THE ROLE OF RAPID DOPAMINE SIGNALING WITHIN THE NUCLEUS ACCUMBENS IN NATURAL AND DRUG REWARD-SEEKING BEHAVIORS

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ABSTRACT

COURTNEY MARIE CAMERON: The role of rapid dopamine signaling within the nucleus accumbens in natural and drug reward-seeking behaviors
(Under the direction of Regina M. Carelli)

Learning about rewards and appropriately directing behaviors to obtain them is critical for survival. These processes are subserved by a distributed network of brain nuclei including the nucleus accumbens (NAc) and its dopaminergic input. *In vivo* electrophysiology studies have repeatedly provided evidence that NAc neurons encode goal-directed behaviors for both natural and drug rewards. Specifically, work from this laboratory has shown that subsets of NAc neurons exhibit largely differential, nonoverlapping firing patterns during operant responding for natural rewards (food, water, or sucrose) versus intravenous cocaine (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). Furthermore, the percentage of NAc neurons that encode goal-directed behaviors for cocaine is dramatically increased following 30 days of cocaine abstinence (Hollander & Carelli, 2005; Hollander & Carelli, 2007). While we have observed rapid dopamine (DA) signaling in the NAc during responding for natural (Roitman *et* al, 2004) and drug (Phillips *et al*., 2003) rewards on a timescale similar to NAc phasic cell firing, it is not known whether this DA signaling acts in a manner analogous to NAc phasic activity. The first set of experiments detailed in this dissertation used electrochemical recording techniques to measure rapid DA release in the NAc core during performance of two different tasks: a sucrose/cocaine or sucrose/food multiple schedule. This design allowed us to compare DA release dynamics in specific locations in the

NAc during operant responding for two natural rewards, versus a natural reward and intravenous cocaine. These experiments revealed that, unlike our prior electrophysiology work, rapid DA release in the NAc was not reinforcer specific during performance of a sucrose/cocaine multiple schedule. In the second set of experiments, we used the same data set obtained from Aim 1 to compare basic shifts in pH in discrete locations in the NAc core and determine if aspects of this signaling differed during each phase of the sucrose/cocaine multiple schedule. Our findings revealed that although increases in pH were observed under both reinforcer conditions, the dynamics of this signaling were significantly different when animals responded for intravenous cocaine versus the natural reward, sucrose. The final set of experiments examined the effects of one month of cocaine abstinence on DA release and uptake dynamics in the NAc core. We found that a month of cocaine abstinence potentiated the peak concentration of electrically evoked DA in the NAc following an acute injection of cocaine. Taken together, the results of these studies indicate that DA signaling in the NAc is highly dynamic and can be influenced by many factors, including the type of reinforcer (natural or drug) being self-administered or the pattern of drug exposure (1 day versus 30 days of abstinence). Furthermore, rapid DA signaling does not interact with NAc cell firing in a simple manner, but instead differentially modulates neuronal activity depending on many factors including reward type, specific afferent-efferent projections, and ongoing behavior.

To my parents

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PREFACE

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

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AUC	area under the curve
BLA	basolateral amygdala
CR	conditioned response
CS	conditioned stimulus
CV	cyclic voltammogram
DA	dopamine
$[DA]_{max}$	peak evoked concentration of dopamine
FR	fixed ratio
FSCV	fast-scan cyclic voltammetry
MSN	medium spiny neuron
NAc	nucleus accumbens
OFC	orbitofrontal cortex
PFC	prefrontal cortex
sem	standard error of the mean
$t_{1/2}$	decay time between peak and half-maximal amplitude
US	unconditioned stimulus
VTA	ventral tegmental area

CHAPTER 1: GENERAL INTRODUCTION

Addiction: A disorder of reward processing

Drug addiction is a complex disorder that results in a multitude of physical, psychological, and interpersonal problems for addicted individuals, as well as wide-ranging societal harms. Despite the complexity of addiction, it is almost universally agreed that this disease is defined by a loss of control over drug-taking and drug-seeking behaviors and the persistence of these behaviors despite their negative consequences (American Psychiatric Association, 1994). Thus, addiction differs in important ways from casual drug use, and the escalation from initial use, to abuse, to addiction produces neuroadaptations that are fundamentally different from those associated with casual use (Sinha, 2013). A better understanding of these addiction-related neuroadaptations is critical for the prevention and treatment of addiction.

