, first described by Herrnstein (1961). The matching function described by Herrnstein (1961) was used to examine the relationship between the distribution of pecking and eating behavior by pigeons on concurrent variable-interval schedules for food; the function derived is as follows:

$$\frac{p_1}{p_1+p_2} = \frac{ke_1}{k(e_1+e_2)} \qquad \text{(Eqn 1)}$$

Where, *p* denotes pecking, *e* denotes eating, and *k* is constant (known as an extinction ratio; Skinner, 1938) that gets cancelled out. Note, the subscripts 1 and 2 represent two distinct options; this is congruent for all following equations within

this section. Simply, the matching function predicts that the relative amount of pecking emitted across the options will be proportional to the relative amount of scheduled eating observed across the options. Additionally, data sets from other studies that were being published at the time corroborated this observed relationship (e.g., Catania, 1962; Blough, 1963; Reynolds, 1963; Brownstein and Pliskoff, 1968). Eventually, the relationship would become known as the "matching law" (Herrnstein, 1970; Baum and Rachlin, 1969; Rachlin, 1971). The matching law is written as:

$$\frac{R_1}{R_1+R_2} = \frac{R_{f1}}{R_{f1}+R_{f2}} \qquad \text{(Eqn 2)}$$

Or

$$\frac{R_1}{R_2} = \frac{R_{f1}}{R_{f2}} \qquad \text{(Eqn 3)}$$

Where, *R* denotes rate of any response and R_f denotes rates of reinforcement. To summarize, the matching law states that the relative rate of any response is proportional to its associated relative rate of reinforcement (Herrnstein, 1970). Aside from the relative rate of reinforcement, other reinforcer dimensions followed this relationship (Baum and Rachlin, 1969; Premack, 1969). Thus, the matching law could be expanded (Rachlin, 1971) and conceptualized as:

$$\frac{T_1}{T_2} = \frac{R_1}{R_2} * \frac{A_1}{A_2} * \frac{I_1}{I_2} * \frac{X_1}{X_2} = \frac{V_1}{V_2} \qquad \text{(Eqn 4)}$$

Where *T* denotes time allocated (i.e., time responding), *R* denotes rate of reinforcement, *A* denotes amount of reinforcement, *I* denotes immediacy of reinforcement, *X* denotes all other undefined reinforcer dimensions, and *V* is the

value of consequent reinforcement; altogether, the matching law was expanded to account for other independent reinforcer dimensions that can determine choice behavior.

However, the matching law is not without its issues. For example, studies examining probabilistic reinforcement (Shimp, 1966), reinforcement dependent on interresponse times (Staddon, 1968), and large contrasts in the range of scheduled times under variable-interval schedules (Fantino, 1969) found results that deviated from matching. Rachlin (1971) noted that under the matching law, it is assumed that the relation between the obtained reinforcement and reinforcement value (i.e., determined by reinforcer dimensions) functioned on a 1:1 scale. However, this was not necessarily the rule for all studies and theorized that reinforcer dimensions should be scaled, resulting in the theorized matching equation:

$$\frac{T_1}{T_2} = \frac{V_1}{V_2} = \log\left(\frac{X_1}{X_2}\right) \qquad \text{(Eqn 5)}$$

Where *X* represents all reinforcer dimensions that differ across the two alternatives. An issue with Rachlin's theorized matching law is that it takes the logarithmic transformation on only one side of the equation, which would imply that reinforcer dimensions are multiplicative in nature (Killeen, 1972). Instead, a logarithmic transformation should be applied to both sides of the equation and can be written as:

$$\log\frac{V_1}{V_2} = \log\left(\frac{X_1}{X_2}\right) \qquad \text{(Eqn 6)}$$

Which would indicate that reinforcer dimensions are additive in nature, similar to other working models of preference supported by data (Tversky, 1969; Killeen, 1972).

Although the matching law has approximated experimental data to a large extent, occasional data sets deviated from the matching law. Deviations from the matching law were described to occur in a few forms: undermatching, overmatching, and bias (Baum, 1974; William, 1979). To account for systematic deviations from matching, the generalized matching law (Baum, 1974; William, 1979) was posited and takes the form as follows:

$$\log\left(\frac{B_1}{B_2}\right) = a * \log\left(\frac{r_1}{r_2}\right) + \log b \qquad \text{(Eqn 7)}$$

Or

$$\frac{B_1}{B_2} = b * \left(\frac{r_1}{r_2}\right)^a \qquad \text{(Eqn 8)}$$

Where, *B* denotes behavior at a given option and *r* denotes rate of reinforcement, and *a* and *b* are empirical constants representing sensitivity and bias, respectively. Sensitivity refers to how well a subject is able discriminate differences in reinforcer dimensions across the given options. For example, overmatching occurs if *a* is greater than 1 and results in “greater” detection (i.e., quicker changes) in response allocation across the given options, undermatching occurs if *a* is less than 1 and results in “lower” detection (i.e., slower changes) in response allocation across the given options, and perfect matching occurs when *a* is equal to 1. Bias refers to a subject’s predisposition for a given option (e.g., innate preference seen within individuals), where bias is seen for the first option if

b is greater than 1, bias against the first option is seen if *b* is less than 1, and if there is no bias when *b* is equal to 1.

Since the development of the matching law and, subsequently, the generalized matching law, in both laboratory and natural settings and in both humans and non-human subjects, matching has been largely generalizable and has allowed for the quantitative analysis of the determinants of choice behavior (e.g., Conger and Killeen, 1974; Houston, 1986; Heyman and Monaghan, 1987; Vollmer and Bourret, 2000; Poling et al. 2011). Moreover, to account for all the possible dimensions of reinforcement that can affect preference, Davison and McCarthy (1988) formally provided the concatenated generalized matching law. The concatenated generalized matching law is as follows:

$$\log\left(\frac{B_1}{B_2}\right) = \left[\sum_{i=1}^{n} a_i \log\left(\frac{X_{i1}}{X_{i2}}\right)\right] + \log b \qquad \text{(Eqn 9)}$$

Where, *B* denotes behavior at a given option, *X* denotes independent reinforcer dimensions (e.g., rate, magnitude, immediacy), and *i* denotes the *i*th reinforcer dimension. Whereas, *a* and *b* are independent empirical constants representing sensitivity, for a given reinforcer dimension, and bias, respectively, which function identically as the same free parameters proposed in the generalized matching law (Eqn 7 and 8; Baum, 1974; William, 1979). In addition, the concatenated generalized matching law allows for multiple dimensions of reinforcement, determined by the experimenter, to be quantitatively studied in relation to one another (Rachlin, 1971). To summarize, the concatenated generalized matching law states that the relative rate of response for a reinforcer is proportional to the relative differences in reinforcer dimensions of the available options, assuming

reinforcer dimensions are multiplicative in nature (Killeen, 1972). Importantly, the free parameters (i.e., sensitivity and bias) within the generalized matching law provides insight into how the given reinforcers interact in relation to one another and how one reinforcer can have more value relative to the other.

Altogether, choice theory views substance use disorders as a product of the valuation of drugs of abuse relative to all other reinforcers that are concurrently available. By understanding how different reinforcer dimensions govern the relative value between drugs of abuse and non-drug reinforcers (Herrnstein and Prelec, 1992; Heyman, 1996, 2013), experimenters should be able to develop pharmacological and behavioral methods to shift preference away from drugs towards non-drug alternatives.

Current State of Drug vs. Non-drug Choice in Rodent Models

One area of interest, in the substance use disorder field, is how qualitatively different reinforcers (e.g., food vs. water, drug vs. non-drug) can interact. A framework that has offered insight into the relationship between qualitatively different reinforcers is behavioral economics, a conceptual framework that ascribes value to a reinforcer and how said value can affect behavior (Rachlin et al. 1976, 1980; Hursh, 1980; Hursh and Roma, 2016). One perspective from behavioral economics that has been applied to substance use disorders research is that reinforcers can function as substitutes, complements, or be independent of one another. Specifically, the concept of “substitutes” or substitution, referring to how qualitatively different commodities (e.g., drug and

non-drug rewards) are interchangeable and can replace one for the other, has been explored as a form of treatment for substance use disorders within the last few decades (e.g., Bickel et al. 1998; Cosgrove et al 2002; Venniro et al. 2016, 2017).

Within drug versus non-drug choice, one drug of abuse that has garnered a lot of attention is cocaine. Preclinical-primate research has been at the forefront in drug versus non-drug choice studies (Aigner and Balster, 1978; Banks et al. 2015) and research has shown that choice for cocaine versus a non-drug alternative (e.g., food) can be shifted towards or away from drug by either increasing or decreasing the magnitude, price, frequency, or delay of a given reinforcer (Woolverton and Nader, 1990; Nader and Woolverton 1991, 1992a, 1992b; Nader et al. 1993; Anderson and Woolverton, 2000; Anderson et al. 2002; Negus, 2003, 2004, 2005a, b; Negus and Mello, 2004; Huskinson et al. 2015; Hutsell et al. 2015), all of which are independent reinforcer dimensions that appear under choice theory. In short, by manipulating the the relative value, determined by the dimensions of reinforcement, the substitutability for the given reinforcers can be changed and choice for the more valuable option, according to the organism, will occur. Furthermore, choice procedures have provided insight into the pattern of behavior seen under single-schedules. For example, the "inverted U-shape" produced by fixed-ratio schedules of reinforcement (Kelleher and Morse, 1968; Spealman and Goldberg, 1978; Katz, 1989), where the doses on the descending limb are hypothesized to be aversive (Roberts and Koob, 1982; Zito et al. 1985), are the doses that produce the greatest preference for

drug. Likewise, drug doses that produce comparable breakpoints in progressive ratio schedules are thought to have the same value (Griffiths et al. 1978, 1979; Richardson and Roberts, 1996); however, higher doses of drug are often associated with greater preference for said drug. In all, choice procedures can dissociate the reinforcing effects of a drug from its rate-altering effects (Banks and Negus, 2012). Preclinical choice procedures have also served as means to test pharmacological agents as possible pharmacotherapeutics for cocaine use disorders by examining how treatments of a compound can further shift choice away from cocaine (e.g., Woolverton and Balster, 1979; Negus, 2003; Negus and Mello, 2004; Thomsen et al. 2008, 2014; Banks et al. 2011, 2013, 2015; Hutsell et al. 2015).

Drug versus non-drug choice studies have also been applied to human clinical research. For example, through contingency management (Jablonksy and DeVries, 1972; Hamner, 1974), a form of behavioral therapy used to reallocate behavior from one alternative in exchange for another, it was demonstrated that money or vouchers can be used to promote abstinence in individuals with cocaine use disorders (e.g., Vandrey et al. 2007; Festinger et al. 2014) and that the magnitude (i.e., monetary value) of the non-drug alternative can increasingly shift choice away from cocaine (e.g., Greenwald et al. 2014). Altogether, these studies demonstrate the effectiveness that a non-drug alternative can have on reducing cocaine use. Additionally, the use of d-amphetamine (Greenwald et al. 2014), bupropion (Stoops et al. 2012) as pharmacotherapies was shown to decrease cocaine choice. Remarkably, contingency management in combination

with pharmacotherapies (e.g., d-amphetamine and bupropion) was demonstrated to further promote abstinence from cocaine in individuals with cocaine use disorders (Grabowski et al. 2001; Poling et al. 2006). Although the utilization of contingency management has proved promising, the biggest issue, much like pharmacotherapies for other substance use disorders (e.g., opioids), regarding contingency management is that once treatment stops the likelihood of relapse increases drastically. In addition, there are no actual approved pharmacotherapeutics for cocaine use disorders and all other pharmacological agents tested have failed; thus, solely relying on a drug to promote continued abstinence is currently unachievable (Moeller and Stoops, 2015).

With issues in relapse and the lack of viable pharmacotherapeutics, research into the neurobiological underpinnings that drive preference for cocaine versus non-drug alternatives have recently shifted towards rodent models in attempts to resolve this issue (Ahmed, 2010; Ahmed et al. 2013; Banks and Negus, 2012, 2017). Within the last decade a growing number of preclinical studies have aimed to develop and determine the necessary parameters to model drug versus non-drug choice in rats. Interestingly, the majority of drug versus non-drug choice procedures done in rodents utilizes a “discrete-trials” choice procedure developed by Lenoir et al. (2007) and has more or less become the prototypical rodent drug versus food choice procedure for all subsequent research (Lenoir and Ahmed, 2008; Cantin et al. 2010; Augier et al. 2012; Kerstetter et al. 2012; Lenoir et al. 2013a, 2013b; Pelloux et al. 2013; Perry et al. 2013, 2015; Tunstall and Kearns, 2014, 2015, 2017; Tunstall et al 2014;

Caprioli et al. 2015, 2017; Madsen and Ahmed, 2015; Vandaele et al. 2016; Vanhille et al. 2015; Kearns et al. 2017; Venniro et al. 2016, 2017; Schwartz et al. 2017; Huynh et al. 2017; Bagley et al. 2017; Freese et al. 2018). The "discrete-trials" choice procedure functions in two phases, a sampling-phase and a choice-phase. Generally, the sample-phase consists of four trials, where a single-lever associated with either drug or food reinforcement (2 trials of each type) is presented in an alternating manner, such that completion of the fixed-ratio response requirement on the available lever results in lever retraction and reinforcement delivery. After the sampling-phase, the choice-phase, typically consisting of twelve trials, begins, where both levers are now extended, and rats have the option to choose between drug and food on a fixed-ratio 1 schedule of reinforcement. Upon completion of the response requirement, both levers are retracted, and reinforcement delivery occurs. In addition, each trial is placed on a limited-hold, such that if an animal does not complete the required response ratio in a set-amount of time, the trial will result in an omission. Of importance, under the "discrete-trials" choice procedure, a constant unit dose of drug (e.g., 25 mg/kg) is being compared against a set amount of non-drug reinforcer (e.g., food pellet, sucrose, or saccharin) within a given session.

The other drug versus food choice procedure used in rodents (Thomsen et al. 2008, 2013, 2014, 2017), which also technically functions as a discrete-trails procedure, was adapted from a choice procedure used in primates (Negus, 2003). This choice procedure also consists of a sampling-phase and a choice-phase; however, this was repeated in five different blocks within a given session.

In each block, a constant dose of drug (e.g., cocaine) is compared against a constant non-drug reinforcer (e.g., sucrose solution). Importantly, unlike the "discrete-trials" choice procedure (e.g., Lenoir et al. 2007), the dose of drug (0 mg/kg to 1 mg/kg) increases as a function of block. During the sampling-phase, one drug and one food reinforcer, independent of one another in time, are passively delivered to the rat. Furthermore, upon drug or food delivery, during the sampling-phase, the corresponding lever is extended to provide an association between the response-outcome contingency. After the forced-sampling phase, both levers are extended and upon completion of a fixed-ratio 5 schedule of reinforcement on either the corresponding drug-lever or food-lever, both levers are retracted, and the reinforcer chosen was delivered. Furthermore, each choice-phase lasted for either 20-minutes or when a total of 15 reinforcers was earned. It should also be noted that within Thomsen et al. (2013), a between-session dose increase was also tested; such that, instead of increasing the dose of cocaine throughout the session, one constant dose was used throughout the entire session for all 5 blocks and increased on subsequent days. Results from within- and between-session dose increases were comparable (Thomsen et al. 2013).

Like human and primate research, rodent choice procedures have demonstrated that the availability of a non-drug alternative can shift choice away from drugs of abuse (e.g., Lenoir et al. 2007; Thomsen et al. 2008, 2013; Cantin, 2010). Since a large majority of studies have used the "discrete-trials" choice procedure (e.g., Lenoir et al. 2007), the current state of preclinical-rodent

research for drug versus non-drug choice is working under the assumption that non-drug alternatives (e.g., sucrose, saccharin, and food) are “better reinforcers” than drugs of abuse (e.g., cocaine, methamphetamine, nicotine, and heroin), where the majority of individual rats, will always choose a non-drug alternative over food (Lenoir et al. 2007; Ahmed et al. 2013). Specifically, non-drug reinforcers are “better reinforcers” since rats will, for the most part, always choose the non-drug alternative regardless of the dose of drug available (e.g., cocaine) and the amount of drug consumed in the past (via escalation procedures; Lenoir et al. 2007; Cantin et al. 2010). Moreover, it has been argued that any dose-dependent preference (e.g., Thomsen et al. 2013) seen in drug versus non-drug choice procedures that vary doses within a session is a byproduct of choosing under the influence (Vandaele et al. 2016). For example, having recently sampled cocaine results in a situation where a rat is choosing while under the influence of cocaine. Consequently, this notion has resulted in the hypothesis that by being under the influence of cocaine, it is likely the rat will choose cocaine again producing an increase in preference for cocaine and through this perpetual process the rat will end up in some persistent state of cocaine taking (Vandaele et al. 2016). In brief, once a certain concentration of cocaine within an organism is reached, a shift from non-drug choice to cocaine choice will occur (Vandaele et al. 2016; Freese et al. 2018). If the hypothesis that drug intake causes drug preference is the mechanism that explains dose-dependent preference, it would also suggest that any dose-dependent choice seen in human (e.g., Stoops et al. 2010) and primate research (e.g., Negus,

2003) is driven solely by the pharmacological effect of the drug and has little to do with the relative valuation of available alternatives.

Despite the number of drug versus non-drug choice studies that have been published over the years, only a few studies have applied choice theory to quantitatively analyze how differences in reinforcer dimensions can influence drug versus non-drug preference (e.g., Anderson and Woolverton, 2000; Anderson et al. 2002; Hutsell et al. 2015). Moreover, the application of the matching relationship could elucidate the current state of drug versus non-drug choice in rodent models, where a non-drug reinforcer is asserted to be "qualitatively" better (i.e., having higher innate value) than drugs of abuse regardless of the features drug reinforcement (Lenoir et al. 2007; Ahmed et al. 2013). Additionally, almost all studies examining drug versus non-drug choice expresses drug choice as the number of drug reinforcers earned divided by total (i.e., drug and non-drug) number reinforcers earned, in which the calculated proportion is the assumed value of drug relative to the non-drug reinforcer. Although this measure is common, it is also representative of the relative reinforcer ratio that the organism earns. Specifically, the relative reinforcer ratio is an often-overlooked factor in choice procedures, and preference between two reinforcers, whether it be between non-drug (e.g., McCarthy and Davison, 1984; Johnstone and Alsop, 2000), drug (e.g., Iglauer and Woods, 1974; Iglauer et al. 1975; Woolverton and Alling, 1999), or even drug versus non-drug (e.g., Anderson and Woolverton, 2000; Anderson et al. 2002) is controlled by the relative frequency of reinforcement.

One method that has been used to control for differential rates of reinforcement across the available options in choice procedures has been non-independent or dependent scheduling (Stubbs and Pliskoff, 1969; McCarthy and Davison, 1984). Under dependent scheduling, access to an alternative is dependent upon sampling on all other alternatives. For example, in McCarthy and Davison (1984), pigeons were tasked to discriminate if a light was considered “bright” or “dull” under a controlled reinforcer ratio (CRR; i.e., dependent) schedule. Briefly, the CRR used functioned such that the relative stimulus frequency (i.e., likelihood that the presented light was “bright” or “dull”) was held constant at (50%), and the relative rate of reinforcement was manipulated at three different variable intervals (VI 30/30, VI 75/19, and VI 19/75); importantly, the CRR schedule functioned such that if a reinforcer was arranged for a correct response for a given option (e.g., identification of the light being “bright”), the schedule associated with the other correct response (e.g., identification of the light being “dull”) became unobtainable until the arranged reinforcer was earned. To summarize, subjects were forced to make correct responses across both options. Results from McCarthy and Davison (1984) demonstrated that pigeons under the CRR schedule demonstrated response biases towards the richer option (e.g., VI 75 option) when the rate of reinforcement was different, and indifference when the rate of reinforcement was equivalent (e.g., VI 30/30); moreover, response biases remained unchanged as the discriminability of the lights decreased, whereas under an uncontrolled reinforcer ratio schedule, where the relative rate of reinforcement is dependent

on the choices made by the subject, demonstrated extreme response bias towards the option that resulted in greatest reinforcement. In all, these data demonstrate that the relative rate of reinforcement affects choice; furthermore, under the CRR, pigeons were able to discriminate the changing luminance levels when the difference in light intensity decreased. To summarize, choice is highly influenced by how often an organism comes in contact with the given alternatives.

Likewise, the relative reinforcer ratio earned by the organism also reflects how much drug an organism has taken, and previous research has suggested that there is a relationship between overall intake history of cocaine and the neural adaptations observed (Freeman et al. 2002; Mantsch et al. 2004; Kufahl et al. 2009; Larson et al. 2010; Besson et al. 2013; Gao et al. 2017). Additionally, drug-induced neuroadaptive changes are hypothesized to drive substance use disorders (Everitt and Robbins, 2005; Volkow et al. 2008, 2011). Thus, it is possible that under drug versus non-drug choice procedures, the supposed neural correlates associated with cocaine preference may be a byproduct of the relative reinforcer ratios earned and not preference (e.g., Guillem and Ahmed, 2017). Altogether, the current drug versus non-drug choice procedures that are being utilized in rodent choice procedures are not without issues.

Summary and Aims

Preclinical self-administration research has provided invaluable insight into the neurobehavioral mechanisms associated with substance use disorder. Although preclinical self-administration research has been prolific, much of the research completed has been conducted under single-schedules, where a drug (e.g., cocaine) reinforcer is the only available alternative. However, outside of the laboratory, other reinforcers (e.g., food, monetary goods, and social relationships) are concurrently available alongside drugs of abuse, and human clinical data support the ability for non-drug alternatives to reduce drug choice (e.g., Foltin et al. 2015; Lile et al. 2016). Because a hallmark of substance use disorders is the disproportionate time spent seeking and taking drugs, instead of pursuing other reinforcing alternatives, understanding the processes that underlie choice of drug versus non-drug alternatives is crucial. Recently, a growing literature has begun to investigate drug versus non-drug choice behavior in rodent models to better understand the neurobehavioral mechanisms that drive preference for a drug over a non-drug reinforcer (Ahmed, 2010, 2013; Banks and Negus, 2012).

Under all current drug versus non-drug choice procedures, rats are given the opportunity to allocate preference across two alternatives (e.g., cocaine versus a palatable non-drug commodity) and through the choices made the relative value for the given options can be assessed. Much like preclinical research completed in primates (e.g., Negus, 2003), the magnitude and price of a given reinforcer determines cocaine or food choice in rats (Thomsen et al.

2013). However, recent research into the determinants that drive drug versus non-drug choice have concluded that drug intake, specifically the presence of cocaine within a rat's system during choice, is the driving mechanism that results in preference for cocaine (Vandaele et al. 2016; Freese et al. 2018). Additionally, all current drug versus non-drug choice procedures also overlook differential rates of reinforcement across each alternative. The rate of reinforcement, or how frequently an animal experiences a given alternative, is also an important dimension of reinforcement that determines preference (Anderson and Woolverton, 2000; Anderson et al. 2002). Moreover, differential rates of reinforcement across options can result in systematic biases making changes on a given alternative difficult to detect due to insufficient experience with said alternative (McCarthy and Davison, 1979, 1981; Johnstone and Alsop, 1999).

In attempts to better investigate the neurobehavioral mechanisms that drive preference for cocaine versus food, the current issues of intake causing preference and reinforcer frequency must be resolved. The first experiment of this dissertation will 1) examine a novel model for cocaine versus food choice that accounts for the current confounds that are present in all other drug versus non-drug choice procedures (i.e., differential rates of reinforcement) and additionally examine how environmental manipulations can influence choice when the relative frequency of reinforcement and consequent total intake is held constant. The second experiment will 2) determine how frequency of reinforcement affects cocaine versus food choice. Additionally, the non-drug alternative used herein is compared to saccharin, the non-drug alternative that is

currently used in the majority of all other choice procedures, to determine if there are any possible differences regarding the non-drug alternative used. Finally, the last two experiments of this dissertation will 3) determine cellular brain activation for cocaine versus food preference when the relative frequency of reinforcement and consequent total intake is held constant and 4) determine cellular brain activation for cocaine versus food preference in cocaine-experienced rats and food-experienced rats. These experiments herein aim to expand the current knowledge regarding the neurobehavioral mechanisms underlying value-based decision-making and extend that knowledge to decision-making scenarios involving drug versus non-drug alternatives.

Chapter 2

Experiment 1: Drug vs. Non-drug Choice under Controlled Reinforcer Ratio Schedules

Previous choice studies (e.g., Lenoir et al. 2007; Cantin et al. 2010; Thomsen et al. 2013) have investigated procedural determinants necessary for a non-drug alternative (e.g., saccharin and sucrose) to effectively compete against a drug of abuse (e.g., cocaine). For example, Lenoir et al. (2007) trained a group of rats on a "discrete-trials" choice procedure for either a 0.25 mg/kg/infusion of cocaine or a maximum of 0.3 ml of a 0.2% saccharin solution. Under these conditions, rats showed exclusive preference for saccharin. When the dose of cocaine was increased (e.g., 0.75 mg/kg/infusion and 1.5 mg/kg/infusion) there were no changes in preference. Furthermore, by adding delays (e.g., 0 to 18s) to saccharin delivery, longer delays resulted in a shift towards cocaine. Interestingly, increasing the price (i.e., ratio requirement) for both options further increased preference for saccharin. Altogether, Lenoir et al. (2007) concluded that a 0.2% saccharin was "qualitatively" better (e.g., having more innate value) than cocaine since the dose of cocaine does not influence preference. Using the "discrete-trials" procedure other studies have also found similar results (e.g., Cantin et al. 2010; Lenoir et al. 2013; Madsen and Ahmed, 2015).

Conversely, under another drug versus non-drug choice procedure based on primate choice protocols (Negus, 2003), dose-dependent preference was demonstrated between cocaine (0.0 mg/kg/infusion to 1.0 mg/kg/infusion) and 56% Ensure in water (Thomsen et al. 2013). Furthermore, adjustments to the

price (i.e., ratio requirement) for a given alternative resulted in orderly shifts in preference towards the cheaper option. Moreover, changes in the concentration of Ensure also resulted in orderly shifts in preference, where lower concentrations resulted in a greater shift in choice for cocaine.

These differences in results regarding the extent that cocaine dose affects preference, lead to the investigation in differences between the two choice procedures. In a series of experiments conducted by Vandaele et al. (2016), it was concluded that the inter-trial interval (ITI) was the key variable that caused these differences in preference seen between the two procedures. Specifically, under the “discrete-trials” procedure (e.g., Lenoir et al. 2007), each trial was separated by a 10-min ITI, whereas the primate-modeled choice procedure (e.g. Thomsen et al. 2013) had a 20-s ITI. It was hypothesized that the programmed ITI affected cocaine concentrations within a rat at the time of choice, and that by shortening the ITI to 1 minute that a large majority of rats that were once showing exclusive preference for saccharin switched to exclusive preference for cocaine. Furthermore, regardless of the state of the rat (i.e., food deprived), rats would choose cocaine continuously if they were under the influence of cocaine. Altogether, it was concluded that cocaine preference is caused by cocaine intake such that there must be drug on board at time of choice to get preference for cocaine. Additionally, it was hypothesized that by taking cocaine, preference for cocaine increases due to the anorectic effects that are associated with cocaine use which subsequently devalues the non-drug alternative. Overall, it was hypothesized that crossing some threshold level of cocaine intake results in a

"locked-in" pattern of drug-taking, regardless of consequences (Vandaele et al. 2016).

Although the hypothesis that intake causes preference may seem plausible, via some threshold reached causing some significant pharmacological aspect of the given drug to take place, another often overlooked confound is the relative rate of reinforcement, or how frequently each reinforcer is experienced during choice. Within these choice procedures rats are limited to a set number of available reinforcers across which they are allowed to distribute their choices; however, a choice for one reinforcer results in the net-loss in availability for the other reinforcer. Thus, the relative rate of reinforcement across reinforcers can become disparate, where repeated choice for one option results in a greater overall loss for the other option. Importantly, frequency of reinforcement is a determinant of choice according to choice theory (McCarthy and Davison, 1988). Thus, it is possible that the current discrepancy in results regarding the dose-dependent effects of cocaine influencing choice (e.g., Lenoir et al. 2007, Cantin et al. 2010 vs. Thomsen et al. 2013) is in part due to differential sampling histories for the given alternatives.

Herein, we utilized a CRR schedule (Stubbs and Pliskoff, 1969; McCarthy and Davison, 1984) for cocaine versus food choice to dissociate preference from intake, while controlling for rate of reinforcement across the two options that will vary under uncontrolled reinforcer ratio (URR) schedules. If the hypothesis that cocaine preference is driven by cocaine intake then, preference and intake should be correlated. Furthermore, if cocaine preference is driven by the

accumulation of cocaine (e.g., Vandaele et al. 2016; Freese et al. 2018), then once a certain threshold of cocaine is reached within a rat, a “locked-in” pattern of drug-taking should take hold such that all choice, regardless of environmental manipulations, should be identical under the under a CRR schedule.