While many different substances can lead to addiction, cocaine abuse in particular has become a significant worldwide health problem. Cocaine is the second most commonly used illicit substance worldwide (UNODC, 2011). Of those individuals who use cocaine, 5% will develop a substance dependence disorder within the first year of use, and 20% of these individuals will become dependent on cocaine long-term (Wagner & Anthony, 2002). This difficulty in remaining drug-free is typical of cocaine addiction. Cocaine-taking behavior is usually characterized by a cycle of bingeing, abstinence, craving, and finally relapse (Gawin,

1991). The propensity to relapse, even after extended periods of abstinence, contributes to the difficulty in treating cocaine addiction.

Cocaine is highly rewarding, and the biological mechanisms underlying cocaine reinforcement have been extensively studied. The primary pharmacological action of cocaine in the brain is blockade of the dopamine, norepinephrine, and serotonin transporters which remove their respective neurotransmitters from the synapse after they have been released (Heikkila *et al.*, 1975; White, 1998). Thus, cocaine increases the levels of dopamine, norepinephrine, and serotonin in the synapse through reuptake inhibition. It has long been hypothesized that the rewarding effects of cocaine are primarily mediated by increases in dopamine neurotransmission (De Wit & Wise, 1977; Roberts *et al.*, 1977).

However, organisms likely did not evolve to process information about drugs such as cocaine. Instead, drugs of abuse likely act on a brain reward circuit that normally processes biologically relevant rewards such as food, water, or sex. Repeated drug use can lead to the dysregulation of reward processes that are highly adaptive under most other circumstances. Therefore, drug addiction can be thought of as a disorder of reward processing. Given the concept of addiction as aberrant reward learning, a clearer understanding of how organisms learn about rewards under normal circumstances can improve our understanding of addiction.

Associative reward learning

Learning about rewards and appropriately directing behavior to obtain them is critical for survival. Animals must associate cues in their environment with the availability of rewards in order to increase their chances of finding rewards in the future. They must also perform the behaviors that are most likely to result in acquisition of a reward. To accomplish this, animals

have evolved associative learning mechanisms. Associative learning is typically divided into two categories: stimulus-outcome and action-outcome.

In stimulus-outcome learning (also called Pavlovian or classical conditioning), an organism learns to associate a previously neutral stimulus (the conditioned stimulus, CS) with a biologically salient outcome (the unconditioned stimulus, US). With multiple CS-US pairings, the CS comes to gain biological salience and can influence behavior in the absence of the US (Pavlov, 1927). During stimulus-outcome learning, presentation of stimuli does not depend on the behavioral actions of the organism; however, Pavlovian stimuli can come to exert control over behavior. This is often adaptive, such as when an animal learns to associate a particular sound with food delivery and subsequent presentation of the sound elicits approach to the area where food was delivered. Pavlovian cue-outcome associations can also become maladaptive. For example, when drug-related stimuli gain salience through conditioning, they can become powerful motivators and overtake the influence of other environmental stimuli, thus driving maladaptive seeking of drug rewards (Robinson & Berridge, 1993; Berridge & Robinson, 1998).

The second type of associative learning is action-outcome learning (also called operant or instrumental conditioning) in which an organism learns to associate a behavioral response with a biologically salient outcome (Thorndike, 1933; Skinner, 1938). This association then results in an increase or decrease in the behavioral response depending on whether the outcome was appetitive or aversive. An outcome that increases the rate of behavioral responding is termed a reinforcer. Action-outcome learning is a *goal-directed* behavior because it is dependent on the consequences of a behavior (the outcome). Thus, devaluation of the outcome causes a decrease in the behavioral response (Adams & Dickinson, 1981; Rescorla, 1992). Like stimulus-outcome

learning, action-outcome learning is typically adaptive but can become maladaptive under certain conditions such as addiction (Belin *et al.*, 2013).

Associative learning processes can be influenced by many factors, but of particular interest for this dissertation is the role of reward type (i.e., natural reinforcers such as food or water versus drug reinforcers such as cocaine). Both natural and drug rewards are reinforcing as they support robust operant behavior. However, drugs rewards differ from natural rewards in important ways. Because drugs do not satisfy any inherent physiological need such as hunger or thirst, they must exert their reinforcing properties through some other means. This has led to the hypothesis that drugs "tap into" a brain reward circuit that evolved to process information about natural reinforcers (Wise, 1997). However, it is also possible that the brain processes natural and drug rewards in fundamentally different ways. While we are far from a consensus on this issue, it is clear that the processing of natural versus drug rewards is intimately linked.