Methods

Subjects

Twenty-four adult male Sprague-Dawley Rats (Harlan Inc.; Indianapolis, IN, USA), weighing approximately 250-275 g on arrival, were used. Rats were individually housed (12:12 hr light:dark cycle) with ad libitum access to food and water in their home cage. During periods of food restriction, rats were maintained at approximately 85% of their free-feeding body weights. All experimentation was conducted during the light phase. All experimental protocols were conducted in accordance to the 2011, *National Research Council: Guide for the Care and Use of Laboratory Animals* (8^{th} edition) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Experiments were conducted in operant chambers (ENV-008CT, MED Associates, St. Albans, VT) enclosed within sound-attenuating compartments (ENV-018MD). Each chamber was connected to a personal computer (SG-502), and all chambers were operated using MED-PC. Within each chamber, a recessed food receptacle (ENV-202R2MA) outfitted with a head-entry detector

(ENV-254-CB) was located on the front response panel of the chamber, two retractable response levers were mounted on either side of the food receptacle (ENV-122CM), and a white cue-light (ENV-221M) was mounted above each response lever. The back-response panel was outfitted with two nosepoke response receptacles (ENV-114BM) directly opposite to front response levers, a house-light (ENV-227M) was located at the top of the back panel between the two nosepoke response receptacles with Sonalert© tones (ENV-223 AM and ENV223-HAM) located on either side of the house-light. Food pellets (45-mg Bio-Serv Precision Pellets; Flemington, NJ) were delivered via a dispenser (ENV-203M-45). Drug infusions were delivered via a syringe pump (PHM-100) through tubing strung through a leash (PHM-110-SAI) that attached to a swivel above the chamber.

Drugs

Cocaine hydrochloride, gifted from the National Institute on Drug Abuse (Bethesda, MD, USA), was mixed in sterile saline (0.9% NaCl).

Establishing Procedures

Magazine shaping

Rats were first trained to retrieve food pellets from the food receptacle for two to three consecutive days. Rats were placed in the operant chambers and given 45 minutes to retrieve and consume 20 food pellets, delivered on a 60-s fixed time schedule.

Lever training

Rats were then trained to lever press on a fixed-ratio (FR) schedule of reinforcement, where completion of the FR requirement on the presented lever would result in lever retraction and delivery of a food pellet. Each session consisted of 30 trials, 15 left-lever and 15 right-lever presentations. Levers were presented individually and pseudo-randomly, where no more than 6 presentations of the same lever would occur in a row. Trials were separated by a 12-s inter-trial interval (ITI). Lever training started on a FR1 for three days, moved onto an FR3 for two days, and ended on an FR5 that lasted for three days.

Orienting response

Next, an orienting response was added. The start of each trial was now signaled by the illumination of the house-light. A contingent response, head-entry into the magazine, would result in the offset of the house-light and extension of either the left or right lever. Each session consisted of 30 trials, 15 left- and 15 right-lever presentations. Levers were presented individually and pseudo-randomly, where no more than 6 presentations of the same lever would occur in a row. Trials were separated by a 12-s ITI. Rats were trained on this response chain for three days.

Catheter surgery

Rats then underwent surgery for implantation of a chronic indwelling jugular catheter. Rats were first anesthetized with a ketamine (Schein, Dublin, OH)/xylazine (Akorn, Inc., Decatur, IL)/acepromazine (Boehringer Ingelheim, St. Joseph, MO; 75/7.5/0.75 mg/kg) mixture at 0.15 ml/100 g body weight (i.p.). Catheters were inserted into the jugular vein, extended under the skin, and exited the body through an incision on the scalp. A cannula was attached to the end of the catheter and secured to the skull using dental acrylic and four jeweler's screws. Animals were given a week to recover after surgery.

Drug self-administration training

Following recovery, rats were then trained to self-administer cocaine (1.0 mg/kg/infusion). Rats were placed on a FR schedule, with an orienting response, for cocaine. Briefly, each trial was signaled by the illumination of the house-light where a head-entry into the magazine would result in the house-light turning off and the extension of a single lever (balanced across animals). Upon meeting the FR requirement, the lever would retract, and rats would receive a 0.1 ml infusion of cocaine, totaling 1.0 mg/kg/infusion; Thomsen et al. 2013) over 5.9s accompanied by the illumination of the cue-light above the lever. Trials were separated by a dark 14.1-s ITI. Sessions lasted for 1 hour and rats started on a FR1 for three days, moved onto a FR3 for two days and ended on a FR5 that lasted for three days.

Food vs. drug lever training

After cocaine-self administration training, rats were placed on a lever discrimination procedure where rats had access to both food pellets and cocaine (1.0 mg/kg/infusion). Each trial began with the illumination of the house-light, where an orienting response into the magazine resulted in the house-light turning off and the extension of the previously trained drug lever or the opposite food lever. Completing the FR5 on the presented lever would result in lever retraction and reward delivery accompanied by the illumination of the corresponding cue-light for 5.9s. Trials were separated by a dark 14.1-s ITI. Sessions ended when 5 of each reinforcer, cocaine and food, were earned. Rats were trained on this schedule for four sessions.

Experiment Proper

Following the establishing procedures, rats were randomly assigned to either the controlled reinforcer ratio (CRR) or uncontrolled reinforcer ratio (URR) schedule for cocaine versus food choice. Both choice procedures functioned similarly in that each session was divided into 5 distinct blocks separated by a dark and empty 2-min inter-block-interval. Additionally, each block was signaled by an accompanying tone pattern (alternating between 40 kHz and 29 kHz) that played continuously at 1.8/0, 1.5/0.3, 0.9/0.9, 0.3/1.5, and 0/1.8 seconds (see Table 1). In each of the 5 blocks, responses on the food lever resulted in the delivery of a single 45-mg food pellet, while responses on the cocaine lever resulted in an infusion of cocaine at varying doses. The dose of cocaine (0,

0.032, 0.10, 0.32, and 1.0 mg/kg/infusion) increased as a function of block. Upon food pellet delivery, the lever would retract and the cue-light above the corresponding lever would turn on for 5.9s in all blocks. Upon cocaine infusion, the cue-light above the corresponding lever would turn on for a varying duration that matched the infusion length (0, 0.189, 0.59, 1.89, and 5.9s) that achieved the dose for the given block. Each trial began with the illumination of the house-light where an orienting response into the magazine would turn off the house-light and extension of the response lever or levers. All responses were scheduled on a fixed-ratio (FR) and required consecutive responding; a changeover in responding would reset the FR count. Upon completion of the FR requirement, levers would retract and reward delivery, signaled by a corresponding cue-light, would occur. Rats were initially trained on a FR1 and were incrementally progressed up to an FR5. All trials were separated by a dark and empty 10-s inter-trial-interval (ITI). Sessions ended upon completion of all 5 blocks.

Controlled Reinforcer Ratio (CRR)

The CRR choice procedure consisted of a total of 3-drug and 3-food trials per block. Both levers (cocaine and food) were extended during each trial. Importantly, during each trial only one of the two reinforcers was randomly made available. Regardless of which lever the rat responded on, the reinforcer that was scheduled for that trial had to be earned to advance onto the next trial. Importantly, using this method, the relative number of cocaine to food reinforcers earned (3 each) is kept constant across all sessions and between all animals

(i.e., the cocaine:food reinforcer frequency ratio is held constant). After completion of all 6 trials, the block would end and enter into the inter-block-interval.

Uncontrolled Reinforcer Ratio (URR)

The URR choice procedure, based on methods in Thomsen et al. (2013), consisted of a sample-phase and choice-phase for each block. Sample-phases consisted of two trials, where a single-random lever that corresponds with either food or cocaine was independently extended. Rats were required to complete each sample-trial to advance. After completion of the sample-trials, the choice-phase started where both levers were extended on trial start. With both levers extended, rats had the opportunity to distribute 6 total choices across the two options within 30 minutes. Upon completion of the FR, both levers would retract, and reward delivery would occur. After 6 total reinforcers within a block were earned or 30 minutes had elapsed, the block would end and enter into the inter-block-interval.

Environmental Manipulations

Following stability, defined as no significant changes in choice performance (i.e., percent choice at end points) for four consecutive days, under baseline conditions on either choice procedure (CRR or URR) all rats were assigned, via Latin square design (baseline first), to the environmental manipulations.

Food restriction

To determine the effects of food motivation on cocaine choice, rats were food restricted and maintained at approximately 85% of their free-feeding body weights during the testing period.

Drug-infusion cue removal

To determine the effects that cocaine-associated cues have on choice, the cue-light signaling cocaine infusion was removed; thus, cocaine delivery went unsignaled across all blocks.

Orienting-response removal

To determine the effects of subject-determined trial initiation on choice, the orienting response was removed. All trials were no longer initiated by a head-entry into the magazine; thus, the house-light was not used, and all trials began immediately with the extension of the response lever or levers.

Each experimental manipulation was tested for a minimum of ten days. Additionally, rats were returned to baseline conditions for a minimum of seven days before being assigned to the next environmental manipulation. Moreover, once completing the assigned choice procedure, rats were switched to the opposite choice procedure and trained to stability and underwent the same series of environmental manipulations. The resulting n-sizes were n=20 for CRR and URR baseline; n=14 for CRR and n=11 URR for food restriction; n=15 CRR and n=10 URR for no drug-infusion cue; n=12 CRR and n=9 URR for no orienting response (i.e., head entry). All attrition was due solely to catheter failure.

Analysis

Choice for cocaine was calculated differently for the URR and CRR choice procedures. In the URR, preference for each block was calculated as the total number of cocaine reinforcers earned divided by the total number of reinforcers earned (see Figure 1; e.g., Lenoir et al. 2007; Thomsen et al 2013). Because the number of reinforcers for both drug and food were kept constant under the CRR, using the same measure would always result in 50% cocaine preference; an alternative preference measure for the CRR was necessary. Preference for the CRR was calculated as the total number of choice responses for cocaine (i.e., responses on the drug lever when drug was not scheduled) divided by the overall number of choice responses for both reinforcers (i.e., responses made on both the drug and the food lever when the respective reinforcer was not scheduled; see Figure 2). To address possible concerns regarding the continuous nature of the choice measure, where choice responses made under the CRR have an unlimited range, versus the discrete measure (i.e., number of reinforcers earned) under the URR, preference for the CRR was also calculated as the proportion of first responses for cocaine made on each trial; both choice measures for the CRR were significantly correlated (Pearson's r = 0.99; $p < 0.05$; see Figure 3). Thus, we settled on using the number of choice responses made (Baum and Rachlin, 1969; Killeen, 1972). Additionally, calculating preference under the URR as the proportion of number of choice responses made for cocaine results in the exact same measure as the number of reinforcers earned since they are the same measure.

To quantitatively analyze choice under the CRR and URR, the generalized matching law (Baum, 1974; Hutsell et al. 2015) was applied. The form of the generalized matching equation used is as follows:

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{M_f}{M_d}\right)^{s_M}} \qquad \text{(Eqn 10)}$$

Where B_d represents behavior for drug, B_f represents behavior for food, and M_d represents the magnitude (i.e., dose) of drug, and M_f represents the magnitude of food. The free parameter s_M represents the sensitivity to change in the relative magnitude between drug and food reinforcers. However, since drug and food are qualitatively different reinforcers, and the relative comparison for drug to food is unknown, the generalized matching equation applied was:

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{a}{M_d}\right)^{s_M}} \qquad \text{(Eqn 11)}$$

Where all variables are the same above except M_f becomes a free parameter *a*; *a* is a scaling constant, which can be conceptualized as the cocaine-food exchange rate that scales food reinforcement into cocaine units. Such that, the unit dose of cocaine that is equivalent to one 45-mg food pellet is the dose that produces 50% drug choice, suggesting that, under the given conditions, food and drug are perfect substitutes. Thus, larger numbers indicate greater relative value for food, and lower numbers greater relative value for cocaine.

Using the data from the same sessions used to calculate choice, estimated whole-body cocaine levels (mg/kg) at reinforcer delivery were also determined according to the following kinetics equation (Weiss et al. 2003):

$$B_n = (B_{n-1} + D)e^{-kt} \quad \text{(Eqn 12)}$$

Where B_n represents current cocaine levels (mg/kg), B_{n-1} represents cocaine levels (mg/kg) from previous infusions, *D* represents the dose of cocaine for the given block, *k* represents the decay constant (0.0383), and *t* represents minutes since last infusion.

All data were analyzed using linear mixed-effects modeling (LME; Gelman and Hill, 2006) and nonlinear mixed-effects modeling (NLME; Pinheiro et al. 2007). All correlations were carried out using Spearman's ρ (Zar, 1972; Hauke and Kossowki, 2011). For all tests, α was set to 0.05.

Percent cocaine choice for baseline conditions were independently analyzed using NLME with schedule (nominal) and dose (continuous) as within-subject factors, and subject as a random factor. Additionally, all percent cocaine choice manipulations were analyzed using NLME with schedule (nominal), condition (nominal), and magnitude (continuous) as within-subject factors, and subject as a random factor. The averaged whole-body cocaine levels (mg/kg) at reinforcer delivery was analyzed with LME with schedule (nominal), condition (nominal) and dose (continuous) as within-subject factors, and subject as a random factor. Correlations between parameter estimates from the generalized matching law (i.e., *a*, cocaine-food exchange rate) and the average estimated whole-body cocaine levels prior to reinforcer delivery during the last block (i.e., 1.0 mg/kg/infusion cocaine) were calculated using Spearman's ρ; the last block was chosen since whole-body cocaine levels are cumulative.

Using data from the same sessions used to calculate the percent choice curves and whole-body cocaine levels, latency to first response was calculated for each block as the time to head-entry, following house-light illumination, added to the time to first lever press during choice trials for baseline, food restriction, and no cocaine cue conditions. However, latency to first response was calculated for each block as time to first lever press during choice trials for the no head-entry condition only, due to the lack of a contingent-orienting response. Latencies were analyzed with LME with schedule (nominal), condition (nominal) and dose (continuous) as within-subject factors, and subject as a random factor. In addition, overall response rates were calculated as the total number responses made on the cocaine or food lever during the duration of the choice trials for each block. Overall response rates were analyzed with LME with schedule (nominal), condition (nominal), reinforcer (nominal), and dose (continuous) as within-subject factors, and subject as a random factor. Additionally, whole-body cocaine levels (mg/kg) at reinforcer intake as a function of trial-by-trial were analyzed with LME with schedule (nominal), condition (nominal) and trial (continuous) as within-subject factors, and subject as a random factor.

Results

Figure 4 illustrates percent choice for cocaine under the (4A) CRR and URR at baseline, including individual choice profiles under the (4B) CRR and (4C) URR. NLME analysis of baseline preference revealed that the CRR produced greater sensitivity to changes in relative reinforcer magnitude (s_M) than

the URR [$F(1,172)=10.47$, $p<0.05$], while there were no significant differences in cocaine-food exchange (a). Thus, while both procedures produced similar dose-dependent increases in cocaine preference, sensitivity to changes in the relative reinforcer magnitude ratio was greater under the CRR schedule.

Figure 5 illustrates percent choice for cocaine across the different environmental manipulations under the (5A) CRR and (5B) URR, along with parameter estimates from the generalized matching equation for (5C) cocaine-food exchange rate (a) and (5D) magnitude sensitivity (s); whereas Figure 6 illustrates individual choice profiles for the environmental manipulations. NLME analysis revealed a significant main effect of condition [$F(3,515)=57.13$, $p<0.05$] and a schedule x condition interaction [$F(3,515)=6.63$, $p<0.05$] on the cocaine-food exchange rate (a), indicating that the substitutability between cocaine food was affected by the different environmental manipulations, and that these differences were schedule-dependent. Post hoc analysis (Bonferroni corrected) indicated that the cocaine-food exchange increased, relative to respective baseline, under the CRR when animals were food restricted, while there was no effect of food restriction under the URR. Post hoc analysis (Bonferroni corrected) also indicated that the cocaine-food exchange increased, relative to respective baseline, under the CRR and URR, while the cocaine-food exchange decreased, relative to respective baseline, under the CRR and URR. Finally, NLME analysis also revealed a significant main effect of schedule [$F(1,515)=3.35$, $p<0.05$] on sensitivity to reinforcer magnitude (s_M), indicating that the sensitivity to the relative magnitude was greater overall under the CRR. Altogether, the results

demonstrated that the effects of environmental manipulations on the relative value between cocaine and food was differentially affected when the reinforcer ratio was controlled (CRR) versus uncontrolled (URR), and the overall sensitivity to changes in relative reinforcer magnitude was better under the CRR.

Figure 7 illustrates latency to first response under the CRR and URR. LME analysis revealed a main effect of dose [$F(1,24.03)=141.66$, $p<0.05$], schedule [$F(1,18.88)=30.52$, $p<0.05$], and condition [$F(3,14.26)=6.38$, $p<0.05$] for latency to first response. LME analysis also revealed a dose x schedule interaction [$F(1,20.84)=29.86$, $p<0.05$] and dose x condition [$F(1,39.62)=5.28$, $p<0.05$] interaction for latency to first response. Altogether, the results revealed that the latency to first response increased as a function of dose.

Figure 8 illustrates overall rates of responding for cocaine and food under the CRR and URR. LME analysis revealed a main effect of dose [$F(1,27.10)=173.48$, $p<0.05$], schedule [$F(1,24.79)=57.42$, $p<0.05$], and reinforcer [$F(1,24.69)=49.76$, $p<0.05$] for overall rates. LME analysis also revealed a significant dose x schedule x condition x reinforcer interaction [$F(3,62.17)=6.12$, $p<0.05$]. Altogether, the results demonstrated that the overall rates of responding for cocaine versus food changed, depending on the condition, as a function of dose across the blocks, where the overall rates of responding for food decreased as the rates of responding for cocaine increased. Moreover, the overall rate of responding under the URR was greater than that of the CRR.

Figure 9 illustrates the whole-body cocaine levels (mg/kg) at reinforcer delivery under the (9A) CRR and (9C) URR averaged for each block, and the

correlations between the averaged individual whole-body cocaine levels (mg/kg) during the last block and individual cocaine-food exchange rates (*a*) under the (9B) CRR and (9D) URR. LME analysis revealed a main effect of dose [$F(1,24.93)=533.32$, $p<0.05$], schedule [$F(1,22.98)=22.38$, $p<0.05$], and condition [$F(3,45.45)=4.96$, $p<0.05$]. LME analysis also revealed a dose x schedule x condition interaction [$F(3,42.03)=3.46$, $p<0.05$], indicating that whole-body cocaine levels increased throughout the session, but increased at different rates between the environmental manipulations, where the URR produced higher whole-body cocaine levels than the CRR. Moreover, under the URR, whole-body cocaine levels increased at different rates, under different manipulations, due to individual subjects-determining when to take drug; on the contrary, under the CRR whole-body cocaine levels were identical across all manipulations. When individual whole-body cocaine levels (mg/kg) during the last block was correlated to individual cocaine-food exchange rates, it was revealed that under the URR a strong correlation was found (Spearman's $\rho = 0.69$, $p<0.05$ for overall URR; Spearman's $\rho = 0.51$, $p<0.05$ for baseline condition only; Spearman's $\rho = 0.66$, $p<0.05$ for food restriction only; Spearman's $\rho = 0.91$, $p<0.05$ for no cocaine cues only; Spearman's $\rho = 0.40$, NS for no head entry condition only), indicating that preference and intake are codependent. However, under the CRR, where the relative ratio of reinforcers earned was kept constant, the correlation between whole-body cocaine levels (mg/kg) and cocaine-food exchange rate (*a*) was eliminated (Spearman's $\rho = 0.01$, NS for overall CRR; Spearman's $\rho = 0.09$, NS for baseline condition only; Spearman's $\rho = 0.32$, NS for food restriction only;

Spearman's ρ = 0.13, NS for no cocaine cues only; Spearman's ρ = 0.30, NS for no head entry condition only); thus, dissociating preference from intake.

Figure 10, which illustrates the whole-body cocaine levels (mg/kg) at reinforcer delivery plotted as a function of trial under the (10A) CRR and (10B) URR for all conditions; whereas, Figure 11 illustrates individual profiles for whole-body cocaine levels for under the CRR and URR. LME analysis revealed a main effect of trial [$F(1,25.44)=593.58$, $p<0.05$], schedule [$F(1,21.22)=56.23$ $p<0.05$], and condition [$F(3,42.46)=3.24$ $p<0.05$], and a trial x schedule x condition interaction [$F(3,32.42)=3.30$ $p<0.05$], indicating that whole-body cocaine levels increased throughout the session, but increased at different rates between the environmental manipulations. Furthermore, the URR produced higher whole-body cocaine levels than the CRR.

Discussion

Although uncommon, dependent scheduling (e.g., CRR) has been applied to non-drug choice studies (Baum and Davison, 2000; Grace et al. 2003; Beeby and White, 2013; Pope et al. 2015) and to drug-drug choice studies (Llewellyn et al. 1976). However, to our knowledge this experiment is the first to successfully apply dependent scheduling to drug versus non-drug choice. Granting that the use of dependent scheduling, via the CRR, resulted in visually-similar results as independent scheduling (i.e., the URR used herein), at baseline, the use of the CRR did so while controlling for frequency of reinforcement; a known variable that affects drug preference (e.g., Anderson and Woolverton, 2000; Anderson et

al. 2002). Under both schedules, dose-dependent preference was observed. Additionally, there were no differences in the cocaine-food exchange rate (*a*; CRR = 0.18 vs. URR = 0.21), at baseline, across the two schedules; these reported values also mirror indifference points (i.e., dose of cocaine where choice for cocaine is at 50%) in previous findings (e.g., Thomsen et al. 2013). However, further analysis, via NLME, revealed that under the CRR, sensitivity to magnitude (s_M) was greater, indicating rats could better discriminate the relative difference between a food pellet and the dose of cocaine since the CRR required subjects to sample both alternatives across all doses (Davison and Baum, 2000).

While both choice procedures produced comparable dose-dependent preference for cocaine, whole-body cocaine levels at reinforcer delivery increased as a function of block for both procedures as well; albeit, rats under the URR reached higher whole-body cocaine levels at the end of the session due to the design of the procedure where the number of reinforcers earned for a given alternative is subject-determined. Correlations between the cocaine-food exchange rate (*a*) and the averaged whole-body cocaine levels during the last block revealed that these two measures were strongly correlated for the URR, suggesting that preference and intake are intertwined. For example, this correlation indicates that a rat with a low cocaine-food exchange rate (*a*) chose cocaine over food earlier within the session, resulting in higher levels of whole-body cocaine levels at the end of the session, supporting the possibility that preference for cocaine is driven by intake of cocaine. On the contrary, the CRR

did not produce this codependency between preference and intake, demonstrating that preference is not necessarily the byproduct of cocaine intake.

Results from the environmental manipulations also provided some interesting insight into cocaine versus food choice under the CRR and URR choice procedures. First, food restriction increased the cocaine-food exchange rate (*a*) under the CRR, but not under the URR. Shifting of the cocaine-food exchange rate, under the CRR, parallels findings seen in demand elasticity in open versus closed economies (Hursh and Roma, 2016), where limited access to non-drug commodities can increase the substitutability of given non-drug reinforcer. Second, removal of the cocaine cue increased the cocaine-food exchange rate under both choice procedures. Previous studies have also examined if removal of exteroceptive cues (e.g., light or infusion-pump sound), associated with drug reinforcement, could affect drug preference, and found that removal of either the light or infusion-pump sound had no effects (Thomsen et al. 2013). However, it is possible that the light and infusion-pump sound functioned as a compound cue, and removal of only one aspect of the compound cue did not affect the exteroceptive signals for cocaine reinforcement (Rescorla et al. 1995; Brandon et al. 2000). Finally, under both procedures, removal of the required head-entry response decreased the cocaine-food exchange rate. Despite there being no differences in latency to first response between the conditions with an orienting response (i.e., baseline, food restriction, and no cocaine cue) and the condition without (i.e., no head entry), the cocaine-lever was essentially extended for a longer duration; providing that the food-lever was

also extended for the same duration since both levers are presented during choice. This observed effect could be attributed to conditioned reinforcement (Kearns et al. 2011; Tunstall and Kearns, 2016) since there is evidence that cocaine-associated cues serve as stronger conditioned reinforcers relative to food-associated cues. Likewise, it has also been demonstrated that decreasing the time (e.g., inter-trial interval) between choice opportunities can promote cocaine choice (Elsemore et al. 1980), such that it is possible that be having the levers extend immediately, instead of self-initiated via a contingent head-entry response, could have decreased the perceived time between choices. In all, these environmental manipulations, for the most part, produced orderly shifts in the substitutability of cocaine versus food. However, one important note is that under the CRR, sensitivity to magnitude (s_M) remained relatively unchanged across manipulations; whereas, under the URR, sensitivity to magnitude varied depending on the environmental manipulation used. Although, the overall results for the environmental manipulations are comparable under the CRR and URR, the CRR was able to do so by keeping the relative rate of reinforcement and whole-body cocaine levels constant across all individuals.

Of further note, both choice procedures produced similar patterns of dose-dependent rates of responding (e.g., latency to first response and overall rates) across all conditions (i.e., environmental manipulations); these results are parallel to previously reported rates of responding using a URR schedule for cocaine versus non-drug choice (e.g., Iglauer and Woods, 1974; Negus, 2003; Thomsen et al. 2013). Moreover, rates of responding for cocaine mirror the

"inverted U-shape" seen under single-schedules; however, preference on the descending limb (i.e., higher doses) was in favor for cocaine, thus suggesting that the U-shape seen may be reflective of the pharmacokinetic properties of cocaine and not value (Tsibulsky and Norman, 1999; Weiss et al. 2003).

Given that the current hypothesis, within the rodent literature, suggests cocaine preference is a byproduct of cocaine intake (e.g., Vandaele et al. 2016; Freese et al. 2018), there are a few critical points that need to be considered. First, this hypothesis relies on evidence demonstrating that the administration of cocaine suppresses feeding behavior (e.g., Balopole et al. 1979; Woolverton et al. 1978). Importantly, it should also be noted that the anorectic effect of cocaine occurs under conditions in which cocaine is administered acutely, but not when it is administered chronically (Woolverton et al. 1978; Foltin and Schuster, 1982; Hoffman et al. 1987; Hughes et al. 1996). Under URR choice procedures with varying cocaine doses, like the one used herein and by Thomsen et al. (2008, 2013, 2017), cocaine is forcibly-sampled before the animal can progress onto choice; thus, in some sense, animals are chronically exposed to cocaine which makes it unlikely that cocaine intake under these conditions produces anorexia. Second, d-amphetamine, a stimulant known to have long-lasting anorectic effects similar to cocaine, has been shown to reduce cocaine preference over food (Thomsen et al. 2013; Banks et al. 2013; Hutsell et al. 2015) and money (Grabowski et al. 2004); if anorectic effects are the cause of non-drug devaluation, then administration of d-amphetamine should increase cocaine preference, not decrease it, according to the hypothesis posited in Vandaele et

al. (2016) and Freese et al. (2018). Another argument that the Vandaele et al. (2016) and Freese et al. (2018) make regarding cocaine preference being a byproduct of cocaine intake is that cocaine preference only occurs when a rat is under the influence of cocaine, thus cocaine preference and intake should be correlated. Data from the experiments herein (i.e., Figure 9B) demonstrate that cocaine preference is dissociable from cocaine intake. Furthermore, under the CRR, environmental manipulations produced differences in preference, determined via cocaine-food exchange rates, while keeping cocaine intake exactly the same across all conditions. (i.e., Figure 5A and Figure 9A); if cocaine preference is influenced by cocaine intake, then cocaine preference under the different manipulations should be identical since whole-body cocaine levels were identical across conditions. Altogether, these results suggest that cocaine preference is not necessarily dependent on cocaine intake.

In all, these results from this experiment herein demonstrate that, under a CRR, cocaine preference and cocaine intake are independent, and dissociable. Moreover, results demonstrated that cocaine preference is influenced by the relative difference in magnitude between cocaine and food, obeying choice theory. Furthermore, this present experiment demonstrates the use of a CRR for cocaine versus food choice, which controls for the relative rate of reinforcement, an overlooked issue in all other drug versus non-drug choice studies.

Table 1. Framework used within the URR and CRR choice procedure. Each session consisted of 5 blocks signaled by a distinct tone pattern. The food alternative was kept constant at one 45-mg food pellet signaled by a 5.9s cue-light, while the dose of cocaine increased as a function of block signaled by a corresponding cue-light.

Block	Block Signal	Food	Food Signal	Cocaine Dose (mg/kg)	Cocaine Signal
1	Solid 40 kHz	1 Food Pellet	5.9s Light	0.0	0s Light
2	40 kHz - 1.5s 29 kHz - 0.3s			0.032	0.189s Light
3	40 kHz - 0.9s 29 kHz - 0.9s			0.10	0.59s Light
4	40 kHz - 0.3s 29 kHz - 1.5s			0.32	1.89s Light
5	Solid 29 kHz			1.0	5.9s Light

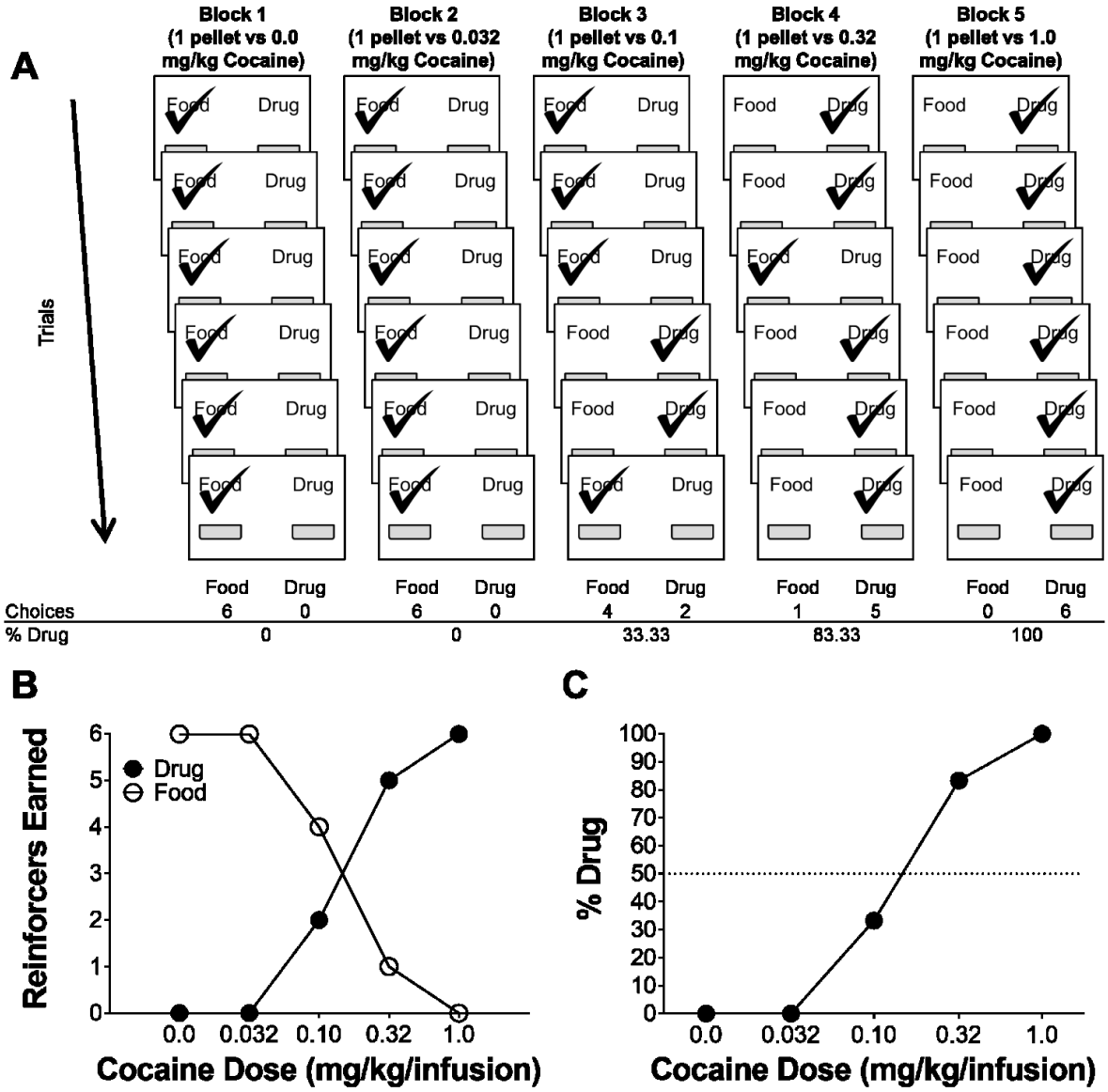

Figure 1. Example session from a single subject under the uncontrolled reinforcer ratio schedule (URR). (**A**) A trial-by-trial (rows) and block-by-block (columns) breakdown during a URR session, where the left lever is associated with food and the right lever is associated with drug. Within each trial, both reinforcers are available and a check mark over the food/drug label represents choice made by the animal. (**B**) Graphical representation of the number of reinforcers earned across blocks as a function of dose. (**C**) Graphical representation of the percent choice for drug via number of drug reinforcers chosen.

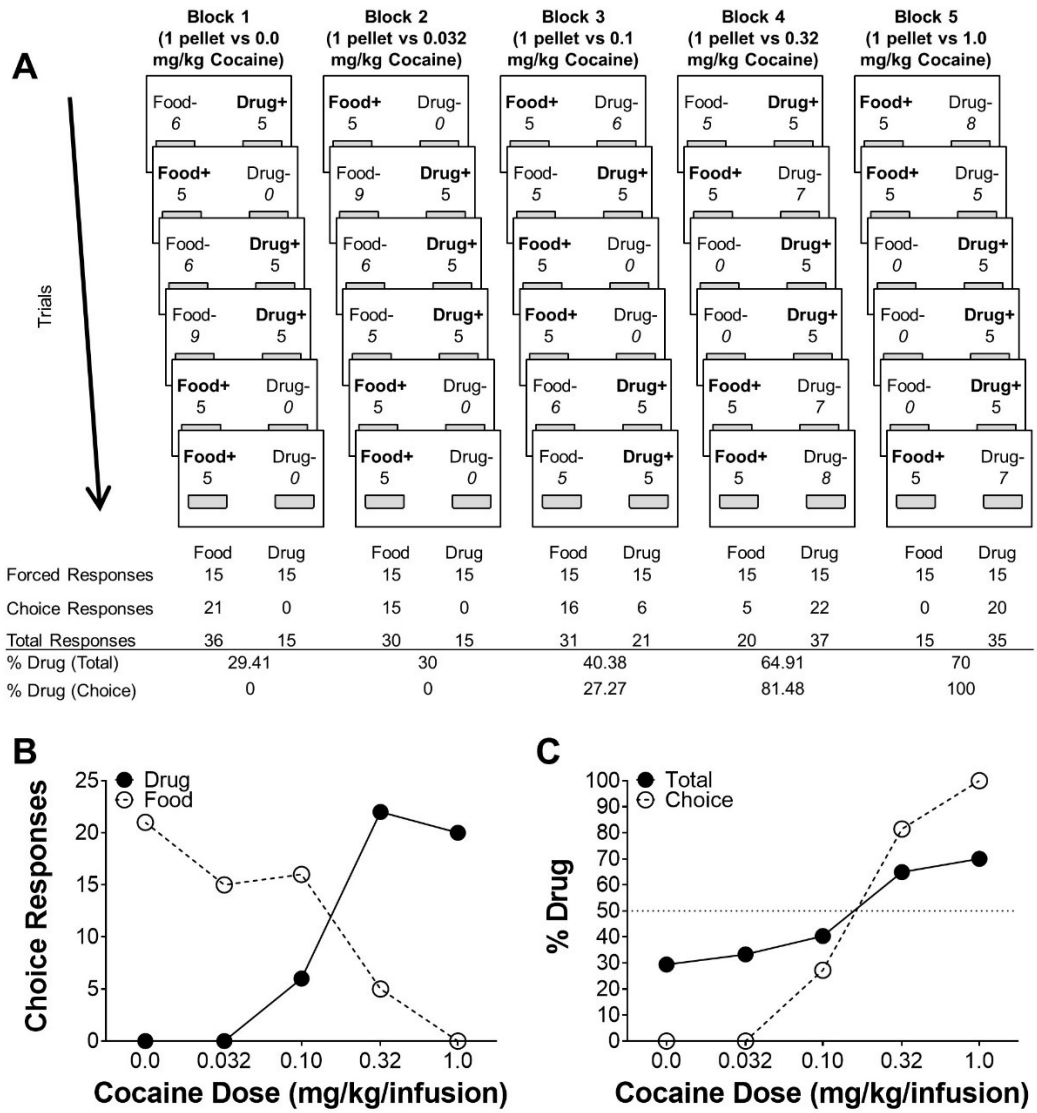

Figure 2. Example session from a single subject under the controlled reinforcer ratio schedule (CRR). (**A**) A trial-by-trial (rows) and block-by-block (columns) breakdown during a CRR session, where the left lever is associated with food and the right lever is associated with drug. Within each trial, only one reinforcer is scheduled, represented by bolded text with (+) sign. The number above each lever, below food/drug labels, represents the number of responses made on that lever. Numbers that are under bolded labels with (+) signs represent forced responses (i.e., responses required to progress the trial); numbers that are under un-bolded labels with (-) signs represent choice responses (i.e., responding on the side where the given reinforcer is unavailable). (**B**) Graphical representation of the number of choice responses (i.e., lever presses when the reinforcer was unscheduled) across blocks as a function of dose. (**C**) Graphical representation of the percent choice for drug accounting for total responses (forced + choice responses) and percent choice for drug according to choice responses.

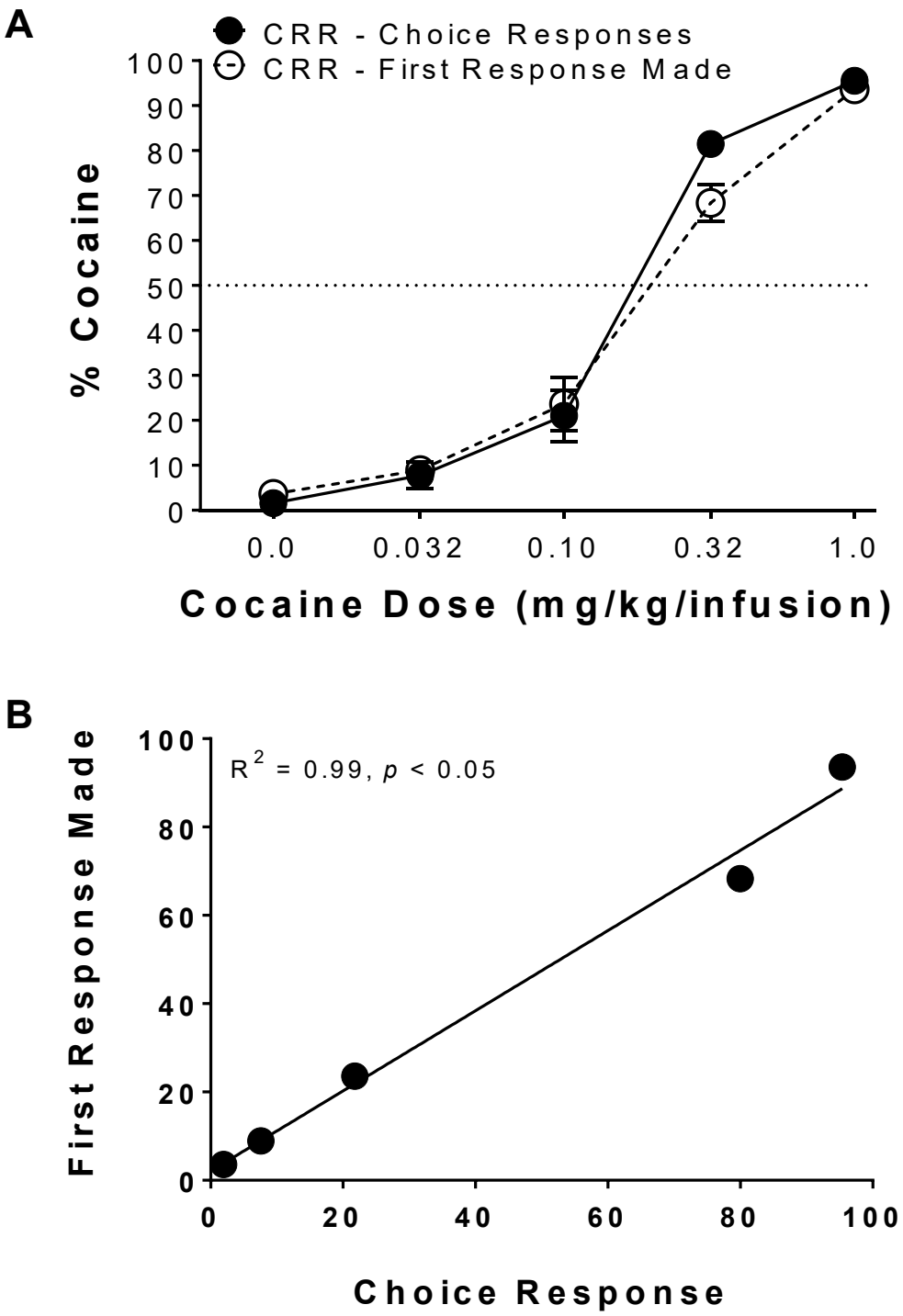

Figure 3. Cocaine versus food choice under the CRR choice procedure. (**A**) Mean (±SEM) percent choice for cocaine calculated via choice responses emitted versus percent choice for cocaine calculated via proportion of first responses made for cocaine. (**B**) Correlation between percent choice for cocaine calculated via choice responses and first response made.

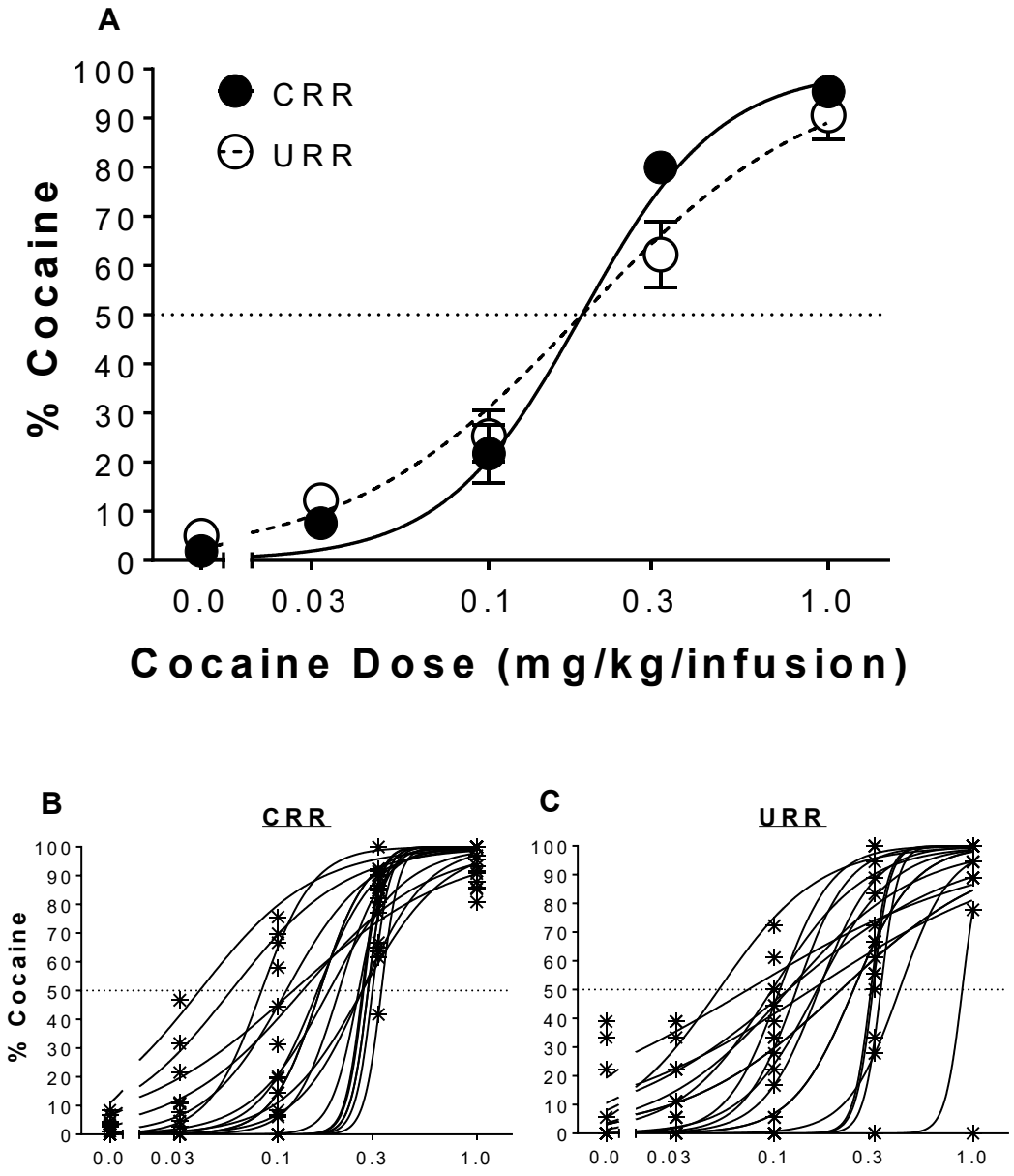

Figure 4. Cocaine versus food choice under the CRR and URR choice procedures. (**A**) Mean (±SEM) percent choice for cocaine under the CRR and URR. Individual choice profiles under the (**B**) CRR and (**C**) URR. Lines are the NLME-determined best fit of Eqn 11.

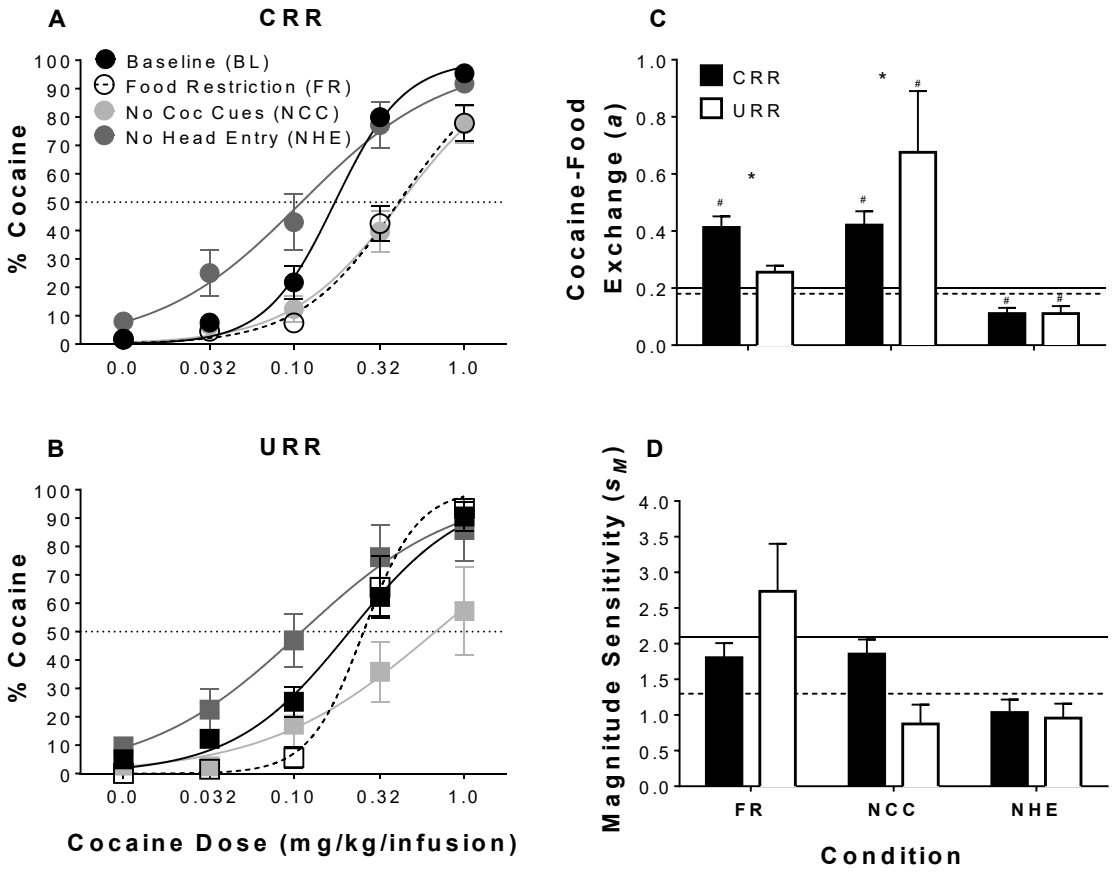

Figure 5. The effects of different environmental manipulations on cocaine versus food choice under the CRR and URR and parameter estimates. Mean (±SEM) percent choice for cocaine under the (**A**) CRR and (**B**) URR for baseline, food restriction, no cocaine cues, and no head entry conditions. Lines are the NLME-determined best fit of Eqn 11. Parameter estimates from the matching equation for (**C**) cocaine-food exchange rate (*a*) and (**D**) sensitivity to magnitude (s_M) under the different schedules and conditions. Note, horizontal lines represent parameter estimates from baseline conditions under the CRR (solid) and URR (dashed).

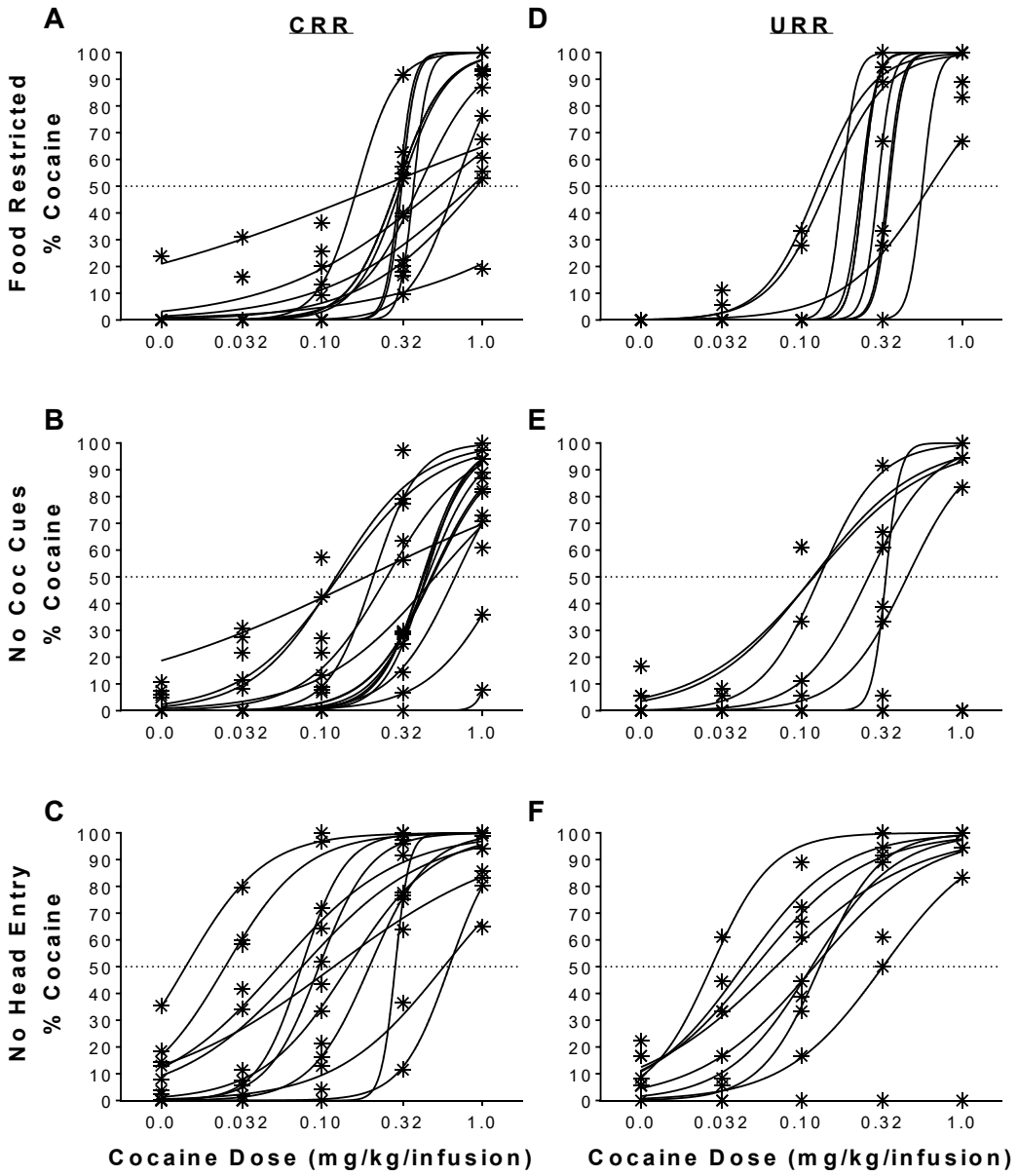

Figure 6. Individual choice profiles for the food restricted (**A**, **D**), no cocaine cues (**B**, **E**), and no head entry (**C**, **F**) conditions under the CRR and URR, respectively. Lines are the NLME-determined best fit of Eqn 11.

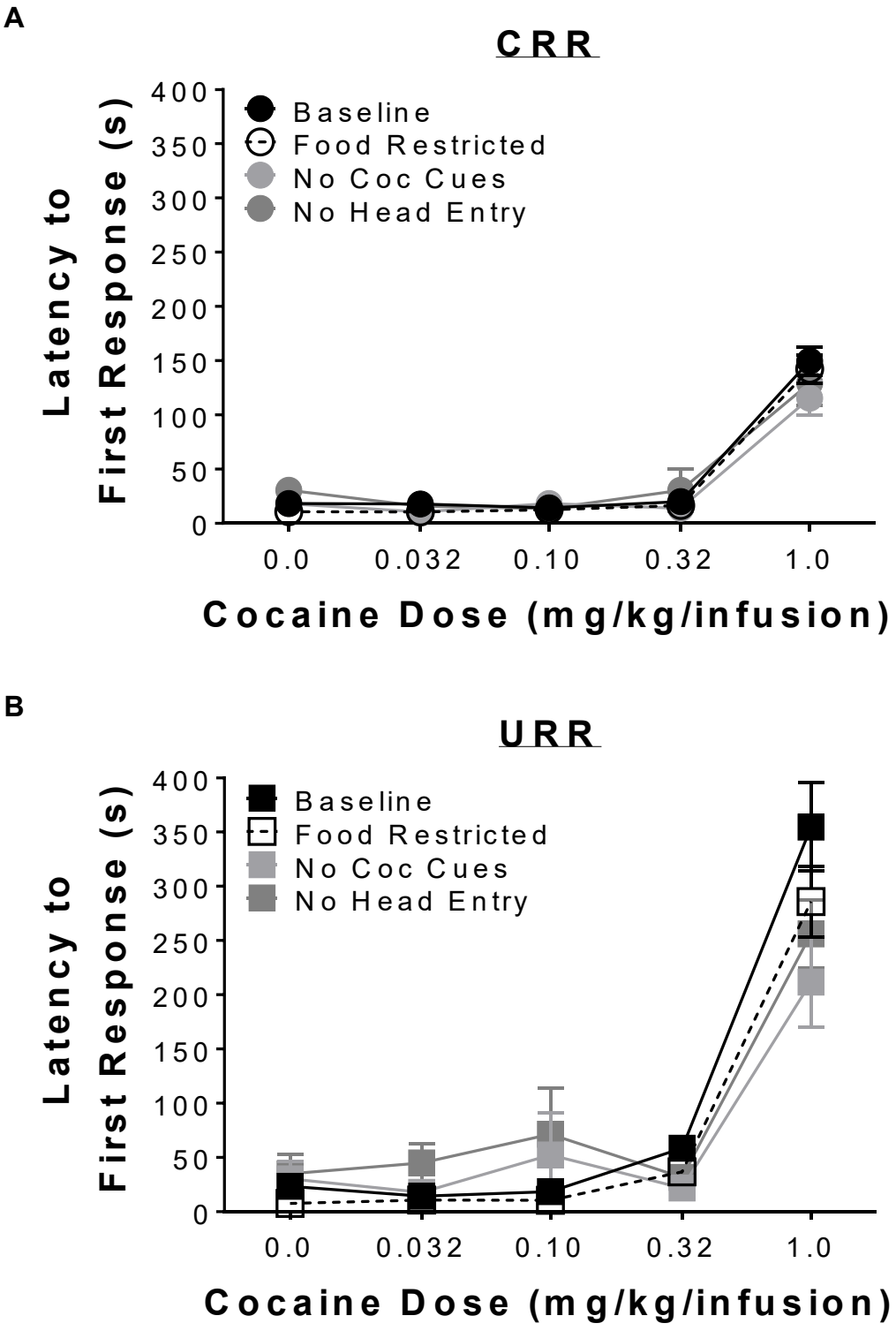

Figure 7. Latency to first response (i.e., lever press during) choice trials under the (**A**) CRR and (**B**) URR for baseline, food restricted, no cocaine cues, and no head entry conditions.