Human cocaine addicts often report anhedonia, dysphoria, and an inability to perceive of anything other than cocaine as potentially pleasurable (Gawin & Kleber, 1986). Additionally, cocaine addicts going through withdrawal have a reduction in striatal dopamine D_2 receptor availability as well as a reduction in dopamine release in the striatum (Volkow, 1993; Volkow *et al.*, 1997; Volkow *et al.*, 1999). It is hypothesized that this hypodopaminergic activity could result in decreased activation of reward circuits by natural reinforcers, causing natural rewards to pale in comparison to drug rewards (Volkow *et al.*, 1999; Volkow *et al.*, 2010). In rats, cocaine experience can alter the hedonic value of a natural reinforcer that predicts access to drug self-administration causing a shift from appetitive to aversive responding during infusion of a sweet tastant (Wheeler *et al.*, 2008; Wheeler *et al.*, 2011). These findings suggest that we can accurately model specific aspects of cocaine addiction in animals, including the devaluation of

natural rewards. The importance of a valid animal model for studying cocaine abuse is discussed in detail below.

Animal model of cocaine-taking: drug self-administration and abstinence

Complex human disorders such as addiction can never be fully replicated in an animal model; however, these models provide an invaluable tool for studying specific aspects of the addiction cycle. As described above, cocaine addiction in humans is characterized by a cycle of drug use, abstinence, and relapse (Gawin, 1991). Perhaps the most valid model for assessing the first phase of this cycle, drug-taking, is operant self-administration (Weeks, 1962; Deneau *et al.*, 1969). This behavioral paradigm allows animals to voluntarily consume drugs or other reinforcing substances, such as food or sucrose, thus eliminating the confound of experimenter-induced substance dependence. Furthermore, operant responding is goal-directed, giving a behavioral readout of an animal's motivation to work for a particular outcome. The self-administration model is well-suited for investigating the processing of natural rewards versus drug rewards. In this laboratory, we have used a multiple schedule self-administration design in which rats respond for a natural reinforcer (e.g., sucrose) versus intravenous cocaine, allowing us to directly compare neural responses, such as cell-firing, across the two reinforcer types.

Self-administration can also be used to examine drug relapse. In the classic reinstatement model, drug self-administration is followed by extinction of the drug-reinforced response. Reinstatement of drug-seeking is then measured in response to a drug prime, presentation of drug-associated cues, or other manipulations (De Wit & Stewart, 1981). However, humans rarely undergo extinction before entering abstinence; therefore, reinstatement does not model this aspect of the addiction cycle. Considering that a major problem in treating cocaine addiction is the high propensity of addicts to relapse even after long periods of abstinence, modeling this

behavior could provide important insights into improving treatment. In fact, research with animal models has consistently shown that drug-seeking behavior (measured by operant responding in the absence of the primary reinforcer) progressively increases with the amount of abstinence from cocaine self-administration (Tran-Nguyen *et al.*, 1998; Grimm *et al.*, 2001; Lu *et al.*, 2004). Thus, this "incubation of craving" phenomenon appears to model the high rates of relapse observed in the human population. Finally, a number of studies (Toda *et al.*, 2002; Kalivas *et al.*, 2003; Lu *et al.*, 2003; Xi *et al.*, 2003) have shown that abstinence-induced changes in behavior are correlated with neuroadaptations in brain areas important for reward processing.

The nucleus accumbens is a key neural substrate of reward learning

As described above, reward learning is a complex process that involves the integration of a variety of external and internal stimuli. As such, this process is subserved by a distributed network of brain nuclei. However, the nucleus accumbens (NAc) is of particular interest because its central location within this network means it is uniquely situated to integrate salient reward-related information and select an appropriate behavioral response. This section will briefly describe the basic structure of the NAc and its afferent/efferent projections before reviewing evidence supporting its role in reward learning.