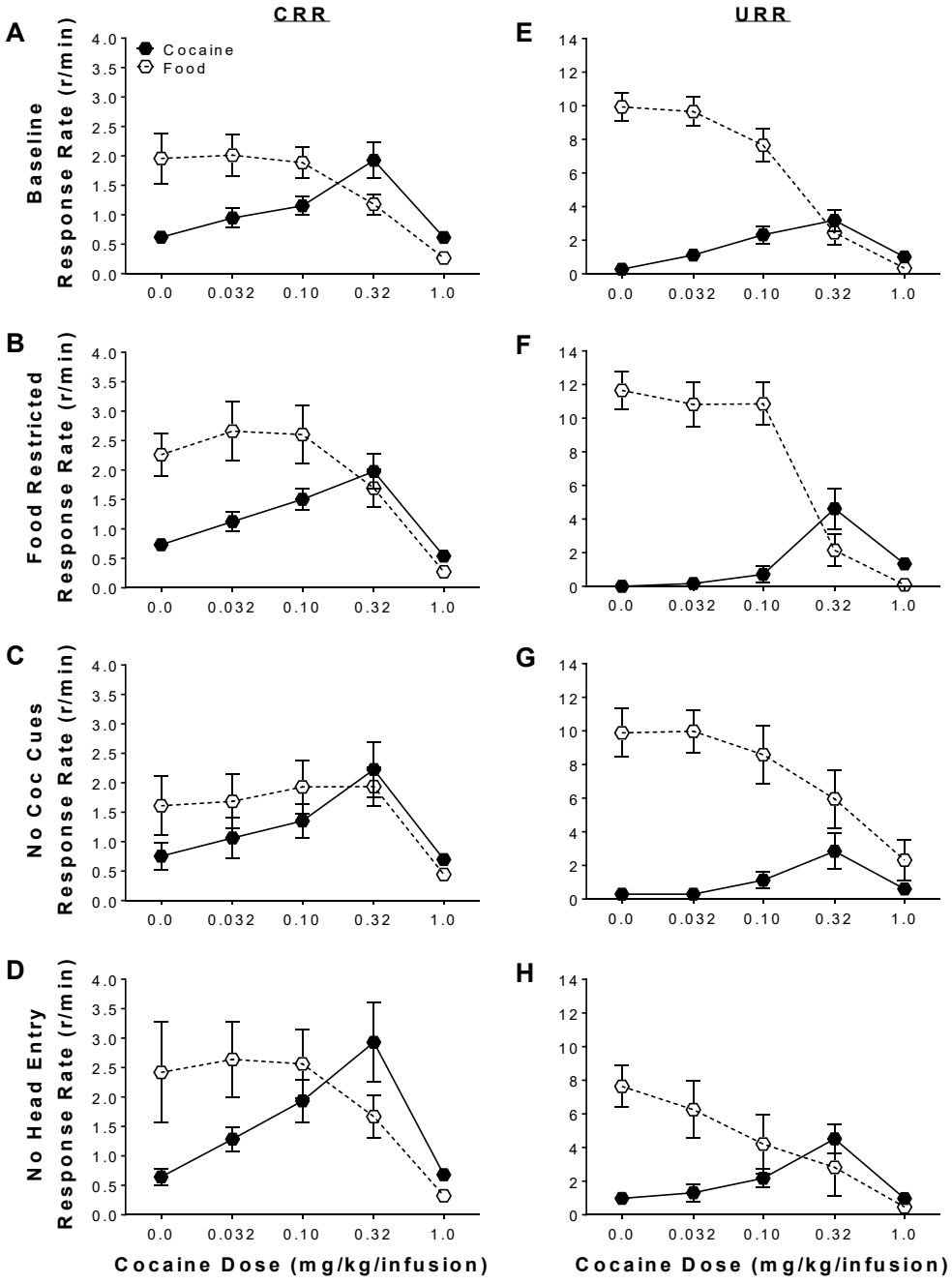

Figure 8. Overall response rates for cocaine and food during choice trials under the CRR and URR for the different manipulations. Mean (±SEM) responses/minute (r/min) for (**A**) baseline, (**B**) food restricted, (**C**) no cocaine cues, and (**D**) no head entry conditions under the CRR. Mean (±SEM) responses/minute (r/min) for (**E**) baseline, (**F**) food restricted, (**G**) no cocaine cues, and (**H**) no head entry conditions under the URR. Note: the y-axis scales between the CRR and URR are different.

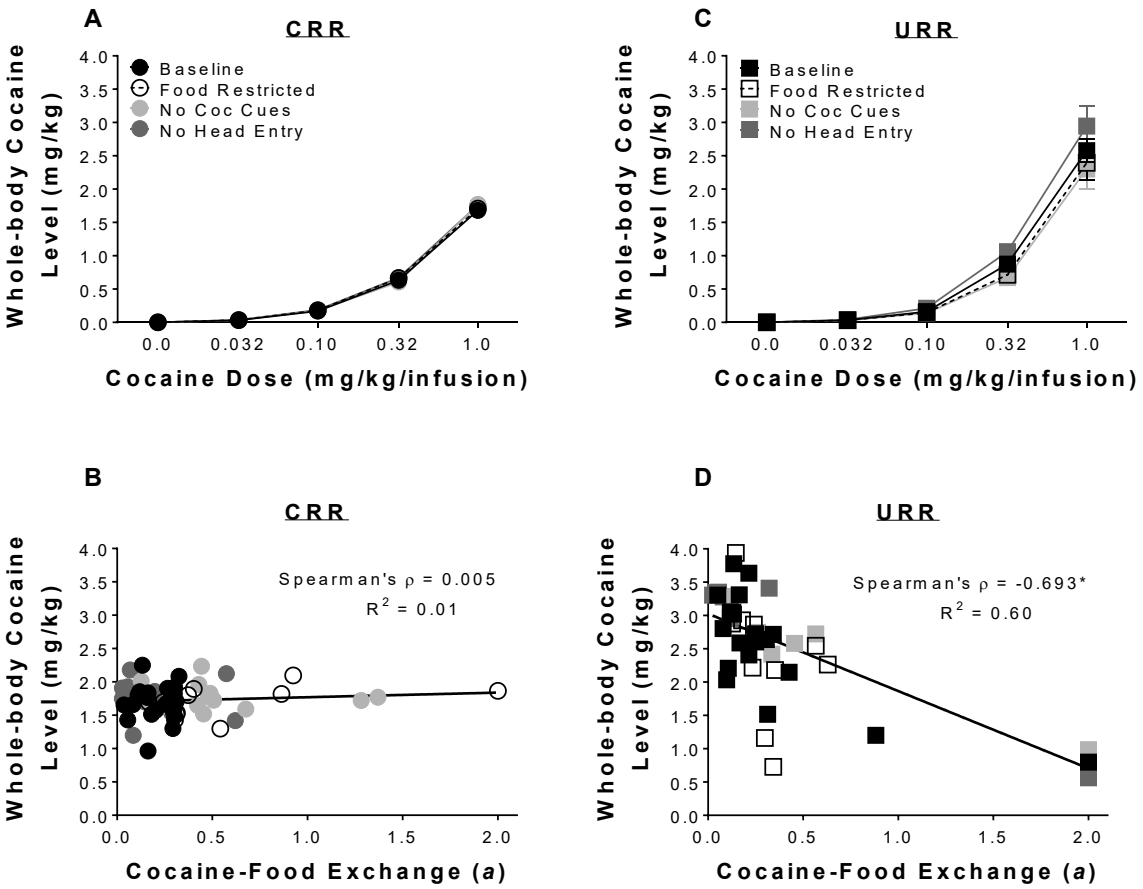

Figure 9. Calculated whole-body cocaine levels (mg/kg) at reinforcer delivery (i.e., amount of cocaine in a rat's system immediately before choosing). Mean (±SEM) whole-body cocaine levels at reinforcer delivery, averaged for each block, under the (**A**) CRR and (**C**) URR. Correlations between individual cocaine-food exchange rates (*a*; constraint set at 2) and individual whole-body cocaine levels reached during choice trials in the last block under the (**B**) CRR and (**D**) URR for the different conditions. * indicates p <0.05.

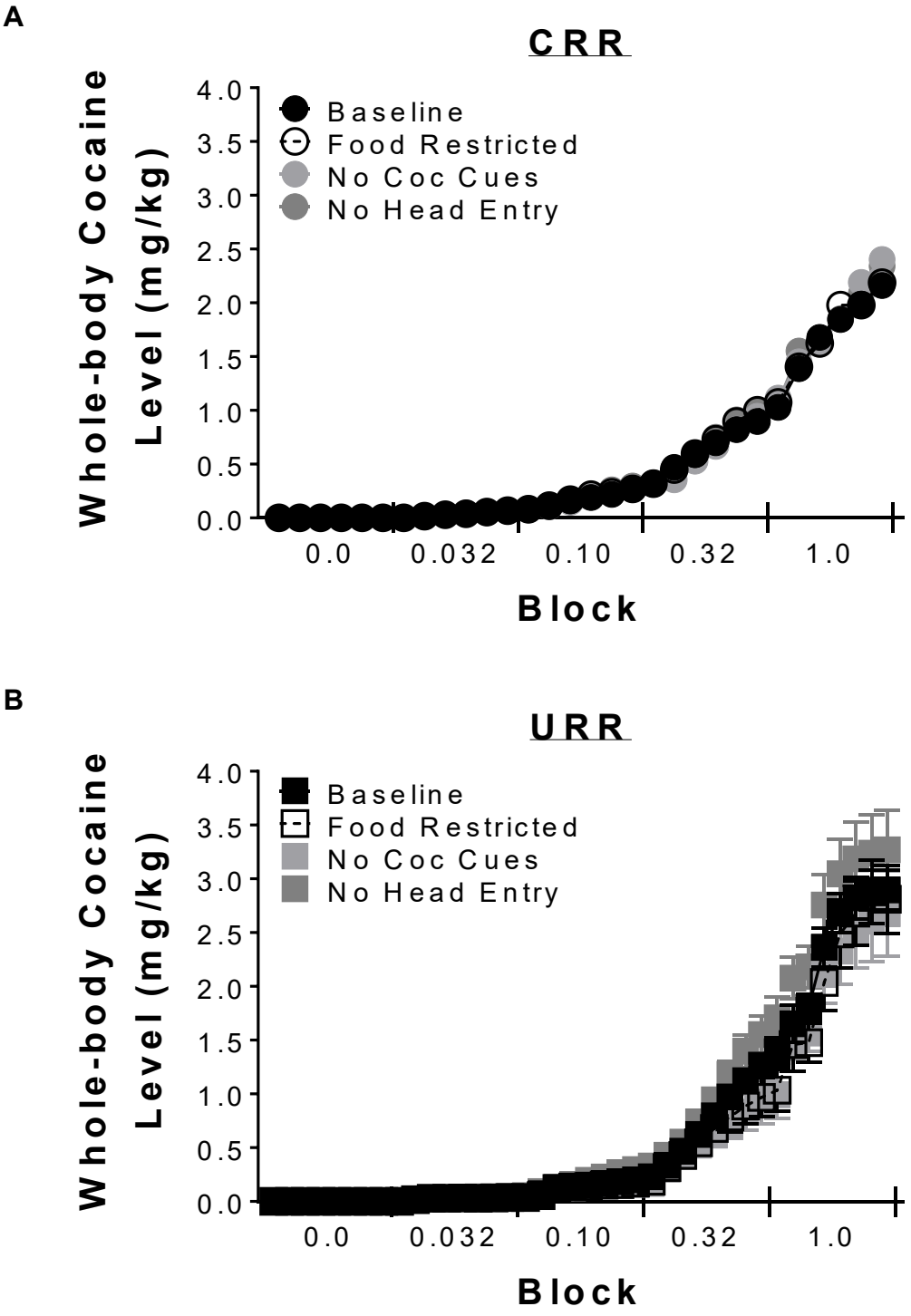

Figure 10. Calculated whole-body cocaine levels (mg/kg) at reinforcer delivery plotted as a function of trial. Mean (±SEM) whole-body cocaine levels at reinforcer across session under the (**A**) CRR and (**B**) URR.

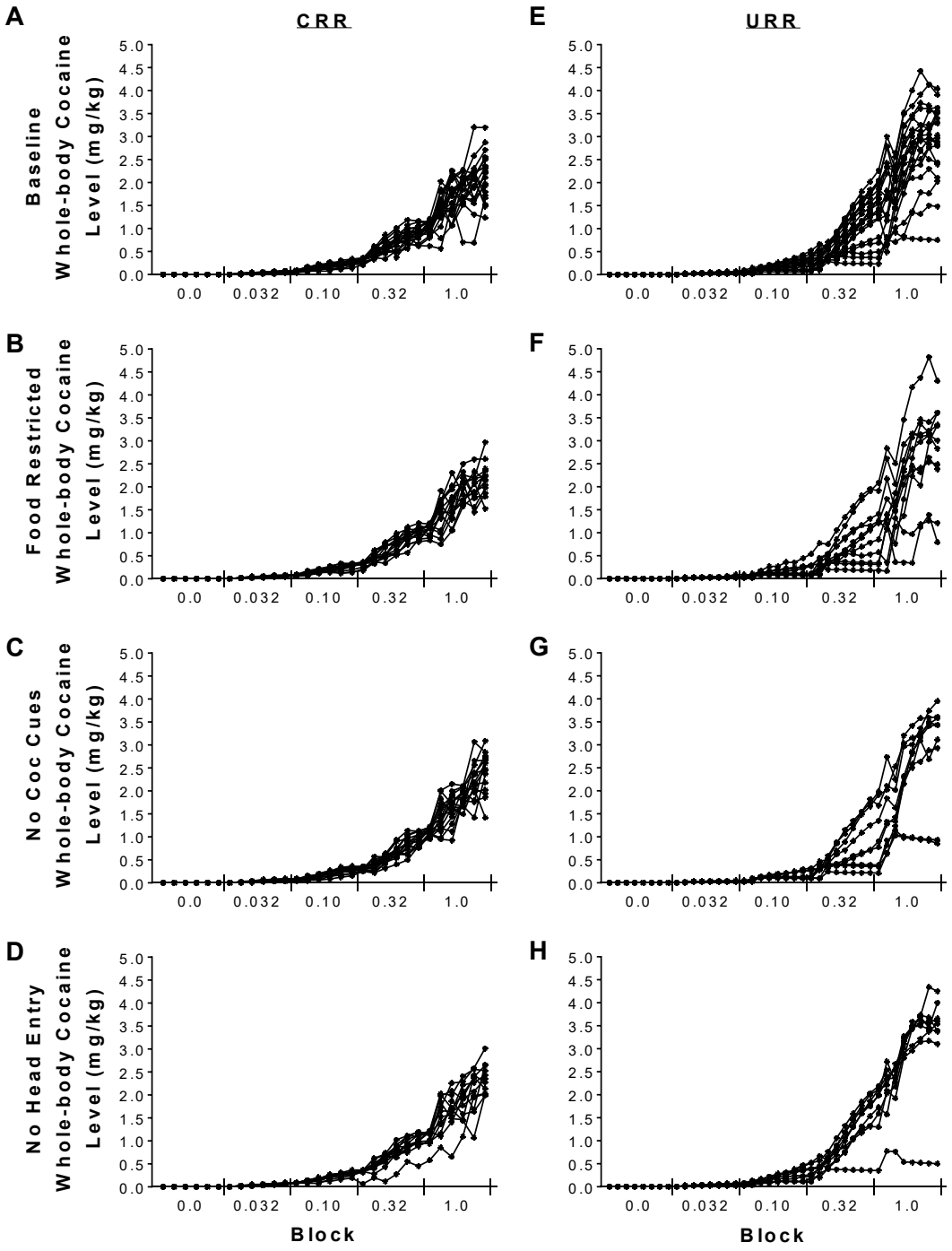

Figure 11. Individual whole-body cocaine levels at reinforcer delivery for each trial during baseline (**A**, **E**), food restriction (**B**, **F**), no cocaine cues (**C**, **G**), and no head entry (**D**, **H**) conditions under the CRR and URR, respectively.

Chapter 3

Experiment 2: Frequency of Drug vs. Non-drug Choice and Quality of Non-drug Alternatives

In Experiment 1, both the controlled reinforcer ratio (CRR) and uncontrolled reinforcer ratio (URR) choice procedures produced dose-dependent preference for cocaine. Importantly, under the CRR greater sensitivity to changes in relative magnitude (s_M) was observed, while preference and intake were dissociated. While the primary goal of using the CRR was to control for the rate of reinforcement, an often-overlooked issue in choice procedures, across the two options, not much can be really said regarding the effects of unequal reinforcer ratios. Providing there is evidence that reinforcer frequency affects drug versus non-drug preference (e.g., Anderson and Woolverton, 2000; Anderson et al. 2002) in monkeys, not much is known regarding this effect in rats.

Using a URR choice procedure, Lenoir et al. (2007) demonstrated that changes in cocaine dose did not affect cocaine preference, while changes to the non-drug alternative (e.g., adding a delay; Cantin et al. 2010) shifted preference towards cocaine. Through these observed results it was concluded that the dose of cocaine had no impact on preference, conflicting with previously-published drug versus non-drug findings (e.g. Nader and Woolverton, 1991; Negus, 2003). Although the URR schedule used in Lenoir et al. (2007) included sampling trials, albeit these trials were optional, it is possible that the number of sampling trials over training days was not sufficient enough for the rats to learn that the dose of cocaine had changed since they never chose cocaine. For example, previous

studies have demonstrated that the rate of reinforcement, or how frequently an organism chooses an option, influences choice (e.g., McCarthy and Davison, 1984) such that it is possible that by choosing food repeatedly rats in Lenoir et al. (2007) have developed a systematic bias towards the food option, resulting in exclusive preference for the non-drug option despite increasing cocaine doses. Likewise, price and delay changes to the non-drug alternative, resulting in preference towards cocaine, could also be a product of the systematic bias (McCarthy and Davison, 1979; Johnstone and Alsop, 1999) that developed through exclusive choice of the non-drug alternative, allowing them to better detect changes to that alternative.

Although Experiment 1 provides strong evidence (i.e., differential preference for cocaine under different environmental manipulations with identical whole-body cocaine levels, and a dissociation between preference and intake) against the hypothesis that cocaine preference is caused by choosing under the influence of cocaine (e.g., Vandaele et al. 2016; Freese et al. 2018), arguments could be made that schedules with within-session increasing cocaine doses, and short ITIs (e.g., herein and in Thomsen et al. 2013), results in the accumulation of enough cocaine that choice is made while under the influence of cocaine. In brief, high doses of cocaine are being chosen since some threshold level of cocaine has been reached with in the animal (Freese et al. 2018). Another argument that could be made is that the non-drug alternative used (i.e., 45 mg food pellet) functions differently than saccharin, since saccharin's intense

sweetness surpasses cocaine reward (Lenoir et al. 2007) and is always preferred over cocaine regardless of dose.

Herein, we utilized a CRR schedule for cocaine versus food choice to determine the effects that the relative rate of reinforcement (i.e., frequency) has on cocaine preference. In addition, whole-body cocaine levels were calculated for across the different reinforcer ratios to determine if there was a certain level that was associated with a switch from the non-drug alternative to cocaine. It is hypothesized that if some threshold (Vandaele et al. 2016; Freese et al. 2018) is the driving mechanism for cocaine preference, then rats, upon reaching some whole-body level should prefer cocaine regardless of differential frequencies of reinforcement for drug and non-drug alternatives. Furthermore, a CRR schedule for food (i.e., a single 45 mg food pellet) versus saccharin (0.2%) was utilized to determine if different non-drug reinforcers were comparable and if this may explain some of the differences observed in choice procedures. It is hypothesized that if saccharin, described to have value that surpasses cocaine's innate value (Lenoir et al. 2007), then under the law of transitivity rats should prefer saccharin over the 45-mg food pellet used.

Methods

Subjects

Twelve adult male Sprague-Dawley Rats (Harlan Inc.; Indianapolis, IN, USA), weighing approximately 250-275 g on arrival, were used. Rats were individually housed (12:12 hr light:dark cycle) with ad libitum access to food and

water in their home cage. All experimentation was conducted during the light phase. All experimental protocols were conducted in accordance to the 2011, *National Research Council: Guide for the Care and Use of Laboratory Animals* (8th edition) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Experiments were conducted in operant chambers (ENV-008CT, MED Associates, St. Albans, VT) enclosed within sound-attenuating compartments (ENV-018MD). Each chamber was connected to a personal computer (SG-502), and all chambers were operated using MED-PC. Within each chamber, a recessed food receptacle (ENV-202R2MA) outfitted with a head-entry detector (ENV-254-CB) was located on the front response panel of the chamber, two retractable response levers were mounted on either side of the food receptacle (ENV-122CM), and a white cue-light (ENV-221M) was mounted above each response lever. The back-response panel was outfitted with two nosepoke response receptacles (ENV-114BM) directly opposite to front response levers, a house-light (ENV-227M) was located at the top of the back panel between the two nosepoke response receptacles with Sonalert© tones (ENV-223 AM and ENV223-HAM) located on either side of the house-light. Food pellets (45-mg Bio-Serv Precision Pellets; Flemington, NJ) were delivered via a dispenser (ENV-203M-45). Drug infusions were delivered via a syringe pump (PHM-100) through tubing strung through a leash (PHM-110-SAI) that attached to a swivel above the

chamber. Saccharin was delivered via a second syringe pump (PHM-100) through tubing (PHM-122-18) that connected to the food receptacle.

Drugs

Cocaine hydrochloride, gifted from the National Institute on Drug Abuse (Bethesda, MD, USA), was mixed in sterile saline (0.9% NaCl).

Establishing Procedures for Cocaine vs. Food Choice

Six rats were trained on the same establishing procedures described in Experiment 1.

Experiment Proper for Cocaine vs. Food Choice

Following establishing procedures, rats were assigned to the controlled reinforcer ratio (CRR) schedule described in Experiment 1 for cocaine versus food choice. Briefly, the CRR choice procedure consisted of 5 distinct blocks, each signaled by an accompanying tone and separated by a dark and empty 2-min inter-block-interval, with a total of 3-drug and 3-food trials per block. In each of the 5 blocks, both levers (cocaine and food) were extended during each trial. Importantly, during each trial only one of the two reinforcers was randomly made available. Regardless of which lever the rat responded on, the reinforcer that was scheduled had to be earned to advance onto the next trial. Responses on the food lever, when scheduled, resulted in the delivery of a single 45-mg food pellet, while responses on the cocaine lever, when scheduled, resulted in an infusion of

cocaine at varying doses (0, 0.032, 0.10, 0.32, and 1.0 mg/kg/infusion as a function of block). Responses on the unscheduled lever were recorded and resulted in no consequences. Upon food pellet delivery, the lever would retract and the cue-light above the corresponding lever would turn on for 5.9s in all blocks. Upon cocaine infusion, the cue-light above the corresponding lever would turn on for a varying duration that matched the infusion length. Each trial began with the illumination of the house-light where an orienting response into the magazine would turn off the house-light and extension of the response lever or levers. All responses were scheduled on a fixed-ratio (FR) and required consecutive responding; a changeover in responding would reset the FR count. Upon completion of the FR requirement, levers would retract and reward delivery, signaled by a corresponding cue-light, would occur. Rats were initially trained on a FR1 and were incrementally progressed up to an FR5. All trials were separated by a dark and empty 10-s inter-trial-interval (ITI). Each block ended upon completion of all 6 trials, and each session ended upon completion of all 5 blocks. Rats were trained on the CRR for 2 weeks.

Manipulation of Reinforcer Frequency

To determine the role that the relative ratio of cocaine to food reinforcers earned has (i.e., frequency) on cocaine preference, the relative distribution of cocaine and food trials in the CRR was manipulated. Half of the rats were randomly placed on a CRR schedule that can be described as cocaine-favorable, consisting of 5-drug trials and 1-food trial. The other half was placed on a CRR

schedule that was food-favorable, consisting of 1-drug trial and 5-food trials. In both conditions the distribution of drug to food trials was randomized. Rats were trained for a minimum of ten days, following stability rats were returned to baseline (3-drug and 3-food) for a minimum of seven days, the assigned to the opposite condition and trained for a minimum of ten days. Upon completion of the experiment, the resulting n-size was 5 across all ratio conditions (1:5, 3:3, and 5:1). Attrition was due to catheter failure.

Establishing Procedures for Saccharin vs. Food Choice

Liquid-magazine shaping

Six rats were first trained to drink out of the food receptacle for three consecutive days. Rats were placed in the operant chambers and given 45 minutes to consume 0.1 ml of saccharin (0.2%), delivered on a 100-s fixed time schedule into a cup built into the food receptacle via syringe pump over 5.9s. Each session consisted of 20 trials.

Magazine shaping for food pellet

Rats were then trained to retrieve food pellets (45-mg Noyes Precision Pellets) from the same food receptacle for three consecutive days. Rats were placed in the operant chambers and given 45 minutes to retrieve and consume a total 20 food pellets. Food pellets were delivered one at a time on a 60-s fixed time schedule.

Lever training with an orienting response

Rats were then trained to press a lever for saccharin and food pellets. The start of each trial was signaled by the illumination of the house-light. A contingent response, head-entry into the magazine, would result in the offset of the house-light and extension of either the left or right lever. Completion of the scheduled FR on the presented lever would result in lever retraction and delivery of a single food pellet or saccharin. Each session consisted of 30 trials, 15 left- and 15 right-lever presentations. Levers were presented individually and pseudo-randomly, where no more than 6 presentations of the same lever would occur in a row. Additionally, each lever was associated with either a single food pellet or 0.1 ml of saccharin (0.2%). Trials were separated by a 12-s inter-trial interval (ITI). Lever training started on a FR1 for two days, moved onto an FR3 for two days, and ended on an FR5 which lasted for three days.

Experiment Proper for Saccharin vs Food Choice

Following establishing procedures, rats were placed on a CRR schedule for saccharin versus food choice. The choice procedure functioned similarly to the CRR schedule for cocaine versus food choice at baseline conditions (3-drug and 3-food trials). Briefly, each session was divided into 5 distinct blocks separated by a dark and empty 2-min inter-block-interval. Additionally, each block was signaled by an accompanying tone pattern (alternating between 40 kHz and 29 kHz) that played continuously at 1.8/0, 1.5/0.3, 0.9/0.9, 0.3/1.5, and 0/1.8 seconds (see Table 1, but instead of cocaine it is saccharin). In each of the

5 blocks, responses on the food lever resulted in the delivery of a single 45-mg food pellet, while responses on the saccharin lever resulted in the delivery of 0.2% saccharin at varying volumes. The volume of saccharin (0, 0.01, 0.03, 0.1 and 0.3 ml/trial) and increased as a function of block. Upon food pellet delivery, the lever would retract and the cue-light above the corresponding lever would turn on for 5.9s in all blocks. Upon saccharin infusion, the cue-light above the corresponding lever would turn on to signal the volume (0, 0.189, 0.59, 1.89, and 5.9s), while the pump would continuously deliver saccharin (0, 0.59, 1.77 ,5.9, and 17.7s) until the desired volume was reached. Each trial began with the illumination of the house-light where an orienting response into the magazine would turn off the house-light and extension of the response lever or levers. All responses were scheduled on a fixed-ratio (FR) and required consecutive responding, where a changeover in responding would reset the FR count. Upon completion of the FR requirement, levers would retract and reward delivery, signaled by a corresponding cue-light, would occur. Rats were initially trained on a FR1 and were incrementally progressed up to an FR5. All trials were separated by a 10-s inter-trial-interval (ITI). All sessions ended upon completion of all 5 blocks. Finally, the relative FR ratio for saccharin versus food was manipulated to 1:3, such that the FR requirement for food was 3 times greater than saccharin.

Analysis

Preference for cocaine versus food choice was calculated exactly as described in Experiment 1 for the CRR, via the total number of choice responses on the cocaine lever (i.e., responses on the drug lever when drug was not scheduled) divided by the overall number of choice responses (i.e., responses made on both the drug and the food lever when the respective reinforcer was not scheduled). For saccharin versus food choice, the same preference calculation was used, but instead of choice responses for cocaine it was choice responses for saccharin.

To quantitatively analyze how the relative ratio of cocaine to food reinforcers experienced affects cocaine preference the concatenated generalized matching law (Baum, 1974; Davison and McCarthy, 1988; Hutsell et al. 2015) was applied. The form of this matching equation is as follows:

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{M_f}{M_d}\right)^{s_M} * \left(\frac{R_f}{R_d}\right)^{s_R}} \quad \text{(Eqn 13)}$$

Where B_d represents behavior for drug, B_f represents behavior for food, and M_d represents the magnitude (i.e., dose) of drug, M_f represents the magnitude of food, R_d represents the frequency of cocaine reinforcement, and R_f represents the frequency of food reinforcement. The free parameter s_M represents the sensitivity to magnitude of cocaine vs. food reinforcement, while s_R represents the sensitivity to relative frequency. Prior to application of this equation, the generalized matching equation used in Experiment 1 (Eqn 11) was first applied to baseline (3-drug:3-food). Application of this equation was used first to determine

the free parameter *a*, thus allowing it to serve as a constant. This was done, since the relative reinforcer ratio at baseline is 1.

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{a}{M_d}\right)^{s_M} * \left(\frac{3}{3}\right)^{s_R}} \qquad \text{(Eqn 14)}$$

Or

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{a}{M_d}\right)^{s_M} * 1} \qquad \text{(Eqn 15)}$$

By allowing *a* to serve as a constant the free parameters, s_M and s_R can be solved. The resulting equation used to determine the role that the relative frequency of cocaine to food reinforcers experienced is as follows:

$$\frac{B_d}{B_d+B_f} = \frac{100}{1+\left(\frac{a}{M_d}\right)^{s_M} * \left(\frac{R_f}{R_d}\right)^{s_R}} \qquad \text{(Eqn 16)}$$

Where *a* was calculated to be 0.22 from Eqn 15. Using the data from the same sessions used to calculate choice, estimated whole-body cocaine levels (mg/kg) at reinforcer delivery were also determined using Eqn 12 from Experiment 1 (Weiss et al. 2003):

All data were analyzed using linear mixed-effects modeling (LME; Gelman and Hill, 2006) and nonlinear mixed-effects modeling (NLME; Pinheiro et al. 2007). For all tests, α was set to 0.05.

Percent cocaine choice for all relative ratio conditions were independently analyzed using NLME with frequency (continuous) and magnitude (continuous) as within-subject factors, and subject as a random factor. Additionally, whole-body cocaine levels (mg/kg) at reinforcer intake as a function of block was

analyzed with LME with ratio condition (continuous) and block (continuous) as within-subject factors, and subject as a random factor.

Percent choice for saccharin was analyzed using LME, due to the shape of the data, with dose (continuous) and price (continuous) as within subject factors, and subject as a random factor.

Results

Figure 12 illustrates (12A) percent choice for cocaine under the CRR for the different relative ratio manipulations and (12B) averaged whole-body cocaine levels at reinforcer delivery. NLME analysis revealed significant effect of sensitivity to magnitude (s_M = 1.68) [$F(1,69)=21.46$, $p<0.05$] and a significant effect of sensitivity to frequency (s_R = 1.06) [$F(1,69)=37.63$, $p<0.05$], altogether indicating that magnitude and frequency of reinforcement are independently affecting cocaine choice. Specifically, sensitivity to magnitude reflects the dose-dependent choice curves, while sensitivity to relative frequency reflects the shifts in the choice curves in Figure 12A. LME analysis on whole-body cocaine levels (mg/kg) at reinforcer delivery averaged for each block revealed a main effect of dose [$F(1,5.29)=3134.80$, $p<0.05$], ratio experienced [$F(2,10.02)=265.47$, $p<0.05$], and dose x ratio interaction [$F(2,10.05)=164.70$, $p<0.05$], indicating that whole-body cocaine levels increased as a function of dose, but increased at different rates depending on the reinforcer ratio experienced.