Nucleus accumbens structure and connectivity

The NAc is largely (~ 95%) composed of inhibitory GABAergic medium spiny neurons (MSNs) (Groves, 1983). MSNs are characterized by a large dentritic arbor that is densely covered in spines (Gerfen, 1988). These spines serve as the site of synapses with other neurons, thus greatly increasing the area and number of available synaptic connections onto MSNs. MSNs are projection neurons, sending axons out of the NAc to downstream structures (Groves, 1983). The remaining 5% of cells are local interneurons that do not project out of the NAc. This

population is composed of both cholinergic and GABAergic interneurons (Gerfen, 1988). Cholinergic interneurons synapse onto MSNs and other cholinergic interneurons (Aosaki *et al.*, 1994; Graybiel *et al.*, 1994; Morris *et al.*, 2004) , while GABAergic interneurons form inhibitory synapses onto MSNs (Kreitzer & Malenka, 2008). The connectivity of interneurons allows for precise regulation of discrete subsets of neurons in the NAc.

The NAc receives convergent glutamatergic input from limbic areas including the prefrontal (PFC) cortex, orbitofrontal cortex (OFC), hippocampus, and basolateral amygdala (BLA) (Zahm & Brog, 1992; Brog *et al.*, 1993). It also receives a dense dopaminergic (DA) input projection from the ventral tegmental area (VTA) (Zahm & Brog, 1992; Brog *et al.*, 1993). In turn, the NAc sends efferent projections to motor areas including the ventral pallidum and lateral hypothalamus (Zahm, 1999). This anatomical organization supports the classical view of the NAc as a limbic-motor integrator that translates incoming reward-related information into motivated behavior through its connections to motor regions (Mogenson *et al.*, 1980).

However, it is unlikely that the NAc as a whole sends a single integrated output to its target structures in order to initiate behavior. Theories of basal ganglia function suggest that the NAc is embedded in a larger system that is organized into several structurally and functionally discrete circuits that are essentially parallel in nature (Alexander *et al.*, 1986; Alexander & Crutcher, 1990). Additionally, it was proposed that the NAc is composed of a collection of functionally heterogeneous "neuronal ensembles" that are characterized by distinct afferent/efferent projections (Pennartz *et al.*, 1994). Within this framework, unique sets of limbic inputs converge on specific ensembles of NAc neurons that then generate output to a particular set of target structures, inducing behavioral effects that are specifically linked with each ensemble.

The role of the NAc in goal-directed behavior

Given the functional connectivity of the NAc as a corticolimbic motor integrator described above, many studies have targeted this brain region to better understand its particular role in reward learning. Pharmacological and lesion studies have repeatedly demonstrated that the NAc plays a direct role in both the appetitive and consummatory phases of goal-directed behaviors (Stratford, 1997; Stratford & Kelley, 1997; Berridge & Robinson, 1998; Kelley, 2004). Furthermore, lesions to the NAc or blockade of dopamine within the NAc disrupt the acquisition and expression of Pavlovian conditioned responses (Di Ciano *et al.*, 2001; Di Chiara, 2002; Dalley *et al.*, 2005), impair the acquisition and expression of learned associations (Balleine, 1994; Di Ciano & Everitt, 2001; Cardinal, 2002; Dalley, 2002; Schoenbaum & Setlow, 2003; Dalley *et al.*, 2005), and alter behavioral responses to reward-paired cues (Nicola *et al.*, 2005). Thus, the NAc is critical for both operant and Pavlovian aspects of reward-related learning.

Additionally, the NAc plays a critical role in the acquisition and persistence of drug-seeking behaviors. Drugs of abuse such as cocaine act not only as primary reinforcers, maintaining instrumental responses to acquire the drug, but can also support the formation of Pavlovian associations with environmental cues predictive of drug availability. These cues can then act as conditioned stimuli (CSs), eliciting Pavlovian approach behaviors. Under other circumstances, these drug-associated cues can become conditioned reinforcers and support instrumental responding even in the absence of the primary reinforcer. Both Pavlovian approach behavior and conditioned reinforcement are forms of drug-seeking, and the NAc is critical for the acquisition of both (Parkinson *et al.*, 1999; Everitt & Robbins, 2000; Di Ciano *et al.*, 2001; Di Ciano & Everitt, 2004a). The NAc is also implicated in the resumption of drug-seeking following extinction of cocaine self-administration (McFarland & Kalivas, 2001; Fuchs *et al.*,

2004; McFarland *et al.*, 2004; Peters & Kalivas, 2006; Bäckström & Hyytiä, 2007; Fuchs *et al.*, 2008). Finally, the NAc likely mediates many of the motivational changes associated with cocaine abstinence, including the persistence in drug-seeking seen following extended abstinence (Kalivas, 2009). Overall, it is clear that the NAc is necessary for normal reward processing and may very well be a critical brain region in the development of aberrant reward processing that characterizes drug addiction.