Figure 13 illustrates saccharin versus food choice. LME analysis revealed no main effects and no interactions. Collectively, these results indicate that

preference for saccharin could not be obtained relative to a single 45-mg food pellet under the given conditions.

Discussion

In accordance with the matching law (Herrnstein, 1961; Killeen, 1972; Baum, 1974, 1979; Davison and McCarthy, 1988) and previous drug versus non-drug studies (Anderson and Woolverton, 2000; Anderson et al. 2002), the relative rate of reinforcement across given alternatives affects preference. In addition to the relative magnitude between cocaine and food reinforcement, when the relative ratio between cocaine and food was in favor for cocaine the choice curve shifted leftwards, relative to baseline, indicating that the relative value for cocaine increased, where the cocaine-food exchange rate is estimated to be 0.07 mg/kg/infusion. Similarly, in addition to the relative magnitude between cocaine and food reinforcement, when the relative ratio between cocaine and food was in favor for food the choice curve shifted rightwards, relative to baseline, indicating that the relative value for cocaine decreased, where the cocaine-food exchange rate is estimated to be 0.56 mg/kg/infusion. Importantly, both these shifts, via relative frequency, maintained similar dose-dependency, via relative magnitude, occurred within-subject. Collectively, these results demonstrated that both magnitude and frequency are independent-variables that determines the relative value for cocaine, and subsequently preference for cocaine.

When whole-body cocaine levels were calculated from this experiment herein, the rate at which whole-body cocaine levels increased was related to how

many cocaine trials were available. Given that the choice procedures used herein produce increasing whole-body cocaine levels throughout the session, and it has been suggested that reaching some level of cocaine intake influences preference for cocaine (Freese et al. 2018) then some whole-body cocaine level should be shared across all cocaine versus non-drug choice studies. Additionally, whole-body cocaine levels at the time of choice were calculated for Lenoir et al. (2007) and Kearns et al. (2017). Briefly, in Lenoir et al. (2007), rats were given a choice between 0.25 mg/kg/infusion of cocaine and a maximum of 0.3 ml of 0.2% saccharin; under these conditions all rats preferred saccharin. Additionally, when the dose of cocaine was increased to 0.75 mg/kg/infusion and 1.5 mg/kg/infusion, with adjustments to the ITI to create comparable levels of cocaine at the time of choice, preference for saccharin remained unchanged. Briefly, in Kearns et al. (2017), a “discrete-trials” choice procedure was utilized as well, but instead a 1.0 mg/kg/infusion of cocaine was compared against a single 45-mg food pellet with a 10-min ITI. Simulations from both (Lenoir et al. 2007 and Kearns et al. 2017) can be seen in Figure 14. To compare whole-body cocaine levels herein with calculated whole-body cocaine levels from Lenoir et al. (2007) and Kearns et al. (2017), the cocaine-food exchange rate for all three tested reinforcer ratios was determined resulting in values of 0.22 mg/kg at baseline (3:3), 0.07 mg/kg when the cocaine to food reinforcer ratio was 5:1, and 0.56 mg/kg at when the cocaine to food reinforcer ratio was 1:5. Next, whole-body cocaine levels associated with these cocaine-food exchange rate values were interpolated (respective intersection between vertical lines and whole-body

cocaine levels in Figure 12B); resulting in whole-body cocaine levels estimated to be at 0.336 mg/kg when the experienced ratio is equivalent, 0.206 mg/kg when the experienced ratio is in favor of cocaine, and 0.619 mg/kg when the experienced ratio is in favor of food at these points. If cocaine preference is determined by cocaine concentrations at time of choice (Freese et al. 2018), then these interpolated whole-body cocaine levels (horizontal lines in Figure 15) should be reflective of the point in time before preference for cocaine should begin. That is, whole-body cocaine levels above the line should be indicative of cocaine preference. For example, when the cocaine and food reinforcer ratio was equivalent, the calculated levels for all cocaine doses used in Lenoir et al. (2007) are all below it (e.g., thick dotted line in Figure 15), indicating that rats in Lenoir et al. (2007) might not have reached some concentration threshold that elicits cocaine preference. However, by manipulating the relative reinforcer ratio, either in favor for cocaine or for food, the hypothetical whole-body cocaine threshold changes; thus, suggesting that cocaine preference is not driven by choosing under while under the influence of cocaine. Importantly, these changes occurred within-subject. Furthermore, whole-body cocaine levels from Kearns et al. (2017), provide some further insight against this notion that cocaine preference is driven by cocaine intake. As mentioned above, Kearns et al. (2017) used 1.0 mg/kg/infusion of cocaine and a single 45-mg food pellet as reinforcers with a 10-min ITI. Under these conditions Kearns et al. (2017) found group differences such that rats either showed preference for cocaine or preference for food. Furthermore, when the ITI was increased to 60 minutes in one of the

experiments, this change did not produce any significant changes in preference. Altogether, data herein and data from Kearns et al. (2017) further argue against the notion that cocaine preference is driven by cocaine intake.

Studies using saccharin (e.g., Lenoir et al. 2007, 2013; Lenoir and Ahmed, 2008; Cantin et al. 2010; Madsen and Ahmed, 2015) as a non-drug alternative have shown that saccharin exclusively promotes non-drug preference regardless of cocaine dose. However, using a food pellet, there seems to be some mixed results or some form of graded response (e.g., Kearns et al. 2017). Thus, to determine if there are any interesting differences between the non-drug alternatives used across studies, saccharin and a food pellet were compared under a CRR schedule. Using a CRR schedule, where the relative rate of reinforcement was kept equivalent across both options, there were no observable volume-dependent preference for saccharin. Furthermore, when the price (i.e., required responses) for a single 45-mg food pellet was tripled, preference was still seen for the food pellet. Altogether, these findings reveal that the relative value of a single 45-mg food pellet was significantly greater than 0.3ml of 0.2% saccharin.

As previously mentioned, and demonstrated herein, rate of reinforcement across given alternatives affects preference (Anderson and Woolverton, 2000; Anderson et al. 2002) and if an animal repeatedly chooses a particular option, changes to a given alternative will likely be undetected unless sampled (McCarthy and Davison, 1979; Johnstone and Alsop, 1999). Thus, in procedures with an optional-sampling phases and uncontrolled reinforcer ratios, the lack of

cocaine contact may explain the lack of dose-dependent preference (e.g., Lenoir et al. 2007, 2013; Cantin et al. 2010). Finally, from the provided individual choice profiles provided in Vandaele et al. (2016), rats are demonstrated to spend the first 10 to 15 minutes choosing saccharin, which results in ~9 to 13.5 ml of saccharin consumed (at maxim) before switching over the cocaine. Thus, it is possible that initial consumption for saccharin causes satiation for saccharin, therefore increasing the likelihood of cocaine choice.

Overall, the results herein demonstrate that, in addition the relative magnitude of cocaine versus food reinforcement, that the relative rate of reinforcement affects cocaine preference. Moreover, the non-drug alternative used herein was demonstrated to have greater relative value than the typical non-drug alternative used (e.g., 0.3ml of 0.2% saccharin).

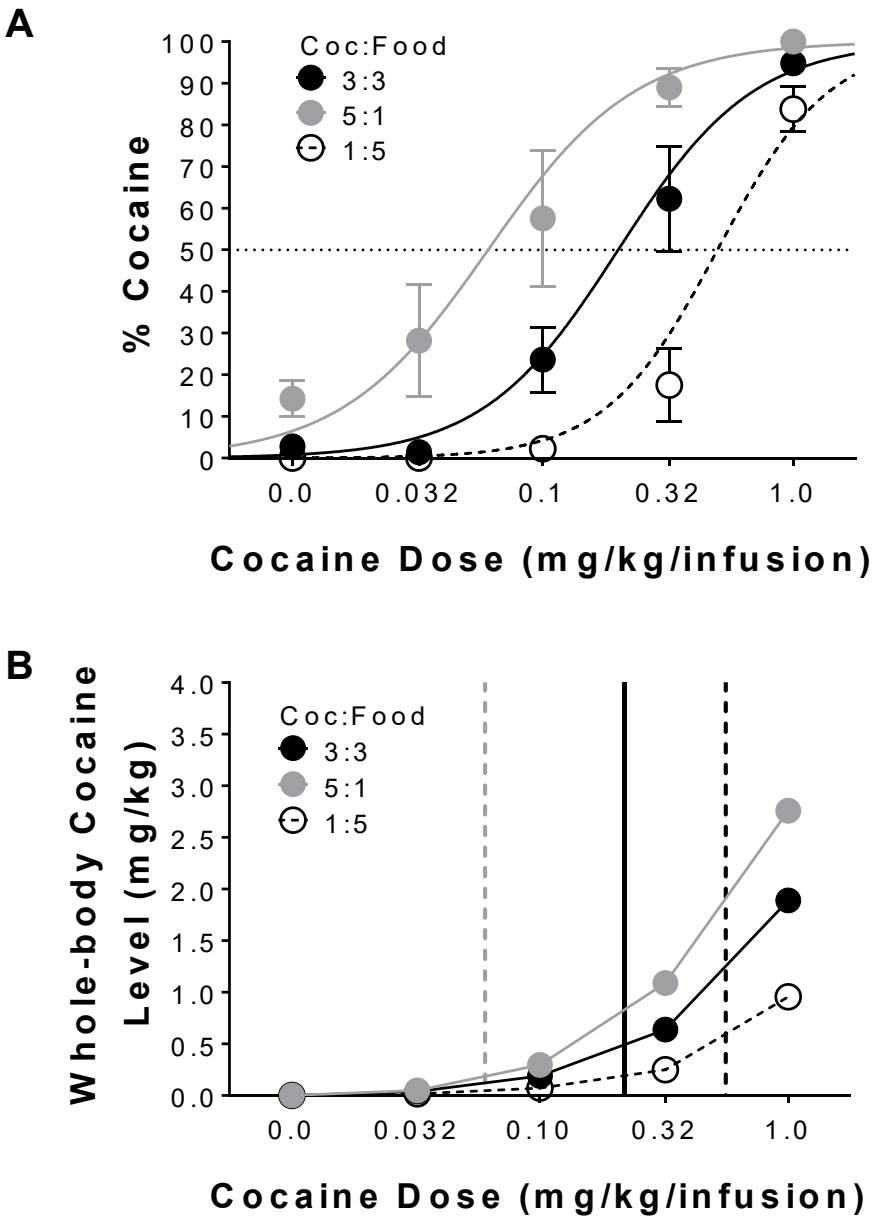

Figure 12. Cocaine choice and calculated whole-body cocaine levels (mg/kg) at different reinforcer ratios under the CRR. (**A**) Mean (±SEM) percent choice for cocaine under the CRR for an equal reinforcer ratio (3:3), a reinforcer ratio in favor of cocaine experience (5:1), and a reinforcer ratio in favor of food experience (1:5). Lines are the NLME-determined best fit of Eqn 16. (**B**) Mean (±SEM) whole-body cocaine levels at reinforcer delivery under the CRR for the 3 tested reinforcer ratios (3:3, 5:1 in favor of cocaine, and 1:5 in favor of food). The vertical lines represent the dose of cocaine that is equivalent to a single 45-mg food pellet for the tested reinforcer ratios; the solid black line corresponds to equal experience, the dotted gray line represents experience in favor of cocaine, and the dotted black represents experience in favor of food.

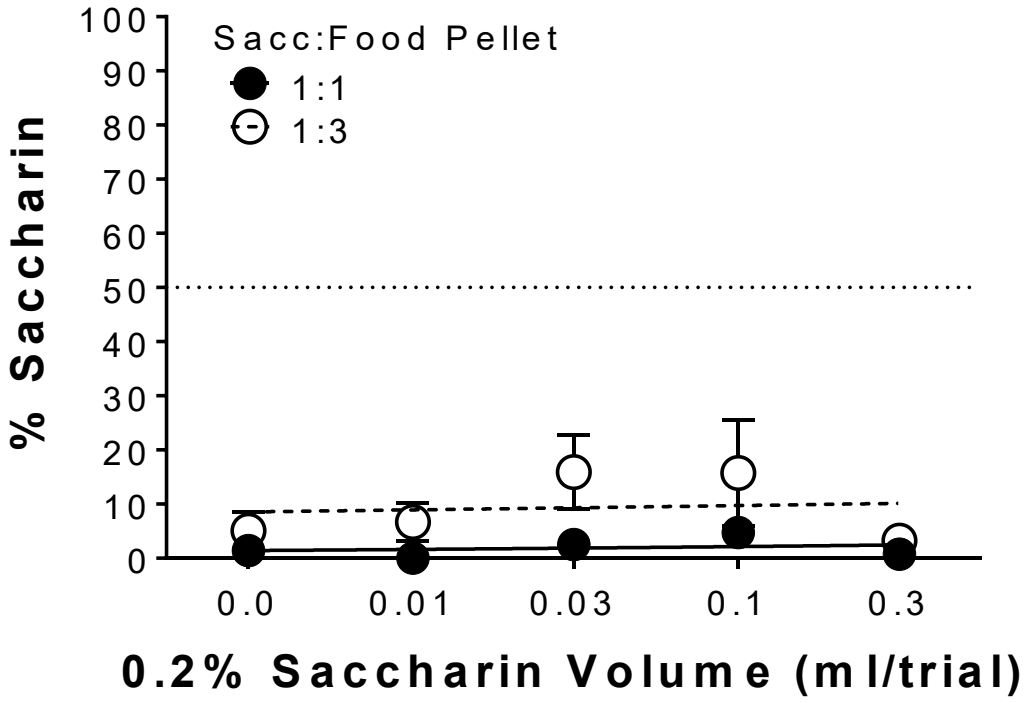

Figure 13. Saccharin versus food choice. Mean (±SEM) percent choice for saccharin when the fixed-ratio requirement for both options was equivalent (1:1), and when the fixed-ratio requirement was increased on the food alternative only (1:3).

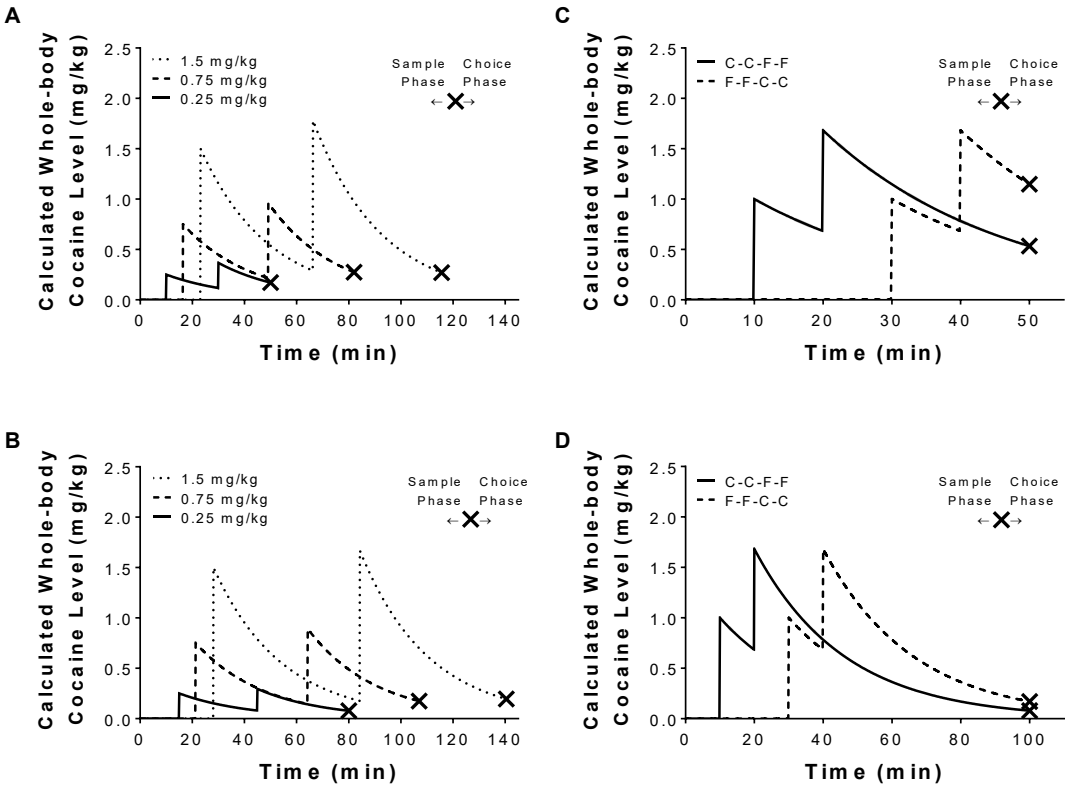

Figure 14. Calculated whole-body cocaine levels (mg/kg) at reinforcer delivery via methods described from other choice studies that utilized a "discrete-trials" choice procedure. Calculated whole-body cocaine levels from the various doses used in Lenoir et al. 2007 if the subject (**A**) responds immediately upon lever presentation or if the subject (**B**) responds prior to the end of the limited-hold. Calculated whole-body cocaine levels from Kearns et al. 2017 for a (**C**) 10-min ITI and for a (**D**) 60-min ITI with 1.0 mg/kg/infusion cocaine. Legends in (**C**, **D**) represent the order of sampling trials that produce the greatest and lowest whole-body concentrations prior to choice. The bolded x represents the point in time when the animal makes its first choice.

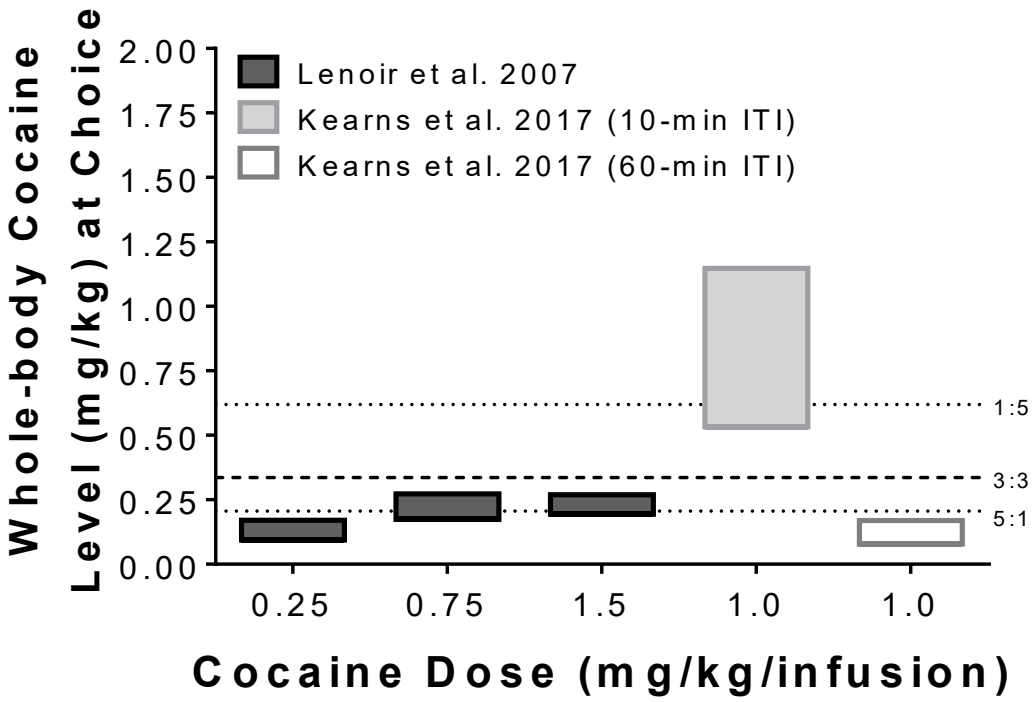

Figure 15. The calculated whole-body cocaine levels from Figure 14, expressed as a range via solid rectangles. The dotted horizontal lines represent estimated whole-body cocaine levels when cocaine and food are equivalent. Theoretically, anything above a given line should result in cocaine choice, while anything below a given line should result in food choice if the concentration of cocaine at choice is what determines preference. The thick black line represents equal reinforcer ratio experience (3:3), the dotted gray line (bottom-most) represents experience in favor of cocaine (5:1), and the dotted black line (top-most) represents experience in favor of food (1:5).

Chapter 4

Drug reinforcers (e.g., cocaine) and non-drug reinforcers (e.g., food) have been shown to share overlapping neurobiological mechanisms (Robbins and Everitt, 1996; Schultz et al. 1997; Volkow et al. 2011). For example, the nucleus accumbens (NAc) is heavily implicated in reward valuation for both drug and non-drug reinforcers (Cardinal et al. 2001; Knutson et al. 2001; Salamone et al. 2007; Stopper and Floresco, 2011), while the orbitofrontal cortex (OFC) has been demonstrated to play an important role in decision-making for both drug and non-drug reinforcers (Gallagher et al. 1999; Wallis, 2007; Volkow et al. 2008; Buckley et al. 2009; Camille et al. 2011; West et al. 2011). Given that the neurobiological process between drug and non-drug reinforcers are shared, insight into these neurobehavioral mechanisms that drive preference for a drug over a non-drug reinforcer should greatly advance knowledge regarding substance use disorders (Ahmed, 2010, 2013; Banks and Negus, 2012). For example, recent studies completed have been using choice procedures as a form of voluntary abstinence to investigate the brain regions (e.g., cortical and ventral tegmental areas) that are supposedly responsible for reinstatement (Pelloux et al. 2013; Caprioli et al. 2015, 2017; Venniro et al. 2017). There have also been studies completed examining OFC activity via electrophysiology in drug-preferring and food-preferring rats (Guillem and Ahmed, 2017; Guillem et al. 2018). However, all these studies completed have used the “discrete-trials” choice procedure (Lenoir et al. 2007), thus, making it possible that the suspected neurobiological mechanisms that drives preference may be confounded by intake.

Experiment 3: Determine Cellular Brain Activation during Cocaine vs. Food Choice

The mesocorticolimbic pathway (Everitt and Robbins, 2005) is involved in reward-learning and processes related to reward-learning, such as decision-making. When drugs of abuse (e.g., methamphetamine) and non-drug reinforcers (e.g., chocolate-flavored pellets) are delivered independently, in a temporal manner, different populations of cells within the nucleus accumbens (NAc), a brain region located in the mesocorticolimbic pathway, are independently activated, as measured by c-fos protein and mRNA expression (Xiu et al. 2014). Similarly, electrophysiological recordings from cells in the NAc have shown that certain cells only respond to cocaine or natural rewards (e.g., water) when presented (Carelli et al. 2000; Carelli, 2002). Within the orbitofrontal cortex (OFC), another brain region located in the mesocorticolimbic pathway, electrophysiological recordings of cells in this area in non-human primates have demonstrated that different OFC cells are involved in the encoding and valuation of different reinforcer types and the choices made between them (Padoa-Schioppa and Assad, 2006, 2008; Padoa-Schioppa, 2013). Collectively, these examples suggest that certain cell populations within a given brain region of the mesocorticolimbic pathway are independently involved with specific reinforcers and features of reinforcement for said reinforcers.

Current research into the neurobiological mechanisms that drive cocaine versus non-drug choice have demonstrated that the number of neurons in the

OFC that encode for cocaine, relative to non-drug reward, is correlated with the number of cocaine choices made by a rat (Guillem and Ahmed, 2017); that is, the more neurons that are involved in cocaine encoding, the more cocaine choice occurs. Although there is evidence suggesting that different OFC and NAc cell populations govern valuation of qualitatively different reinforcers, there are currently very few studies examining this relation in drug versus non-drug choice. One difficulty in studying the neurobiological mechanisms that underlie drug-related decision-making is the positive feedback relationship between choices and experienced reinforcement under choice procedures where the relative rate of reinforcement is subject-determined (e.g., Ahmed et al. 2013); there is a direct relation between the amount of drug and non-drug reinforcers earned through the choices that an individual makes. Importantly, differential self-administration histories with drug reinforcers can cause differences in neural adaptations and associated value (Nestler, 2001; Hyman et al. 2006; Moal and Koob, 2007; Kalivas and O'brien, 2008; Koob, 2012), making it difficult to dissociate the effects of drug intake from drug preference. Thus, it is possible that OFC cell firing in response to cocaine in a cocaine-preferring rat, is a byproduct of the schedule used (e.g., Guillem and Ahmed, 2017); that is, greater neural activity for cocaine is a direct result of taking more cocaine overall.

The controlled reinforcer ratio (CRR) schedule described in Experiment 1 and Experiment 2, demonstrated its ability to separate preference from intake, while controlling for differential rates of reinforcement across cocaine and food reinforcers. Furthermore, use of the CRR results in equivalent experience in

cocaine versus food choice across all session across all subjects, thus limiting variability in drug exposure. Importantly, under the CRR choice procedure, individual differences in the cocaine-food exchange rate was observed (Figure 4B and Figure 9B). Given that these individual differences are not correlated with cocaine intake, if the brain is involved in the mediation of preference then there should be individual differences in neuronal activity associated with individual differences in preference.

It is hypothesized that a subset of cells in the OFC and NAc will be independently activated in response to cocaine; likewise, another subset of cells in the OFC and NAc will also be independently activated in response to food. Furthermore, if the relative number of neurons involved in cocaine versus food reinforcement is related to preference, it is predicted that the relative activation of these separate populations (ratio of cocaine to food populations activated) will be negatively correlated with individual preferences for cocaine (i.e., cocaine-food exchange rate, a). Specifically, animals with a greater preference for cocaine (lower a) will have a higher percentage of cocaine activated cells, while animals with a lower preference cocaine (higher a) will have a lower percentage of cocaine activated cells. Moreover, it is also hypothesized that the number of cells that activate in response to both cocaine and food (i.e., overlapped) will be negatively correlated with the sensitivity (s_M) parameter. Specifically, individuals with high sensitivity (e.g., good discrimination) will have a lower number of overlapped cells, while individuals with low sensitivity (e.g., poor discrimination) will have a higher number of overlapped cells.

Methods

Subjects

Twelve adult male Sprague-Dawley Rats (Harlan Inc.; Indianapolis, IN, USA), weighing approximately 250-275 g on arrival were used. Rats were individually housed (12:12 hr light:dark cycle) with ad libitum access to food and water in their home cage. All experimental protocols were conducted in accordance to the 2011, *National Research Council: Guide for the Care and Use of Laboratory Animals* (8^{th} edition) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Experiments were conducted in operant chambers (ENV-008CT, MED Associates, St. Albans, VT) enclosed within sound-attenuating compartments (ENV-018MD). Each chamber was connected to a personal computer (SG-502), and all chambers were operated using MED-PC. Within each chamber, a recessed food receptacle (ENV-202R2MA) outfitted with a head-entry detector (ENV-254-CB) was located on the front response panel of the chamber, two retractable response levers were mounted on either side of the food receptacle (ENV-122CM), and a white cue-light (ENV-221M) was mounted above each response lever. The back-response panel was outfitted with two nosepoke response receptacles (ENV-114BM) directly opposite to front response levers, a house-light (ENV-227M) was located at the top of the back panel between the two nosepoke response receptacles with Sonalert© tones (ENV-223 AM and

ENV223-HAM) located on either side of the house-light. Food pellets (45-mg Bio-Serv Precision Pellets; Flemington, NJ) were delivered via a dispenser (ENV-203M-45). Drug infusions were delivered via a syringe pump (PHM-100) through tubing strung through a leash (PHM-110-SAI) that attached to a swivel above the chamber.

Drugs

Cocaine hydrochloride, gifted from the National Institute on Drug Abuse (Bethesda, MD, USA), was mixed in sterile saline (0.9% NaCl).

Establishing Procedures

Magazine shaping

Rats were first trained to retrieve food pellets from the food receptacle for two consecutive days. Rats were placed in the operant chambers and given 45 minutes to retrieve and consume 20 food pellets, delivered on a 60-s fixed time schedule.

Lever training

Rats were then trained to lever press on a fixed-ratio (FR) schedule of reinforcement, where completion of the FR requirement on the presented lever would result in lever retraction and delivery of a food pellet. Each session consisted of 30 trials, 15 left- and 15 right-lever presentations. Levers were presented individually and pseudo-randomly, where no more than 6

presentations of the same lever would occur in a row. Trials were separated by a 12-s inter-trial interval (ITI). Lever training started on a FR1, which lasted for three days, moving onto an FR3 for two days, and ending on an FR5 which lasted for three days.

Orienting response

Next, an orienting response was added. The start of each trial was now signaled by the illumination of the house-light. A contingent response, head-entry into the magazine, would result in the offset of the house-light and extension of either the left or right lever. Each session consisted of 30 trials, 15 left- and 15 right-lever presentations. Levers were presented individually and pseudo-randomly, where no more than 6 presentations of the same lever would occur in a row. Trials were separated by a 12-s ITI. Rats were trained on this response chain for five days.

Catheter surgery

Rats then underwent surgery for implantation of a chronic indwelling jugular catheter. Rats were first anesthetized with a ketamine (Schein, Dublin, OH)/xylazine (Akorn, Inc., Decatur, IL)/acepromazine (Boehringer Ingelheim, St. Joseph, MO; 75/7.5/0.75 mg/kg) mixture at 0.15 ml/100 g body weight (i.p.). Catheters were inserted into the jugular vein, extended under the skin, and exited the body through an incision on the scalp. A cannula was attached to the end of

the catheter and secured to the skull using dental acrylic and four jeweler's screws. Animals were given a week to recover after surgery.