NAc neurons encode aspects of motivated behavior: Evidence from electrophysiological studies

In vivo electrophysiological methods provide a unique perspective of neural function because they illuminate the precise correlation of neuronal activity and behavioral events. These methods have been employed to study the cellular mechanisms underlying natural reward and drug-seeking behaviors. Studies have repeatedly demonstrated that NAc neurons exhibit patterned changes in activity (excitations and inhibitions) before, during, and after operant responses for natural or drug rewards (Carelli & Deadwyler, 1994; Pennartz *et al.*, 1994; Nicola & Deadwyler, 2000; Carelli, 2002; Nicola *et al.*, 2004). This patterned firing activity is not limited to operant responding for reinforcers. NAc neurons also exhibit distinct patterned changes in firing rate to Pavlovian conditioned cues that that predict sucrose delivery (Roitman *et al.*, 2005) or that predict sucrose availability and elicit approach behaviors (Day *et al.*, 2006). However, cue-evoked activation of NAc neurons is not unique to sucrose or natural rewards. Several studies demonstrate that NAc neurons are also activated by stimuli associated with the intravenous delivery of cocaine (Carelli, 2000; Hollander & Carelli, 2007).

Importantly, studies from this laboratory have repeatedly shown that NAc neurons exhibit differential, nonoverlapping firing patterns during operant responding for natural rewards (food, water, or sucrose) versus cocaine (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski,

2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). Thus, in contradiction to the notion that abused substances 'tap into' a natural reward circuit, these findings suggest that goal-directed behaviors for natural or drug rewards are largely guided by separate neural circuits in the NAc. Furthermore, only neurons that encode goal-directed behaviors for cocaine are activated by cocaine-associated stimuli (Carelli & Ijames, 2001). Additionally, distinct populations of NAc cells are differentially activated during intra-oral infusion of rewarding (sucrose) versus aversive (quinine) tastants (Roitman *et al.*, 2005). Together these findings suggest that the NAc is composed of distinct microcircuits that include groups of cells with different functional properties.

However, the encoding of goal-directed behaviors by the NAc is highly dynamic and is influenced not only by the type of reinforcer, but also the pattern of drug exposure (Hollander & Carelli, 2005; Hollander & Carelli, 2007). As described previously, cocaine self-administration followed by a period of drug abstinence leads to an increase in behavioral responding for the drug as well as drug-associated stimuli, termed 'incubation of craving' (Tran-Nguyen *et al.*, 1998; Grimm *et al.*, 2001; Lu *et al.*, 2004). Work from this laboratory has demonstrated a neurophysiological correlate of this behavioral phenomenon. Following one month of cocaine abstinence, the percentage of NAc core (but not shell) neurons encoding cocaine-seeking behaviors dramatically increases (2 fold) during resumption of cocaine self-administration (Hollander & Carelli, 2005). Subsequent studies also showed that the percentage of NAc core (not shell) neurons that encode information about cocaine-associated stimuli increases following abstinence, and neurons are more activated during extinction responding (Hollander & Carelli, 2007). Finally, we have shown that the nonoverlapping pattern of NAc cell-firing for a natural versus a drug reinforcer is largely maintained even after one month of cocaine abstinence;

however, the percentage of cells that selectively encode cocaine-related information is increased (Cameron & Carelli, 2012). Together these studies suggest that the increased encoding of cocaine-seeking behaviors in the NAc following abstinence may perturb the normal balance between encoding of natural and drug reinforcers, perhaps representing a neural correlate of the transition to drug dependence.

Rapid dopamine signaling in the nucleus accumbens

The mesolimbic DA projection from the ventral tegmental area (VTA) to the NAc has been extensively studied as a neural substrate of reward, but the specific function of DA transmission within the NAc remains controversial. A common feature of natural rewards as well as many abused substances, including cocaine, is to increase mesolimbic DA (Di Chiara & Imperato, 1988), and a traditional hypothesis posits that NAc DA mediates the hedonic impact of food and drug rewards (Wise & Bozarth, 1982; Wise & Rompre, 1989). However, numerous findings challenge the validity of this hypothesis. NAc DA levels are increased during operant responding to avoid an aversive stimulus (Salamone, 1994), and depletion or antagonism of DA or NAc activity impairs behavioral performance on tasks motivated by aversion rather than reward (Salamone, 1994; Di Chiara, 2002; Huang & Hsiao, 2002; Schoenbaum & Setlow, 2003). The importance of mesolimbic DA in learning about aversive events clearly demonstrates that DA does not mediate the pleasurable hedonic aspects of natural or drug reinforcers as proposed in the original "anhedonia" hypothesis.