Drug self-administration training

Following recovery, rats were then trained to self-administer cocaine (1.0 mg/kg/infusion). Rats were placed on a FR schedule, with an orienting response, for cocaine. Briefly, each trial was signaled by the illumination of the house-light where a head-entry into the magazine would result in the house-light turning off and the extension of a single lever (balanced across animals). Upon meeting the FR requirement, the lever would retract, and rats would receive a 0.1 ml infusion of cocaine, totaling 1.0 mg/kg/infusion; dose from Thomsen et al. 2013) over 5.9s accompanied by the illumination of the cue-light above the lever. Trials were separated by a dark 14.1-s ITI. Sessions lasted for 1 hour and rats started on a FR1 for three days, moved onto a FR3 for two days and ended on a FR5 which lasted for three days.

Food vs. drug lever training

After cocaine-self administration training, rats were placed on a lever discrimination procedure where rats had access to both food pellets and cocaine (1.0 mg/kg/infusion). Each trial began with the illumination of the house-light, where an orienting response into the magazine resulted in the house-light turning off and the extension of the previously trained drug lever or the opposite food lever. Completing the FR5 on the presented lever would result in lever retraction

and reward delivery accompanied by the illumination of the corresponding cue-light for 5.9s. Trials were separated by a dark 14.1-s ITI. Sessions ended when 5 of each reinforcer, cocaine and food, were earned. Rats were trained on this schedule for five sessions.

Experiment Proper

Controlled Reinforcer Ratio (CRR) for Cocaine vs. Food Choice

Following establishing procedures, rats were assigned to the controlled reinforcer ratio (CRR) schedule described in Experiment 1 for cocaine versus food choice. Briefly, the CRR choice procedure consisted of 5 distinct blocks, each signaled by an accompanying tone pattern (alternating between 40/29 kHz at 1.8/0, 1.5/0.3, 0.9/0.9, 0.3/1.5, and 0/1.8 seconds) and separated by a dark and empty 2-min inter-block-interval. Each block consisted of a total of 3-drug and 3-food trials. In each of the 5 blocks, both levers (cocaine and food) were extended during each trial. Importantly, during each trial only one of the two reinforcers was randomly scheduled. Regardless of which lever the rat responded on, the reinforcer that was scheduled had to be earned to advance onto the next trial. Responses on the unscheduled lever were recorded and resulted in no consequences. Responses on the food lever, when scheduled, resulted in the delivery of a single 45-mg food pellet, while responses on the cocaine lever, when scheduled, resulted in an infusion of cocaine at varying doses (0, 0.032, 0.10, 0.32, and 1.0 mg/kg/infusion as a function of block). Upon food pellet delivery, the lever would retract and the cue-light above the

corresponding lever would turn on for 5.9s in all blocks. Upon cocaine infusion, the cue-light above the corresponding lever would turn on for a varying duration (0, 0.189, 0.59, 1.89, and 5.9s) that matched the infusion length. Each trial began with the illumination of the house-light where an orienting response into the magazine would turn off the house-light and extend both levers. All responses were scheduled on a fixed-ratio (FR) and required consecutive responding; a changeover in responding would reset the FR count. Upon completion of the FR requirement, levers would retract and reward delivery, signaled by a corresponding cue-light, would occur. Rats were initially trained on a FR1 and were incrementally progressed up to an FR5. All trials were separated by a dark and empty 10-s inter-trial-interval (ITI). Each block ended upon completion of all 6 trials, and each session ended upon completion of all 5 blocks. Rats were trained on the CRR for 28 days. The resulting n-size was 10, where attrition was due to catheter failure.

Cellular Activation for Cocaine Preference and Food Preference

Two days after the last CRR training session, rats underwent two sessions for cellular activation. Activation consisted of two distinct phases, activation for food preference and activation for cocaine preference; food and cocaine activation phases were presented in a counterbalanced order across individuals. Both activation phases started with a 5-min dark period and consisted of two reinforcer-specific trials. For food activation, after the dark period, the house-light turned on and the accompanying tone pattern (solid 40 kHz; same as the first

block in the CRR) was played. A head-entry into the magazine would turn off the house-light and the extend the food-lever only. Completion of a FR1 on the presented food-lever would result in lever retraction and delivery of a 45-mg food pellet, signaled by a 5.9-s cue-light. After a 2-min ITI, the house-light turned on signaling the start of the second trial. Cocaine activation functioned similarly, such that, after the dark period, the house-light turned on and the accompanying tone pattern (solid 29 kHz; same as the last block in the CRR) played. A head-entry into the magazine would turn off the house-light and extend the drug-lever only. Completion of an FR1 on the drug-lever would result in lever retraction and delivery of 0.1 ml of 1.0 mg/kg/infusion of cocaine over 5.9s, signaled by a 5.9-s cue-light. After a 2-min ITI, the house-light turned on again signaling the start of the second trial. After rats finished the two reinforcer-specific trials for cocaine or food, rats were returned to their home cage, sans food and water, for 90 minutes (McClung and Nestler, 2004; Xiu et al. 2014). Afterwards, rats returned to the operant chambers to complete the opposite activation phase (e.g., food if previous activation was cocaine, and vice versa).

Dual-labeling FISH and FIHC

To determine which cells were activated by cocaine vs. food preference, the immediate early gene c-fos was targeted and labeled due to its expression indicating neuronal activity (e.g., neuronal firing; Dragunow and Faull, 1989; Herrera and Jenkins, 1996; Day et al. 2008; VanElzakker et al. 2008). By exposing rats to conditions where preference for cocaine and preference for food

was observed, the timeline in which form of c-fos is expressed, as mRNA or protein, can be utilized to determine neuronal activity via the form of c-fos labeled. Cellular activation of c-fos in the OFC and NAc were labeled using fluorescent immunohistochemistry (FIHC) and fluorescent in situ hybridization (FISH). Specifically, the reinforcer that was presented first will be associated with c-fos protein expression, labeled via FIHC, and the reinforcer that was presented second will be associated with c-fos mRNA expression, labeled via FISH. Thus, specific FIHC or FISH activation is indicative of specific activation to cocaine and food preference, while overlap in FIHC and FISH labeling is indicative of cellular activation common to both reinforcers.

Immediately after the last trial of the second phase of activation, rats were returned to their home cage, sans food and water. Fifteen minutes (Trotha et al. 2014; Xiu et al. 2014) later, rats were given an overdose of a ketamine/xylazine/acepromazine mixture (same formula used for anesthesia during catheter implantation), and transcardially perfused with cold phosphate-buffered saline (PBS) followed by 4% cold paraformaldehyde in PBS. Following perfusion, brains were extracted and placed in a 4% paraformaldehyde solution at 4 ºC overnight, followed by immersion in 30% sucrose solution dissolved in diethylpyrocarbonate (DEPC)-treated water for approximately 48 hours at 4 ºC. Brains were then frozen in tissue-embedding matrix and stored at -80 ºC until slicing. Brains slices containing the OFC (ranging from approximately +4.5 mm to +3.5 mm AP) and NAc (ranging from approximately +1.7 mm to 0.7 mm; Paxinos and Watson 1998) were collected on a cryostat (Ag Protect Leica CM 1860,

Leica Biosystems, USA) at 45 µm. Every fourth slice underwent FISH/FIHC treatment. Probes for c-fos were purchased from Addgene (Plasmid #8966; pcDNA3-FLAG-Fos WT via John Blenis) and constructed by the University of Kentucky's Center for Molecular Medicine – Protein Core.

Free-floating brain sections were washed with 1x PBS (DEPC-treated) for 10 minutes, followed by a 10-min wash in 2% H_2O_2 (vol/vol) in 1x PBS (DEPC-treated), then another 10-min wash in 1x PBS (DEPC-treated) at room temperature. Next, free-floating brain sections were treated with 0.3% Triton X-100 (vol/vol) in 1x PBS (DEPC-treated) for 20 minutes, then treated in 0.25% acetic anhydride (vol/vol) in 0.1 M triethanolmine (pH 8) for 10 minutes, followed by two washes of 1x PBS (DEPC-treated) for 10 minutes each. Afterwards, brain sections were treated in a hybridization solution (50% formamide, 5x saline-sodium citrate (SSC), 0.3 mg/ml yeast tRNA, 100 µg/ml heparin, 1x Denhardt's solution, 0.1% Tween 20, 0.1% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 5 mM EDTA, in DEPC treated water), followed by incubation in hybridization solution with c-fos anti-sense probes for approximately 18 hours at 65 °C. Following hybridization, brain sections were rinsed briefly in DEPC-treated water and washed twice in 2x SSC for 15 minutes each at 60 °C. Next, brain sections were treated with 2 µg/ml RNase A in 2x SSC at 37 °C for 30 min, followed by a brief rinse in DEPC treated water, and washed twice in in 0.2x SSC at 60 °C for 30 minutes each. Brain slices were then washed three times in 1x PBS (DEPC-treated water) containing 0.05% Tween 20 (PBT) for 10 minutes, blocked with 10% sheep serum (vol/vol) in PBT for 1 hour, and incubated with

digoxygenin antibody (1:500, Roche 11207733910) at 4 °C overnight. On the third day, sections were washed three times in PBT for 10 minutes each before being incubated in an amplification solution with cyanine 3 tyramide (PerkinElmer NEL744B001KT) for 20 minutes. Following incubation, brain sections were washed twice in 1x PBS for 10 minutes.

Immediately following FISH treatment, slices were washed twice in 1x PBS-T (0.1% Triton X-100 in 1x PBS) for 15 minutes each. Afterwards, brain sections were blocked with 3% donkey serum in PBS-T for 60 minutes, and then incubated in the same solution with rabbit c-fos antibody (EnCor RPCA-c-fos-AP) for approximately 36 hours at 4 °C. Following incubation, brain slices were washed three times in PBS-T for 15 minutes each and incubated in 3% donkey serum in PBS-T with the secondary antibody Alexa Fluor 488 (Invitrogen A11034) for 2 hours. After incubation slices were washed in PBS-T three times for 10 minutes each and then PBS for 15 minutes. Finally, slices were mounted on slides, given 24 hours to dry, and cover-slipped with VectaShield (Hardest w/DAPI), and stored at 4 °C. See Figure 16 and 17 for representative images. Additionally, twelve (6 OFC and 6 NAc) slices from random subjects (includes subjects from Experiment 4) were taken and underwent control FISH/FIHC treatment (i.e., use of sense probes during FISH and omission of primary antibody during FIHC; Figure 18). See Table 2 for cell counts.

Analysis

Choice under the CRR was expressed as a percent choice for cocaine, calculated via the total number of choice responses on the cocaine lever (i.e., responses on the cocaine lever when cocaine was not available) divided by the overall number of choice responses (i.e., number of responses on the cocaine lever when cocaine was not available added to the number of responses on the food lever when food was not available). Additionally, the generalized matching law used in Experiment 1 (Eqn 11) was applied to the choice data. Furthermore, estimated whole-body cocaine levels (mg/kg) at reinforcer delivery was also determined according to the following kinetics equation (Eqn12; Weiss et al. 2003).

All data were analyzed using linear mixed-effects modeling (LME; Gelman and Hill, 2006) and nonlinear mixed-effects modeling (NLME; Pinheiro et al. 2007). For all tests, α was set to 0.05. Percent cocaine choice was independently analyzed using NLME with magnitude (continuous) as a within-subject factor and subject as a random factor. The averaged whole-body cocaine levels (mg/kg) at reinforcer delivery was analyzed with LME with dose (continuous) as a within-subject factor and subject as a random factor. Correlations between parameter values from the general matching law (i.e., *a*, cocaine-food exchange rate) and the average estimated whole-body cocaine levels prior to reinforcer delivery during the last block (i.e., 1.0 mg/kg/infusion cocaine) were calculated using Spearman's ρ; the last block was chosen since whole-body cocaine levels at this time point would be dependent on previous blocks.

FISH/FIHC images were obtained using a C2+ laser scanning confocal microscope (Nikon Instruments Inc, Melville, NY). Images were taken at 20x objective. Images were taken in a single XY plane (1.2 mm x 1.2 mm) with Z plane of 10 µm (z-stacks at 2 µm). Images were coded and counted in a blind fashion. Cells were counted in ImageJ. Positive protein signals were identified as solid round- or oval-shaped with a diameter of 6 to 10 µm; positive mRNA signals were identified as round- or oval-shaped clusters (Fontenete et al. 2016) forming a diameter of 6 to 10 µm. Overall counts for protein and mRNA labeled cells were analyzed via LME with reinforcer (nominal), brain region (nominal), and label (nominal) as within-subject factors, and subject as a random factor. Cell counts were also expressed as percent cocaine c-fos+ cells, calculated as the number of c-fos positive cells via cocaine activation divided by the total number of cells activated via cocaine and food activation. Percent cocaine c-fos+ cells were analyzed with LME with brain regions (nominal) as a within-subject factor and subject as a random factor. Correlations between parameter values from the general matching law (i.e., *a*, cocaine-food exchange rate) and percent cocaine c-fos+ cells were calculated using Pearson's r; correlation between sensitivity to magnitude (s_M) and overlapped cells was also calculated using Pearson's r.

Results

Figure 19A illustrates percent choice for cocaine under CRR (see Figure 19B for individual profiles). NLME analysis revealed that the cocaine-food exchange rate (*a*) was 0.36 and sensitivity to magnitude (s_M) was 1.97.

Figure 20A illustrates the averaged whole-body cocaine levels (mg/kg) at reinforcer delivery under CRR for each block, and the correlations (Figure 20B) between the averaged individual whole-body cocaine levels (mg/kg) during the last block and individual cocaine-food exchange rates (*a*). LME analysis revealed a main effect of dose [$F(1,9)=92.57$, $p<0.05$], indicating that whole-body cocaine levels increased throughout the session. Furthermore, there was no correlation (Spearman's $\rho = 0.35$, NS) between whole-body cocaine levels (mg/kg) during the last block and cocaine-food exchange rates (*a*). Altogether, these results mirror baseline CRR conditions seen in Experiment 1, where preference was dissociated from intake.

Figure 21A illustrates c-fos+ cells in the OFC and NAc for cocaine and food. LME analysis revealed a main effect of brain region [$F(1,8)=10.59$, $p<0.05$], indicating that there were more c-Fos+ cells in the OFC than the NAc, and main effect of label [$F(1,8)=11.71$, $p<0.05$], indicating that there were more mRNA labeled cells than protein. However, since the order of cocaine and food activation was counterbalanced, percent cocaine c-fos+ cells was calculated. Figure 21B represents averaged percent cocaine c-fos+ cells in the OFC and NAc. LME analysis revealed no significant differences in percent cocaine c-fos+ cells between the OFC and NAc.

Figure 22 illustrates correlations between individual cocaine-food exchange rates (*a*) and individual percent cocaine c-fos+ cells in the (22A) OFC and (22B) NAc, and the correlation between sensitivity to magnitude (s_M) and overlapped cells in the (22C) OFC and (22D) NAc. Analysis revealed no

correlations between *a* and percent cocaine c-fos+ cells in the OFC (Pearson's r = 0.08, NS) or NAc (Pearson's r = 0.24, NS). Analysis also revealed no correlations between s_M and overlapped cells in the OFC (Pearson's r = 0.21, NS) and NAc (Pearson's r = 0.11, NS).

Discussion

Under the CRR schedule for cocaine versus food choice, where the relative rate of reinforcement for cocaine and food was held constant across the two reinforcers, rats in the present experiment produced dose-dependent preference; comparable to results seen in Experiment 1 (i.e., CRR baseline conditions). Likewise, there was no correlation between individual whole-body cocaine levels (mg/kg) during the last block and individual cocaine-food exchange rates (*a*); demonstrating again that preference is independent of intake.

When c-fos+ cells were labeled and counted, a similar pattern of independent populations of cells activated by cocaine and food was observed (Carelli et al. 2000; Carelli, 2002; Padoa-Schioppa and Assad, 2006; Padoa-Schioppa, 2013; Xiu et al. 2014). Like previous findings examining c-fos expression following cocaine self-administration (Thiel et al. 2010), results demonstrated that there were more c-fos+ cells in the OFC than the NAc. Since the labeling of c-Fos+ cells were dependent on the order in which rats underwent cocaine and food activation, c-Fos+ cell counts were transformed into percent cocaine c-fos+ cells (c-fos+ cells activated by cocaine divided by c-fos+ cells

activated by cocaine and food). Interestingly, results revealed there were no significant differences in percent cocaine c-fos+ cells in the OFC and NAc. Furthermore, there were no correlations seen between parameter estimates between individual cocaine-food exchange rates (*a*) and percent cocaine c-fos+ cells in either the OFC and NAc; there were also no correlations between sensitivity to relative magnitude (s_M) and the number of overlapped cells in either brain regions. These findings suggest that individual differences seen in preference are independent of neuronal activity, measured via c-fos expression, in the OFC and NAc for cocaine and food when the relative rate of reinforcement was kept constant across all individuals during choice training. Altogether, these results herein demonstrate that by keeping the relative reinforcer ratio of cocaine to food reinforcers constant across all subjects, the relative distribution of cocaine to food cells activated by conditions that produce cocaine and food preference was not correlated with individual preference.

Table 2. Protein and mRNA labeled c-fos+ cell in the OFC for Experiment 3 and Experiment 4. Label control represents random slices from Experiment 3 and 4 that underwent FISH treatment using sense probe for c-fos and FIHC treatment without primary antibody for c-fos. Activation control represents 3 rats (minimum of 14 days of CRR training under equivalent conditions) undergoing blank activation sessions (i.e., exposure to operant chamber for 10-min each); brains underwent same FISH/FIHC treatment described in Experiment Proper sections.

	OFC (c-fos+ Cells per mm^2)		NAc (c-fos+ Cells per mm^2)	
	Protein	mRNA	Protein	mRNA
Experiment 3	129.59 ± 13.56	162.56 ± 36.60	36.33 ± 7.02	95.71 ± 20.36
Experiment 4	96.23 ± 11.84	87.70 ± 53.80	53.80 ± 5.88	67.21 ± 14.49
Label Control	0.00 ± 0.00	3.30 ± 0.48	0.28 ± 0.26	4.31 ± 2.24
Activation Control	24.8 ± 5.25	2.23 ± 0.85	14.30 ± 1.10	5.48 ± 2.84

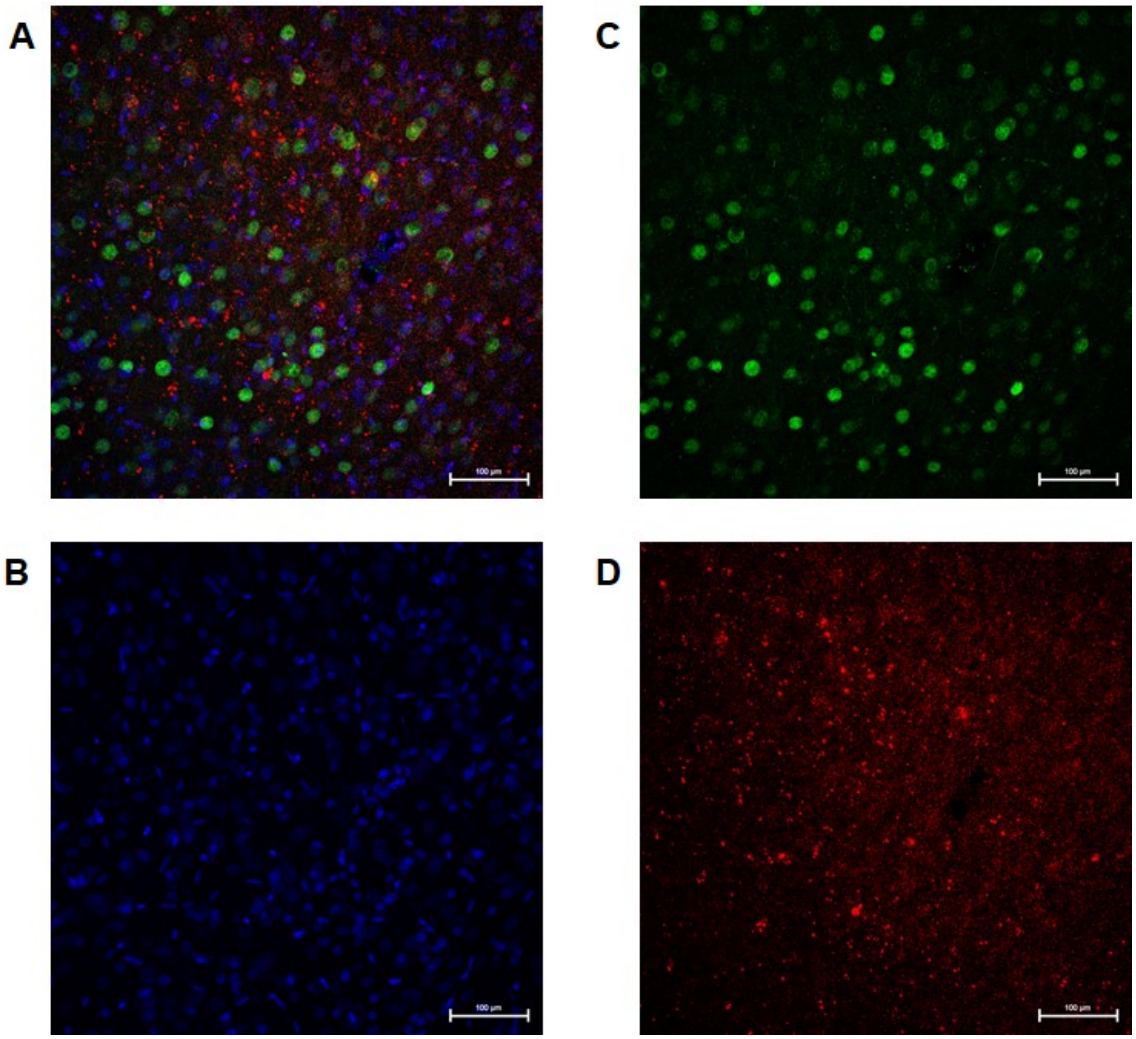

Figure 16. Fluorescent in situ hybridization (FISH) and fluorescent immunohistochemistry (FIHC) c-Fos staining in the OFC. (**A**) Combined FISH/FIHC staining with DAPI. (**B**) DAPI staining. (**C**) FIHC staining for cocaine. (**D**) FISH staining for food. Note: image presented is one-fourth (0.6 mm x 0.6 mm) of full area used for analysis.

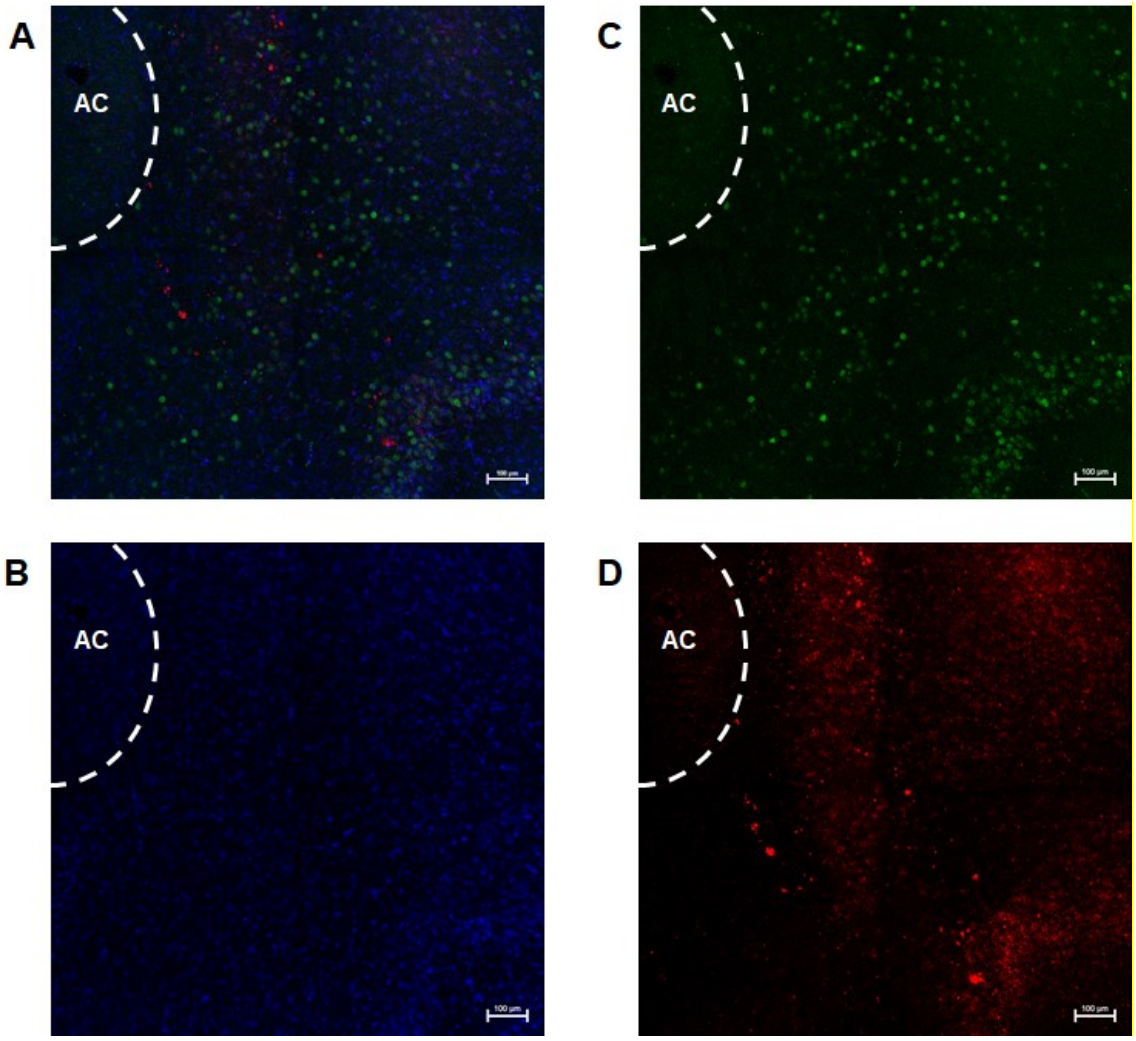

Figure 17. FISH/FIHC c-Fos staining in the NAc. (**A**) Combined FISH/FIHC staining with DAPI. (**B**) DAPI staining. (**C**) FIHC staining for cocaine. (**D**) FISH staining for food. AC stands for anterior commissure.

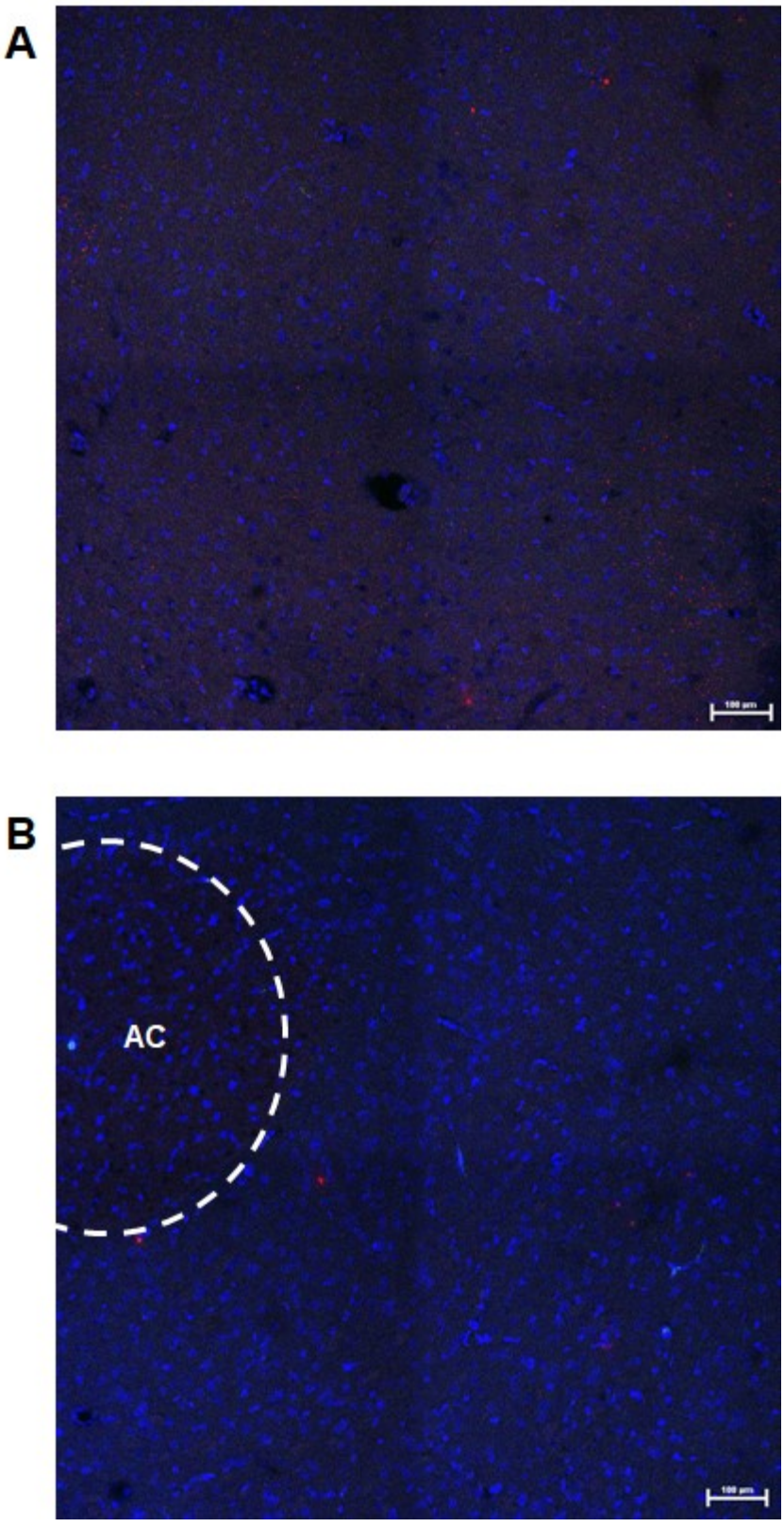

Figure 18. Control FISH/FIHC c-Fos staining in the (**A**) OFC and (**B**) NAc. AC stands for anterior commissure.

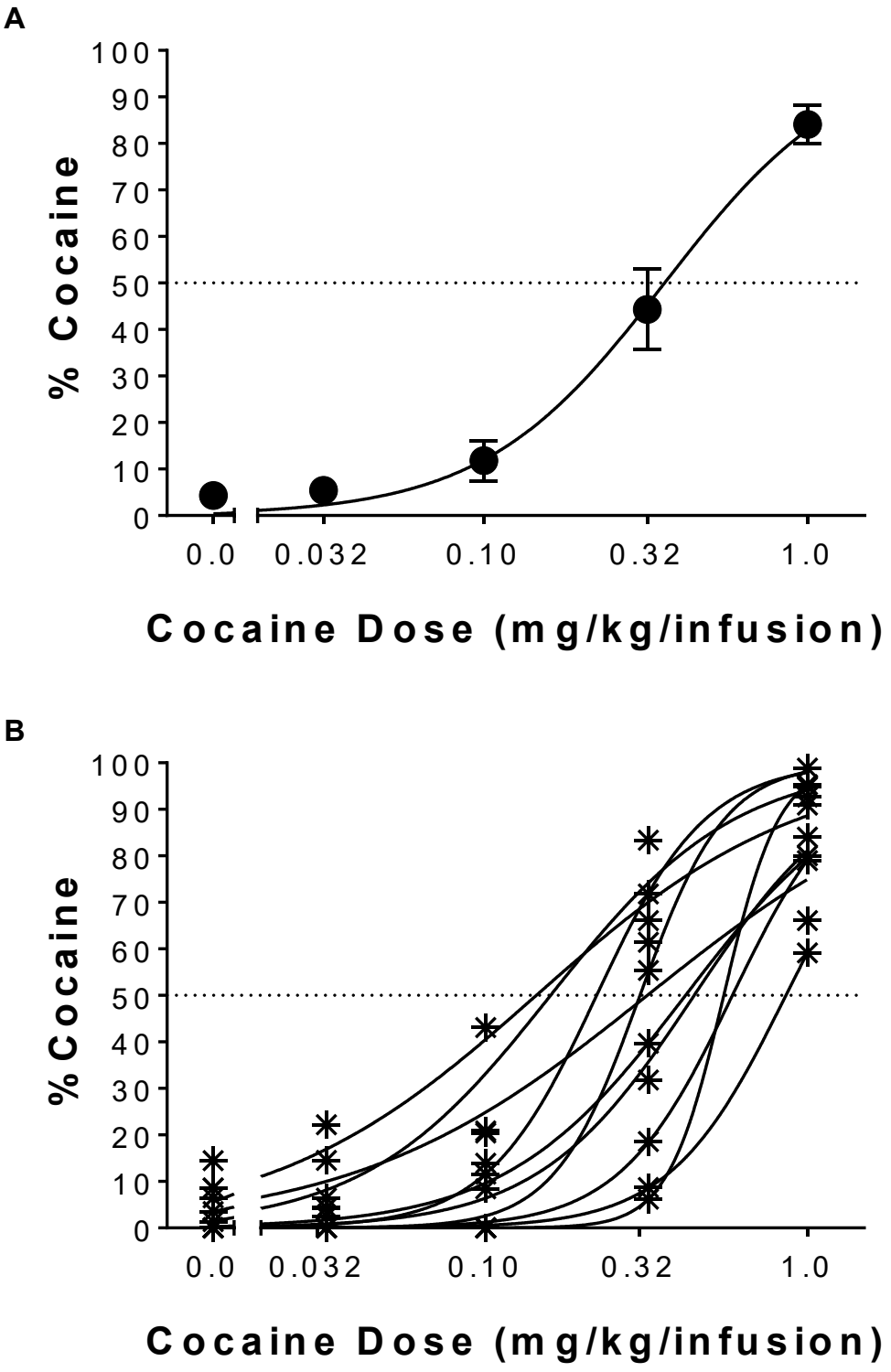

Figure 19. Cocaine versus food choice under the CRR. (**A**) Mean (±SEM) percent choice for cocaine and (**B**) individual choice profiles. Lines are the NLME-determined best fit of Eqn 11.

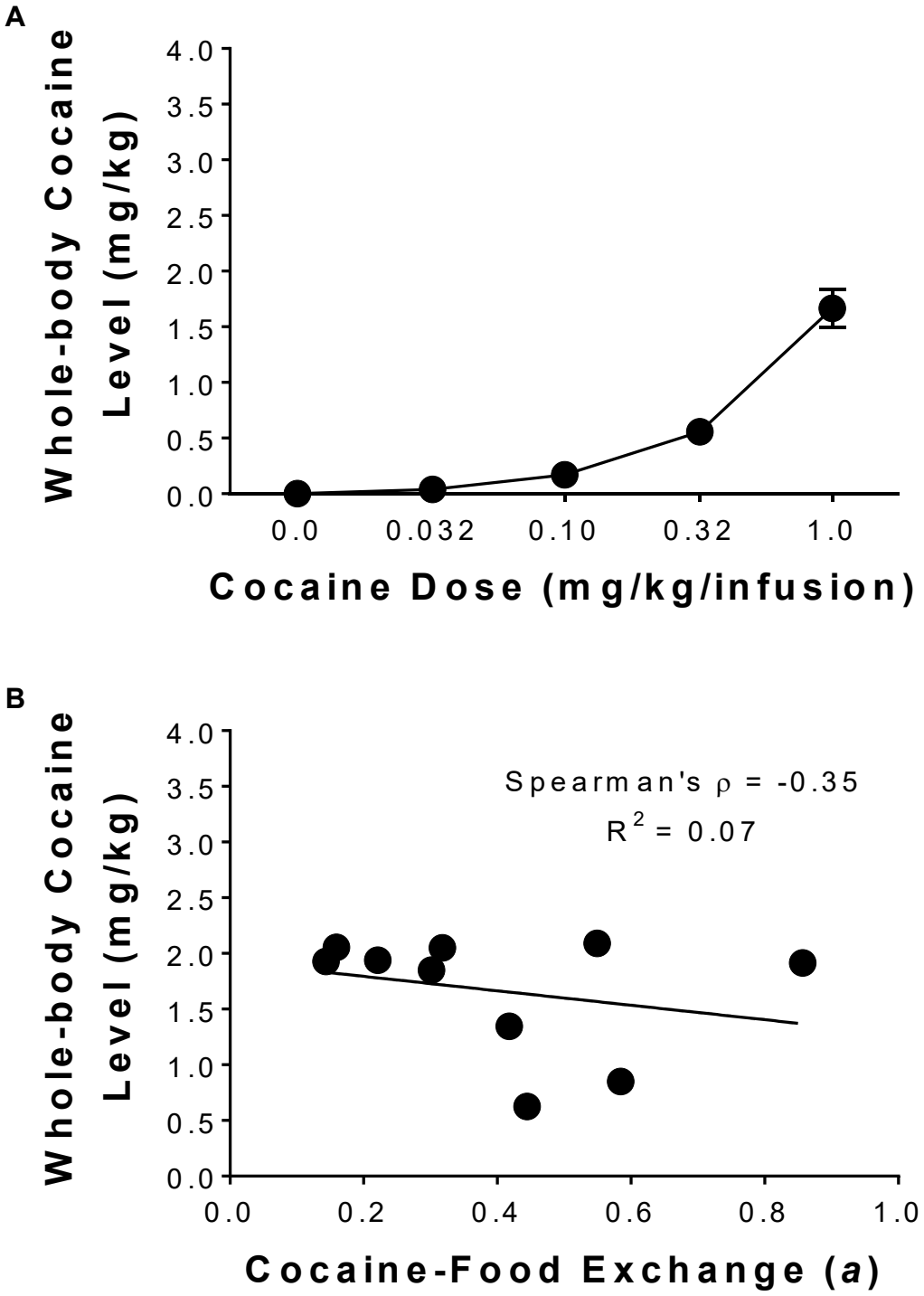

Figure 20. Calculated whole-body cocaine levels (mg/kg) at reinforcer delivery (i.e., amount of cocaine in a rat's system immediately before choosing). (**A**) Mean (±SEM) whole-body cocaine levels at reinforcer delivery, averaged for each block. (**B**) Correlation between individual cocaine-food exchange rates (*a*) and individual whole-body cocaine levels reached during the last block.

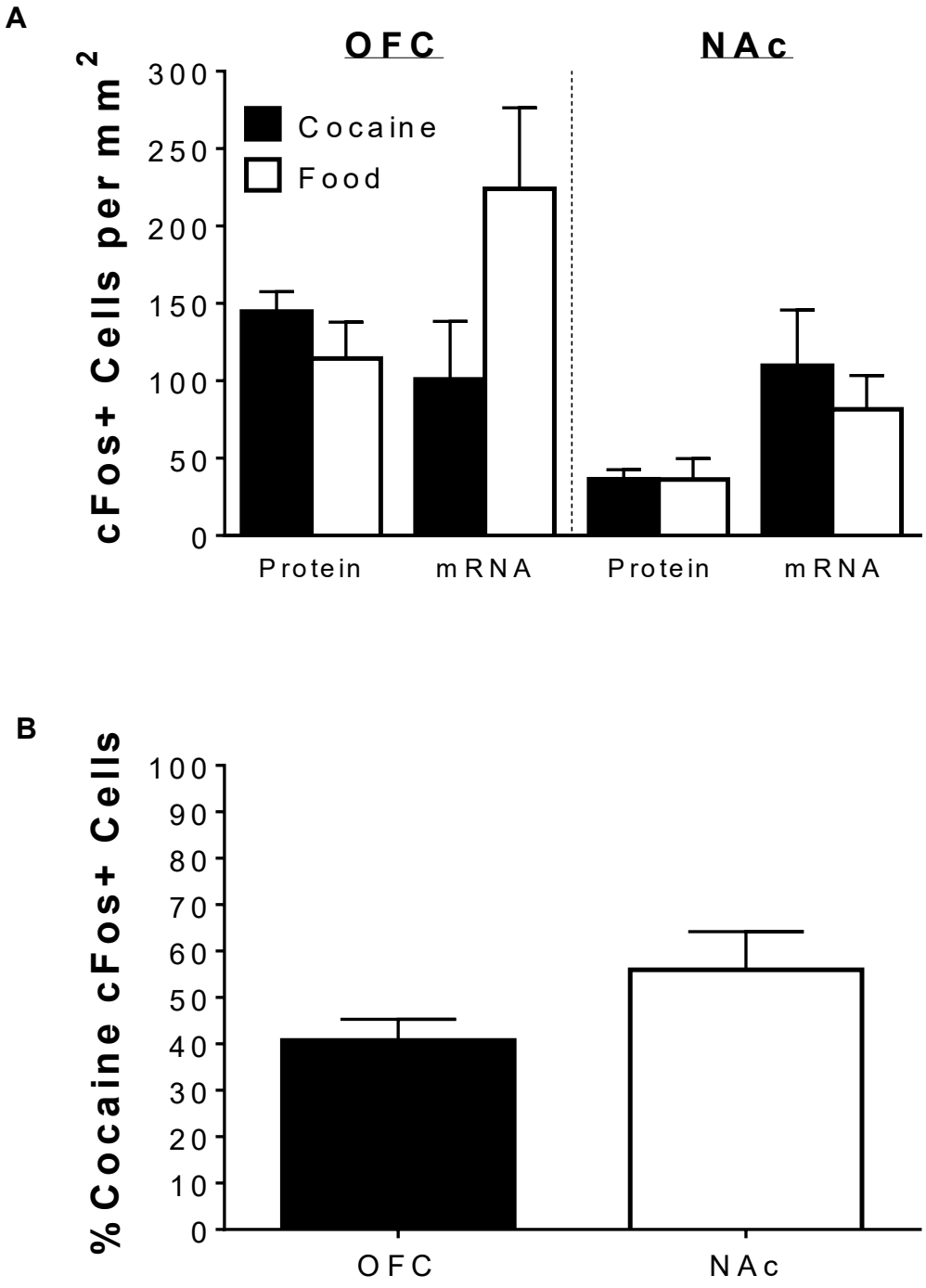

Figure 21. Overall cell counts and percent cocaine c-Fos+ cells in the OFC and NAc. (**A**) Mean (±SEM) c-Fos+ cells labeled via fluorescent in situ hybridization and fluorescent immunohistochemistry. (**B**) Mean (±SEM) percent cocaine c-Fos+ cells, calculated via cocaine c-Fos+ cells divided by cocaine and food c-Fos+ cells.

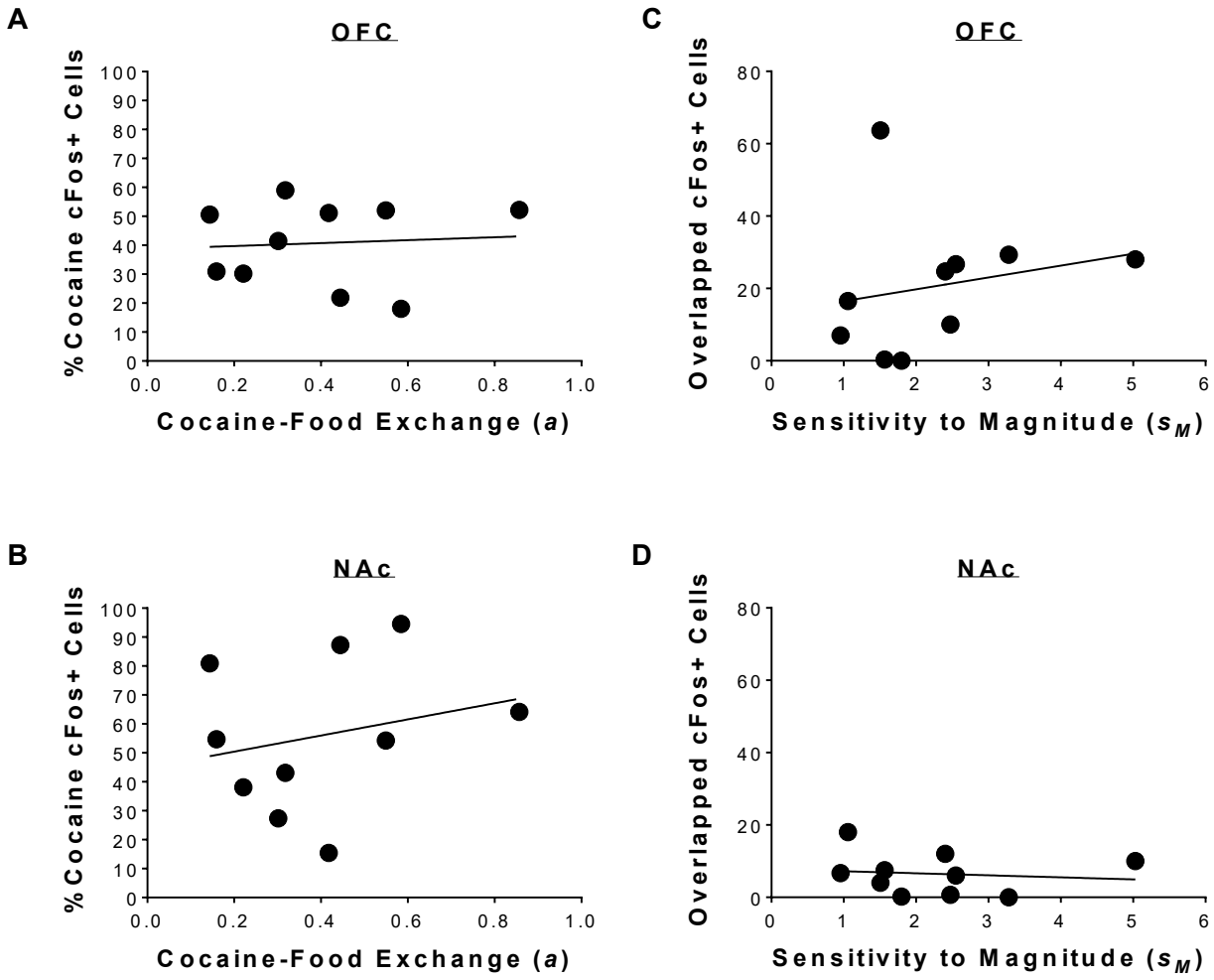

Figure 22. Correlations between parameter estimates via the generalized matching law and cell counts via FISH/FIHC. Correlation between cocaine-food exchange rates (*a*) and percent cocaine c-Fos+ cells in the (**A**) OFC and (**B**) NAc. Correlation between sensitivity to magnitude s_M and overlapped cells in the (**C**) OFC and (**D**) NAc.

Chapter 5

Experiment 4: Determine Cellular Brain Activation during Cocaine vs. Food Choice under Different Reinforcer Ratios

Previous studies have demonstrated that cocaine self-administration is correlated with neuronal activity via c-fos expression (Larson et al. 2010; Zahm et al. 2010; Gao et al. 2017). Moreover, previous studies have also revealed that c-fos expression remained unchanged between rats with differential histories (10 days vs 60 days at FR1) in sucrose-pellet consumption (Gao et al. 2017). Studies have also shown that rats with a greater overall history (i.e., 6-hour daily sessions) of past cocaine self-administration have greater neuroadaptive changes than animals with a less extensive history (i.e., 1-hour daily sessions; Wolf, 2010, 2016). If past cocaine intake influences neuronal activity, it is possible that the electrophysiological measures associated with cocaine preference seen in Guillem and Ahmed (2017) could be a byproduct of overall cocaine intake due to the “discrete-trials” schedule used. That is, under uncontrolled reinforcer ratios schedules, where the relative ratio of cocaine to food reinforcers earned is subject-determined, differences in cocaine intake will occur across individual subjects.

Previous findings herein (i.e., Experiment 2) demonstrated that the rate at which an individual experienced cocaine and food during choice determines preference. In Experiment 2, manipulations to the relative ratio of cocaine to food reinforcers available produced orderly shifts in preference. Specifically, going to a cocaine-rich environment (5:1) produced greater preference for cocaine and

going to a food-rich environment (1:5) produced greater preference for food. Moreover, preference reversals were seen within individuals, while maintaining dose-dependency. Furthermore, the previous experiment (i.e., Experiment 3) demonstrated that individual preference for cocaine (*a*) was independent of c-fos expression for cocaine relative to c-fos expression for food when cocaine intake was held constant across all individuals. Altogether, making it a possibility that previous reports examining the neurobiological mechanisms that underlie drug preference is confounded by drug intake.

By manipulating the relative ratio of cocaine to food reinforcers available, it is hypothesized rats placed into a cocaine-favorable condition (5:1 cocaine to food) will demonstrate preference for cocaine, while rats placed into a food-favorable condition (1:5 cocaine to food) will demonstrate preference for food; a replication of Experiment 2. Moreover, if neuronal activity, via c-fos expression, is related to cocaine intake, then rats that experience greater cocaine intake should show greater c-fos expression than rats with lesser cocaine experience. It is hypothesized that under 5:1 cocaine to food conditions there will be a greater number of cocaine activated cells relative to food activated cells when compared to rats under 1:5 cocaine to food conditions in the OFC and NAc.

Methods

Subjects

Twenty-four adult male Sprague-Dawley Rats (Harlan Inc.; Indianapolis, IN, USA), weighing approximately 250-275 g on arrival were used. Rats were

individually housed (12:12 hr light:dark cycle) with ad libitum access to food and water in their home cage. All experimental protocols were conducted in accordance to the 2011, *National Research Council: Guide for the Care and Use of Laboratory Animals* (8th edition) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Experiments were conducted in operant chambers (ENV-008CT, MED Associates, St. Albans, VT) enclosed within sound-attenuating compartments (ENV-018MD). Each chamber was connected to a personal computer (SG-502), and all chambers were operated using MED-PC. Within each chamber, a recessed food receptacle (ENV-202R2MA) outfitted with a head-entry detector (ENV-254-CB) was located on the front response panel of the chamber, two retractable response levers were mounted on either side of the food receptacle (ENV-122CM), and a white cue-light (ENV-221M) was mounted above each response lever. The back-response panel was outfitted with two nosepoke response receptacles (ENV-114BM) directly opposite to front response levers, a house-light (ENV-227M) was located at the top of the back panel between the two nosepoke response receptacles with Sonalert© tones (ENV-223 AM and ENV223-HAM) located on either side of the house-light. Food pellets (45-mg Bio-Serv Precision Pellets; Flemington, NJ) were delivered via a dispenser (ENV-203M-45). Drug infusions were delivered via a syringe pump (PHM-100) through

tubing strung through a leash (PHM-110-SAI) that attached to a swivel above the chamber.

Drugs

Cocaine hydrochloride, gifted from the National Institute on Drug Abuse (Bethesda, MD, USA), was mixed in sterile saline (0.9% NaCl).

Establishing Procedures

The same establishing procedures described in Experiment 3 was used.

Experiment Proper

Controlled Reinforcer Ratio (CRR) for Cocaine vs. Food Choice

Following establishing procedures, rats were assigned to the controlled reinforcer ratio (CRR) schedule described in Experiment 1 for cocaine versus food choice. Briefly, the CRR choice procedure consisted of 5 distinct blocks, each signaled by an accompanying tone pattern (alternating between 40/29 kHz at 1.8/0, 1.5/0.3, 0.9/0.9, 0.3/1.5, and 0/1.8 seconds) and separated by a dark and empty 2-min inter-block-interval. Each block consisted of a total of 3-drug and 3-food trials. In each of the 5 blocks, both levers (cocaine and food) were extended during each trial. Importantly, during each trial only one of the two reinforcers was randomly scheduled. Regardless of which lever the rat responded on, the reinforcer that was scheduled had to be earned to advance onto the next trial. Responses on the unscheduled lever were recorded and

resulted in no consequences. Responses on the food lever, when scheduled, resulted in the delivery of a single 45-mg food pellet, while responses on the cocaine lever, when scheduled, resulted in an infusion of cocaine at varying doses (0, 0.032, 0.10, 0.32, and 1.0 mg/kg/infusion as a function of block). Upon food pellet delivery, the lever would retract and the cue-light above the corresponding lever would turn on for 5.9s in all blocks. Upon cocaine infusion, the cue-light above the corresponding lever would turn on for a varying duration (0, 0.189, 0.59, 1.89, and 5.9s) that matched the infusion length. Each trial began with the illumination of the house-light where an orienting response into the magazine would turn off the house-light and extend both levers. All responses were scheduled on a fixed-ratio (FR) and required consecutive responding; a changeover in responding would reset the FR count. Upon completion of the FR requirement, levers would retract and reward delivery, signaled by a corresponding cue-light, would occur. Rats were initially trained on a FR1 and were incrementally progressed up to an FR5. All trials were separated by a dark and empty 10-s inter-trial-interval (ITI). Each block ended upon completion of all 6 trials, and each session ended upon completion of all 5 blocks. Rats were trained on the CRR for 14 days.

Manipulation of Frequency

Following training on the CRR under equivalent conditions (3-food and 3-drug trials per block), rats were matched for performance and placed on a CRR schedule that was either cocaine-favorable, consisting of 5-drug trials and 1-food

trial per block, or food-favorable, consisting of 1-drug trial and 5-food trials per block. Rats were trained on cocaine- and food-favorable conditions for 14 days. Upon completion CRR training, the resulting n-sizes were: n=9 for CRR cocaine-favorable (5:1) and n=8 for CRR food-favorable (1:5). All attrition was due to catheter failure.

Cellular Activation for Cocaine Preference and Food Preference

Two days after the last CRR training session, rats underwent two sessions for cellular activation. Activation sessions were identical to the procedures described in Experiment 3. Briefly rats were either placed in an activation session for cocaine preference or food preference; 90 minutes later rats were placed in the opposite condition (food if cocaine was first and vice versa) for activation.

Dual-labeling FISH and FIHC

Immediately after the last trial of the second phase of activation, rats were returned to their home cage, sans food and water. Fifteen minutes later, rats were given an overdose of a ketamine/xylazine/acepromazine mixture, and transcardially perfused. Brains were then frozen in tissue-embedding matrix and stored at -80 ºC until slicing. Brains slices containing the OFC (ranging from approximately +4.5 mm to +3.5 mm AP) and NAc (ranging from approximately +1.7 mm to 0.7 mm; Paxinos and Watson 1998) were collected on a cryostat (Ag Protect Leica CM 1860, Leica Biosystems, USA) at 45 µm. Every fourth slice

underwent FISH/FIHC treatment. FISH/FIHC procedures were identical to the described Experiment 3.

Analysis

Preference for cocaine versus food choice was expressed as percent choice for cocaine, via the total number of choice responses on the cocaine lever (i.e., responses on the drug lever when drug was not scheduled) divided by the overall number of choice responses (i.e., responses made on both the drug and the food lever when the respective reinforcer was not scheduled).

Following stability under baseline conditions, the generalized matching equation (Eqn 11) was first applied to the choice data. Next, to quantitatively analyze how the relative frequency of cocaine to food reinforcers experienced affects cocaine preference the concatenated generalized matching equation (Eqn 16; Baum, 1974; Davison and McCarthy, 1988; Hutsell et al. 2015) was applied. Furthermore, the cocaine-food exchange rate (*a*) under equivalent conditions (3:3), prior to frequency manipulation, was calculated to be 0.32 from Eqn 11. Using data from the same session used to determine choice, estimated whole-body cocaine levels (mg/kg) at reinforcer delivery were also determined using a kinetics equation (Eqn 12; Weiss et al. 2003).

All data were analyzed using linear mixed-effects modeling (LME; Gelman and Hill, 2006) and nonlinear mixed-effects modeling (NLME; Pinheiro et al. 2007). For all tests, α was set to 0.05. Percent cocaine choice for all relative ratio conditions were independently analyzed using NLME with frequency (continuous)

and magnitude (continuous) as within-subject factors, and subject as a random factor. Additionally, whole-body cocaine levels (mg/kg) at reinforcer intake as a function of block was analyzed with LME with frequency (continuous) and block (continuous) as within-subject factors, and subject as a random factor.

FISH/FIHC images were obtained using a C2+ laser scanning confocal microscope (Nikon Instruments Inc, Melville, NY). Images were taken at 20x objective. Images were taken in a single XY plane (1.2 mm x 1.2 mm) with Z plane of 10 µm (z-stacks at 2 µm). Images were coded and counted in a blind fashion. Cells were counted in ImageJ. Positive protein signals were identified as solid round- or oval-shaped with a diameter of 6 to 10 µm; positive mRNA signals were identified as round- or oval-shaped clusters (Fontenete et al. 2016) forming a diameter of 6 to 10 µm. Overall counts for protein and mRNA labeled cells were analyzed via LME with reinforcer (nominal), brain region (nominal), and label (nominal) as within-subject factors, cocaine:food ratio (nominal) as a between-subject factor, and subject as a random factor. Cell counts were expressed as percent cocaine c-fos+ cells calculated as the number of c-fos positive cells via cocaine activation divided by the total number of cells activated via cocaine and food activation. Percent cocaine c-fos+ cells for each brain region was analyzed with LME with cocaine:food ratio (nominal) as a between-subject factor and subject as a random factor. Correlations between parameter values from the general matching law (i.e., *a*, cocaine-food exchange rate) and percent cocaine c-fos+ cells were calculated using Pearson's r.

Results

Figure 23A illustrates percent choice for cocaine prior to frequency manipulation. NLME analysis revealed there were no significant differences between groups. Moreover, NLME analysis revealed that the cocaine-exchange rate (*a*) was 0.32. Figure 23B illustrates whole-body cocaine levels (mg/kg) under the CRR when cocaine to food reinforcer ratios were equivalent. LME analysis revealed there were no significant differences between groups. Altogether, these results indicate that there were no differences between groups prior to being assigned to a cocaine- or food-favorable condition.

Figure 24 illustrates (24A) percent choice for cocaine under the CRR for the different relative ratio manipulations and (24B) averaged whole-body cocaine levels at reinforcer delivery. NLME analysis revealed significant effect of sensitivity to magnitude (s_M = 2.11) [$F(1,67)=142.20$, $p<0.05$] and a significant effect of sensitivity to frequency (s_R = 1.32) [$F(1,67)=26.83$, $p<0.05$], altogether indicating that relative difference in magnitude for cocaine and food reinforcement, and frequency of reinforcement are independently affecting cocaine choice. LME analysis on whole-body cocaine levels (mg/kg) at reinforcer delivery averaged for each block revealed a main effect of dose [$F(1,13.44)=87.13$, $p<0.05$], ratio [$F(1,8.39)=23.81$, $p<0.05$], and dose x ratio interaction [$F(1,13.83)=22.38$, $p<0.05$], indicating that whole-body cocaine levels increased as a function of dose, but increased at different rates depending on the reinforcer ratio experienced.

Figure 25A illustrates c-fos+ cells in the OFC and NAc for cocaine and food under the ratio manipulations. LME analysis revealed a main effect of region

[$F(1,13)=13.18$, $p<0.05$], indicating that there were more c-Fos+ cells in the OFC than the NAc. Since the order of cocaine and food activation was counterbalanced, percent cocaine c-fos+ cells were calculated. Figure 25B represents averaged percent cocaine c-fos+ cells in the OFC and NAc under the ratio manipulations. LME analysis revealed a main effect of cocaine:food ratio [$F(1,15)=5.08$, $p<0.05$] in the OFC, indicating that the percent cocaine c-fos+ cells in the cocaine-favorable group was greater than the food-favorable group. LME analysis revealed no significant differences in percent cocaine c-fos+ cells in the NAc.

Discussion

Using the CRR choice schedule for cocaine versus food choice to experimentally control for the relative ratio of cocaine to food reinforcers experienced, results yielded findings that paralleled previous findings herein (Experiment 2) and by others (Anderson and Woolverton, 2000; Anderson et al. 2002). When rats were matched by performance (Figure 23) and placed into a cocaine-favorable or food-favorable condition, rats adjusted preference accordingly. Specifically, rats placed into the cocaine-favorable condition (5:1) shifted preference towards cocaine, while rats placed into a food-favorable condition (1:5) shifted preference towards food. Additionally, when whole-body cocaine levels (mg/kg) were examined it was revealed that all reinforcer ratios produced increasing whole-body cocaine levels as a function of block.

Expectedly, the rate at which whole-body cocaine levels increased was related to the relative rate of reinforcement.

When c-fos+ cells were labeled and counted, a similar pattern of independent populations of cells were that activated to cocaine and food reinforcement was observed (Carelli et al. 2000; Xiu et al. 2014); results also demonstrated that there were more c-fos+ cells in the OFC than the NAc in general (Thiel et al. 2010). When c-fos+ cell counts were calculated as percent cocaine c-fos+ cells, analysis revealed that rats in the 5:1 cocaine to food condition had greater neuronal activity in the OFC relative to rats in the 1:5 cocaine to food condition. However, there were no differences seen in the NAc.

These results are reflective of the electrophysiological findings seen in Guillem and Ahmed (2017), the only other cocaine versus food choice study examining neural activity in rats. Guillem and Ahmed (2017), demonstrated that the number of neurons in the OFC that encoded cocaine reward was correlated with individual preference for cocaine (measured as the number of cocaine reinforcers chosen relative to total reinforcers chosen, which is also identical to the relative rate of reinforcement for cocaine and saccharin). However, the findings herein suggest otherwise, and that neuronal activity in the OFC, measured via c-fos expression (Dragunow and Faull, 1989; Herrera and Jenkins, 1996; Day et al. 2008; VanElzakker et al. 2008), is instead determined by overall cocaine intake.

Of note, previous studies examining c-fos expression following cocaine self-administration have also demonstrated a negative correlation between

cocaine intake and c-fos activity in NAc (e.g., Larson et al. 2010; Gao et al. 2017). Results herein showed no significant differences in c-fos expression for cocaine between cocaine-experienced and food-experienced groups in the NAc. However, it should be noted that rats in the 5:1 cocaine to food condition experienced approximately 2x overall cocaine intake (calculated as the overall intake during baseline training and frequency manipulation) than rats in the 1:5 cocaine to food condition (~162 mg/kg vs. ~81 mg/kg). Whereas, rats in Gao et al. (2017), which showed a negative correlation in c-fos expression and cocaine intake, had approximately a 5x difference (~480 mg/kg vs. ~90 mg/kg; estimates from Figure 1 in Gao et al. 2017) in cocaine history; making it possible that with prolonged training under the CRR at different reinforcer ratios could eventually result in differences in c-fos expression in the NAc. In all, the findings herein revealed that neuronal activity in the OFC is dependent on overall cocaine intake and not reflective of individual preferences for cocaine.

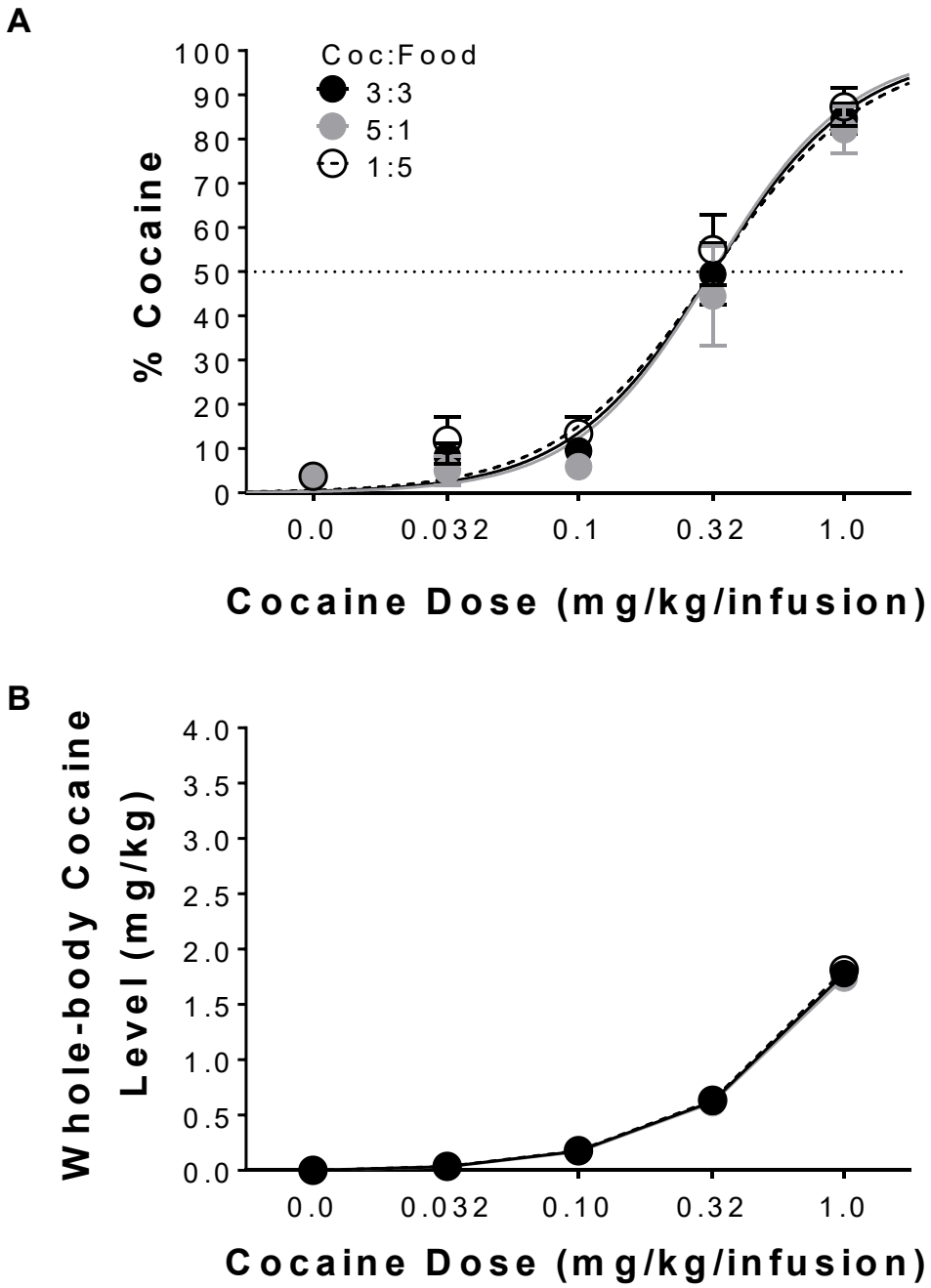

Figure 23. Cocaine versus food choice under the CRR when the ratio of cocaine to food reinforcers was equivalent prior to undergoing ratio manipulation. (**A**) Mean (±SEM) percent choice. Lines are the NLME-determined best fit of Eqn 11. (**B**) Averaged whole-body cocaine levels (mg/kg) at reinforcer delivery when the ratio of cocaine to reinforcers was equivalent. Note: the 3:3 condition combines data for both 5:1 and 1:5 groups.

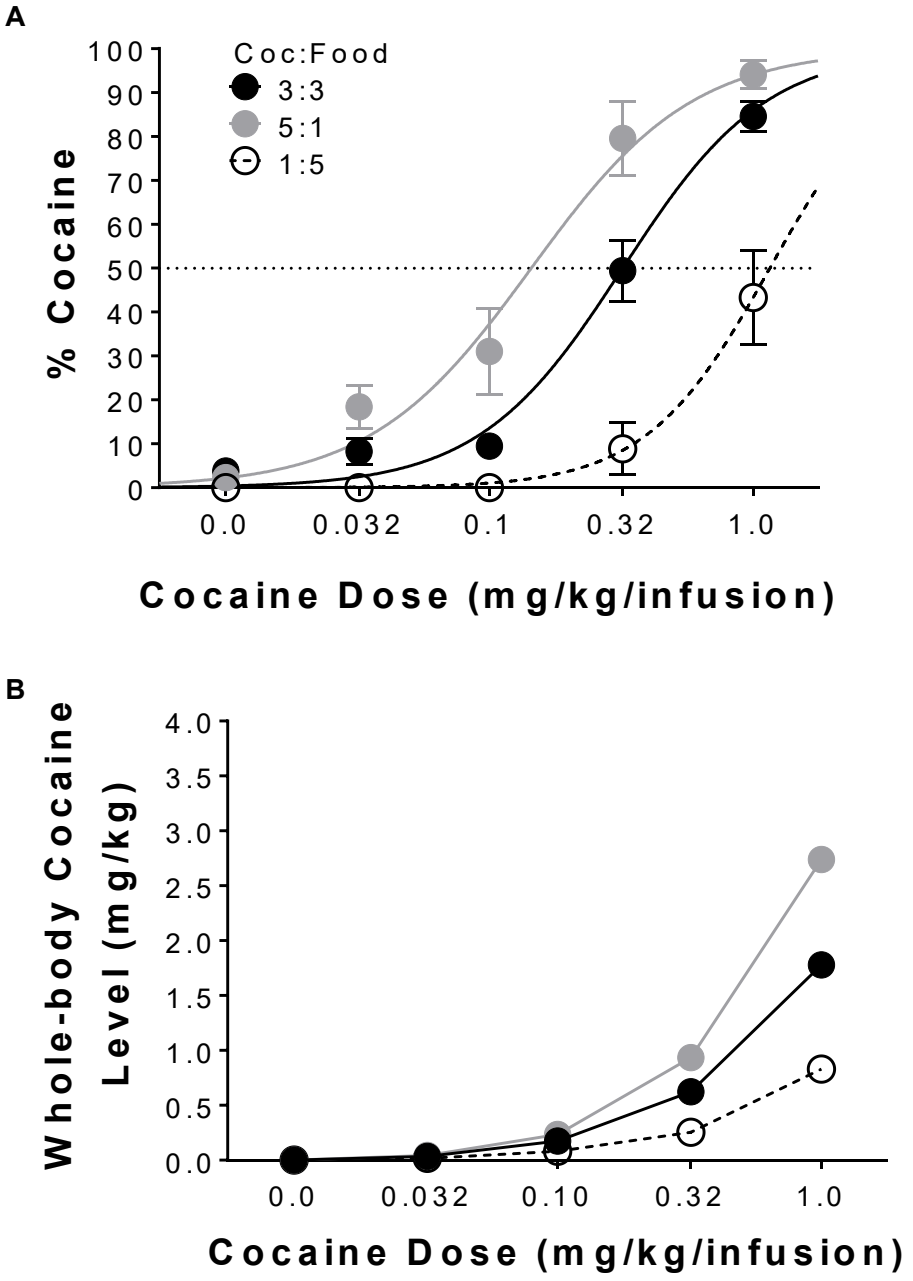

Figure 24. Cocaine choice and calculated whole-body cocaine levels (mg/kg) at different reinforcer ratios under the CRR. (**A**) Mean (±SEM) percent choice for cocaine under the CRR prior to ratio manipulation (3:3), a reinforcer ratio in favor of cocaine (5:1), and a reinforcer ratio in favor of food (1:5). Lines are the NLME-determined best fit of Eqn 16. (**B**) Mean (±SEM) whole-body cocaine levels at reinforcer delivery under the CRR for prior to manipulation (3:3) and after manipulation (5:1 in favor of cocaine and 1:5 in favor of food).

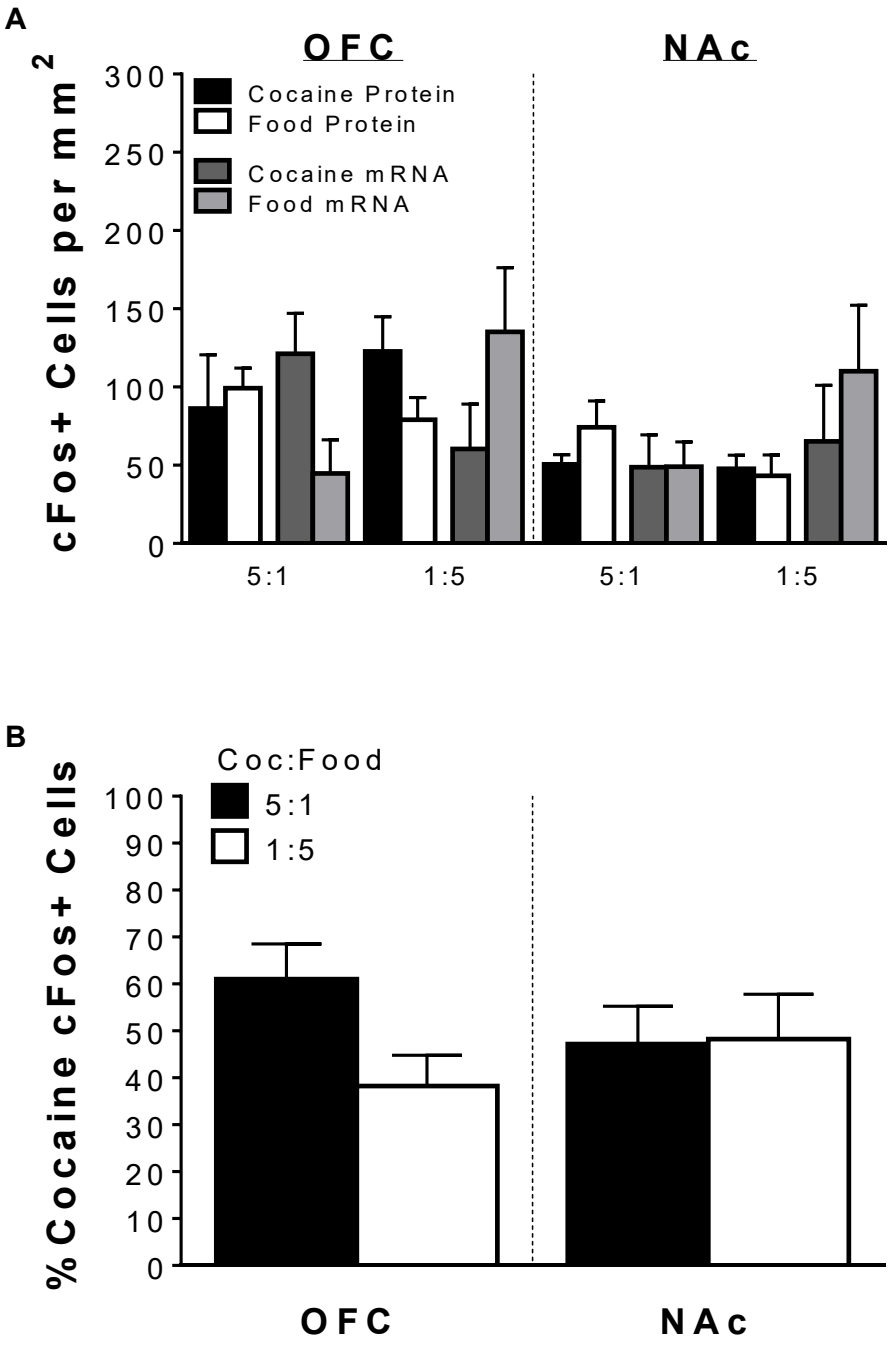

Figure 25. Overall cell counts and percent cocaine c-Fos+ cells in the OFC and NAc for the different ratio manipulations. (**A**) Mean (±SEM) c-Fos+ cells labeled via FISH and FIHC. (**B**) Mean (±SEM) percent cocaine c-Fos+ cells, calculated via cocaine c-Fos+ cells divided by cocaine and food c-Fos+ cells.

Chapter 6

General Discussion

Altogether, the goal of these experiments was to investigate the neurobehavioral mechanisms that underlie preference for cocaine, while controlling for differential rates of reinforcement across individuals. Within these experiments, a novel choice procedure (i.e., controlled reinforcer ratio; CRR) was introduced in attempts to remedy the confound seen in all other drug versus non-drug choice studies where preference is intertwined with intake. Results revealed that like prototypical choice procedures (i.e., uncontrolled reinforcer ratio, URR; Negus, 2003; Thomsen et al. 2013), the CRR produced dose-dependent preference. Although both choice schedules displayed similar shifts in preferences to environmental manipulations, the CRR did so while keeping the relative rate of reinforcement for cocaine and food constant. Of note when whole-body cocaine levels during the last block were correlated with individual cocaine-food exchange rates (*a*), via the generalized matching law (Killeen, 1972; Baum, 1974, 1979; Davison and McCarthy, 1988), it was revealed that under a URR schedule whole-body cocaine levels and cocaine-food exchange rates were correlated, suggesting preference and intake are confounded. However, under the CRR whole-body cocaine levels and cocaine-food exchange rates were not correlated, demonstrating a dissociation between preference and intake. Additionally, it was also revealed that when the relative frequency of cocaine to food reinforcers was manipulated under the CRR in favor of cocaine or food, preference shifted accordingly within subject. Moreover, these shifts in preference were reversible. When compared to other cocaine versus food

studies (e.g., Lenoir et al. 2007; Kearns et al. 2017), it was revealed that preference was not associated with reaching some theoretical threshold level of cocaine, as seen by the varying range of whole-body cocaine levels when cocaine and food preference were equivalent under the varying reinforcer ratio manipulations. In all, these results challenge the hypothesis that cocaine intake causes cocaine preference (Vandaele et al. 2016; Freese et al. 2018). Instead, the results follow choice theory, and all previous choice studies demonstrating that value is determined by the differences in relative reinforcer dimensions (Rachlin, 1971; Killeen, 1972; Baum, 1974; William, 1979; Davison and McCarthy, 1988). Finally, application of the generalized matching law revealed that relative reinforcer magnitude and frequency, independent dimensions of reinforcement, determines the relative value of cocaine.

Given that differential histories in drug intake can result in differential neural adaptations across subjects (Moal and Koob, 2007; Kalivas and O'brien, 2008), studies investigating the underling neurobehavioral mechanisms that drive drug versus non-drug choice are also afflicted by the issue of preference being confounded with intake. Specifically, this confound makes it difficult to determine if any neuroadaptations observed are linked with drug usage or drug preference. Utilizing the CRR choice procedure that allows for a dissociation between preference and intake, the second half of these experiments attempted to elucidate the role the orbitofrontal cortex (OFC) and nucleus accumbens (NAc) have in cocaine versus food choice. The OFC and NAc, brain regions within the reward pathway (Everitt and Robbins, 2005), were chosen due to their

associated role in governing reward-related processes in relation to decision-making (Salamone et al. 2007; Schoenbaum and Shaham, 2008; Padoa-Schioppa, 2013). Following training on the CRR, under equal reinforcer ratios (3:3), c-fos, a marker for neuronal activity (Herrera and Jenkins, 1996; Cruz et al. 2015) was targeted to measure neuronal activity for cocaine and food preference. Using the timeline in which c-fos is expressed as mRNA and protein (Xiu et al. 2014), both preference for cocaine related neuronal activity and preference for food related neuronal activity was labeled using FISH/FIHC staining. Results revealed that the number of c-fos+ cells related to cocaine activation relative to c-fos+ cells related to food activation was not correlated with behavioral measures for cocaine versus food preference in either the OFC or NAc. Furthermore, following CRR training under a 5:1 cocaine to food condition and a 1:5 cocaine to food condition, it was revealed that under the 5:1 cocaine-to food condition, a greater number of c-fos+ cells activated in response to cocaine relative to c-fos+ cells activated in response to food preference within the OFC and not NAc. Collectively, these results suggest that OFC activity for cocaine, relative to food, is related to greater cocaine intake and not preference.

These findings herein are contrary to the only other cocaine versus food choice study examining neuronal activity in the OFC, where it was demonstrated that the relative number of cocaine encoding cells identified, via electrophysiological recordings, is reflective of cocaine preference (e.g., Guillem and Ahmed, 2017). Instead, data herein suggests that the relative increases in neuronal activity for cocaine are related to overall cocaine intake. Furthermore,

data within these experiments demonstrate that the relative rate of reinforcement, or how frequently an organism comes into contact with given alternatives, for cocaine versus food determines cocaine preference. Given that all other drug versus non-drug studies use uncontrolled reinforcer ratio schedules, procedures where the relative frequency of drug to food contact varies, drug intake becomes a confound, making it difficult to dissociate if any neural mechanisms identified to underlie decision-making processes are reflective of preference or drug intake. Thus, application of a CRR choice procedure can better isolate and identify the neural mechanisms that underlie preference, while eliminating the confound of drug intake.

Despite the current lack of studies investigating neuronal activity involved in cocaine versus food choice which to compare, previous electrophysiological studies have repeatedly demonstrated that there are specific subsets of neurons in the OFC involved in encoding valuation of non-drug reinforcers and the decision processes leading up to the choices made (Padoa-Schioppa and Assad, 2006, 2008; Roitman and Roitman, 2010; Padoa-Schioppa, 2013). There are also findings suggesting that the OFC does not necessarily only encode value, but also encode dimensions of reinforcement (e.g., delay; Roesch et al. 2006). Recordings from the NAc have also suggested that the NAc is more responsive towards stimuli that modulate or predict reward (Knutson 2001; Roitman et al. 2004; Cardinal and Howes, 2005; Salamone et al. 2005). Altogether, it is possible that these different phases or features that lead to decision-making are all being captured by the FISH/FIHC labeling methods used herein; especially,

since the lever, cue-light, tone, and actual reinforcer were all presented during the activation phases. Interestingly, there are imaging studies showing that cocaine use increases neuronal activity in the OFC in relation to cocaine-related cues (Childress et al. 1999; Volkow and Fowler, 2000; Schoenbaum and Shaham, 2008); thus, it is possible that the increase in relative cocaine c-fos activity in the 5:1 cocaine to food condition may be related to the cocaine cues presented. Although there are studies demonstrating a negative correlation between cocaine self-administration and c-fos activity in the NAc (Larson et al. 2010; Gao et al. 2017), there are also studies suggesting that increased cocaine self-administration is correlated with increased NAc activity for cocaine-cues (Risinger et al. 2005); thus, it is also possible that NAc c-fos activity measured herein may be muddled by the activation procedure as well.

Like previous studies, the findings herein demonstrated that there are distinct populations of cells within the OFC and NAc that activate in response to cocaine or food (Carelli et al. 2000; Carelli, 2002; Xiu et al. 2014). Moreover, these distinct populations of cells, measured via c-fos expression, could be identified in future studies to investigate neural ensembles involved in drug preference (Cruz et al. 2015). For example, future studies using the CRR to isolate preference from intake could examine glutamatergic signaling within the limbic regions to determine which population of neurons are more likely to respond to drug-related preference (Everitt and Robbins, 2005; Cohen and Greenberg, 2008). Likewise, using a CRR choice procedure could aid in elucidating the role that medium spiny neurons have in the nucleus accumbens

that relates to differential reinforcers (Betran-Gonzalez et al. 2008; Lobo et al. 2010).

Overall, these results herein demonstrate that non-drug alternatives function as economic substitutes for cocaine, and under certain conditions the substitutability (i.e., cocaine-food exchange rate) can be shifted. Moreover, while the CRR choice procedure used herein can control for differential drug to non-drug intake, the CRR could also be used to model certain environmental scenarios. For example, low socioeconomic status is a predictor for substance use disorders (Galea et al. 2004; Walker and Druss, 2012; Redonnet et al. 2016), and within low socioeconomic environments there often is a lack of alternative reinforcers (e.g., job opportunities and social interactions), relative to drugs of abuse (e.g., number of liquor stores in low socioeconomic neighborhoods). By using a CRR choice procedure and modeling situations with low rates for non-drug alternatives (i.e., cocaine- or food-skewed reinforcer frequency ratios), behavioral interventions and pharmacological treatments can be put to the test to see how effectively they can shift preference in situations where preference is biased towards drug. In all, these findings provide impetus for using a CRR schedule when it comes to studying drug versus non-drug choice.

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Curriculum Vitae

Jonathan Chow

Education

Ph.D. Candidate – University of Kentucky 2014 to Present
Lexington, KY
Behavioral Neuroscience & Psychopharmacology
Mentor: Joshua S. Beckmann
Co-mentor: Michael T. Bardo
Dissertation: Cocaine choice: a novel procedure for investigating neuronal activation mediating cocaine preference

Master of Science – University of Kentucky 2012 to 2014
Lexington, KY
Behavioral Neuroscience & Psychopharmacology
Mentors: Michael T. Bardo & Joshua S. Beckmann
Master's Thesis: Examining memory consolidation and reconsolidation in an appetitive Pavlovian learning task

Bachelor of Science – University of Kentucky 2008 to 2012
Lexington, KY
Major: Psychology
Minor: Chemistry
Mentors: Michael T. Bardo & Joshua S. Beckmann
Honor's Thesis: The role of D1 and D2 dopamine receptors on incentive salience attribution and cocaine self-administration

Professional Positions

Fall 2018	Research Assistant, Psychology, University of Kentucky, Supervisor: Dr. Joshua S. Beckmann
Spring 2018- Fall 2017	Teaching Assistant, PSY 456, University of Kentucky Supervisor: Dr. Joshua S. Beckmann
Summer 2017	Instructor on Record, PSY 100
Spring 2017	Teaching Assistant, PSY 459, Supervisor: Dr. Lynda Sharrett-Field Grader, PSY 312 Instructor on Record: Logan Fields
Fall 2016	Research Assistant, Psychology, University of Kentucky, Supervisor: Dr. Joshua S. Beckmann

Spring 2015	Adjunct Professor, Centre College Supervisor: Dr. KatieAnn Skogsberg & Dr. Melissa Burns-Cusato
Fall 2016- Fall 2014	T32 Predoctoral Trainee, University of Kentucky, Supervisors: Dr. Linda P. Dwoskin, Dr. Michael T. Bardo, & Dr. Joshua S. Beckmann
Fall 2017, Fall 2014	Guest Lecturer, University of Kentucky
Spring 2013	Research Assistant, Psychology, University of Kentucky, Supervisor: Dr. Michael T. Bardo
Fall 2013	Teaching Assistant, PSY 456, University of Kentucky Supervisor: Dr. Michael T. Bardo
Spring 2012- Fall 2012	Research Assistant, Psychology, University of Kentucky, Supervisor: Dr. Michael T. Bardo

Awards, Fellowships, Grants

Preparing Future Faculty Certificate, University of Kentucky, Fall 2015

NIDA Predoctoral Traineeship, Department of Pharmaceutical Sciences and the National Institute of Drug Abuse, University of Kentucky, Fall 2014 to Fall 2016 (T32 DA016176)

University of Kentucky – RCTF, Psychology, Fall 2014

Publications

1. **Chow, J. J.**, & Beckmann, J. S. (2018). NMDA receptor blockade specifically impedes the acquisition of incentive salience attribution. *Behavioural brain research, 338, 40-46.*

2. Hofford, R. S., **Chow, J. J.**, Beckmann, J. S., & Bardo, M. T. (2017). Effects of environmental enrichment on self-administration of the short-acting opioid remifentanil in male rats. *Psychopharmacology*, 1-8.

3. **Chow, J. J.**, Smith, A. P., Wilson, A. G., Zentall, T. R., & Beckmann, J. S. (2017). Suboptimal choice in rats: incentive salience attribution promotes maladaptive decision-making. *Behavioural Brain Research, 320,* 244-254.

4. Smith, A. P., Bailey, A. R., **Chow, J. J.**, Beckmann, J. S., & Zentall, T. R. (2016). Suboptimal choice in pigeons: Stimulus value predicts choice over frequencies. *PloS one*, *11*(7), e0159336.

5. **Chow, J. J.**, Nickell, J. R., Darna, M., & Beckmann, J. S. (2016). Toward isolating the role of dopamine in the acquisition of incentive salience attribution. *Neuropharmacology*, *109*, 320-331.

6. Darna, M., **Chow, J. J.**, Yates, J. R., Charnigo, R. J., Beckmann, J. S., Bardo, M. T., & Dwoskin, L. P. (2015). Role of serotonin transporter function in rat orbitofrontal cortex in impulsive choice. *Behavioural Brain Research*, *293*, 134-142.

7. Beckmann, J. S., & **Chow, J. J.** (2015). Isolating the incentive salience of reward-associated stimuli: value, choice, and persistence. *Learning & Memory*, *22*(2), 116-127.

Manuscripts in Submission/Preparation

Beckmann, J.S., **Chow, J. J.**, & Hutsell, B. A., (submitted). Cocaine-associated decision-making: dissociating preference from intake

Chow, J. J., & Beckmann, J. S., (in preparation). Modulating remifentanil choice.

Jonathan Jenn-Sheng Chow
Student's Signature

November 12, 2018
Date