More recent theories have posited that the mesolimbic DA system provides a signal that facilitates learning about rewards. One of the most influential of these theories has come from electrophysiological recordings of midbrain dopamine neurons. A majority of these neurons

exhibit brief increases in activity when rewards are delivered unexpectedly (Mirenowicz & Schultz, 1994; Hollerman & Schultz, 1998; Pan *et al.*, 2005). However, when a cue comes to fully predict reward delivery, DA neurons exhibit transient increases in firing rate during the presentation of the cue rather than during delivery and consumption of the reward (Schultz *et al.*, 1997). Thus in direct contradiction to the "anhedonia" hypothesis, midbrain DA neurons do not fire upon receipt of reinforcer, but rather during presentation of a cue that predicts its arrival. The current hypothesis based on these observations proposes that DA neurons provide a "prediction error" signal. According to this hypothesis, as conditioned stimuli become valid reward predictors, reward delivery does not constitute a violation of expectancy and therefore does not produce phasic dopamine cell firing as an unexpected reward would.

Others suggest that NAc DA mediates the attribution of incentive salience to conditioned cues, facilitating behavioral responses to environmental cues that have come to possess motivational significance through their association with primary reinforcers (Robinson & Berridge, 1993; Berridge & Robinson, 1998). In support of this hypothesis, studies have demonstrated that NAc DA is required for the execution of Pavlovian approach behaviors and for conditioned cues to excite operant responding (Pavlovian-to-Instrumental Transfer) (Everitt *et al.*, 1999; Di Ciano *et al.*, 2001; Everitt *et al.*, 2001; Parkinson *et al.*, 2002; Robbins & Everitt, 2002; Di Ciano & Everitt, 2004b). Furthermore, increasing DA in the NAc potentiates responding for conditioned reinforcement (Wyvell & Berridge, 2000; Wyvell & Berridge, 2001). According to this theory, the predictive value of a CS is not sufficient to engage the mesolimbic DA system; the CS must also be attributed with incentive salience. In support of this idea, it has recently been reported that NAc DA release is higher in rats that have attributed incentive salience to a reward-predictive cue compared to those that have not, and intact dopamine

transmission is only required for learning about CSs if they have been attributed with incentive salience (Flagel *et al.*, 2011). This theory of DA function may have particular relevance to the type of aberrant learning that takes place in addiction. Thus, the normal attribution of incentive salience is adaptive under most circumstances, but because almost all abused drugs increase mesolimbic DA, they also increase the salience attributed to drug-related stimuli. Eventually these stimuli become powerful motivators, overtaking the influence of other environmental stimuli and driving maladaptive seeking of drug rewards

Dopamine acts as a neuromodulator on nucleus accumbens cell firing

As discussed in detail above, MSNs in the NAc receive glutamatergic input from cortical and limbic regions. Dopamine neurons projecting from the VTA synapse onto the necks and spines of MSNs and are located adjacent to glutamatergic synapses (Voorn *et al.*, 1986; Groves *et al.*, 1994). This synaptic organization suggests a critical role for DA in modulating glutamatergic input from other brain regions. Studies have shown that DA release does not have direct excitatory or inhibitory actions on MSNs, but instead acts as a neuromodulator, differentially influencing the activity of glutamatergic afferents rather than exerting global actions across all NAc neurons (O'Donnell *et al.*, 1999; Nicola *et al.*, 2000).

Several properties of NAc MSNs also support the role of DA as a neuromodulator. First, a vast majority of MSNs in the NAc express only one subtype of DA receptor (Bertran-Gonzalez *et al.*, 2008). DA receptors are G-protein coupled receptors and are classified as either "D_1-like" (D_1 and D_5) or "D_2-like" (D_2, D_3, and D_4) (Kebabian & Calne, 1979). D_1 and D_2 receptors are coupled to different G-proteins and result in divergent intracellular signaling cascades (Girault & Greengard, 2004; Snyder, 2011). Importantly, in the NAc core 53% of MSNs express D_1 receptors exclusively, 41% exclusively express D_2 receptors, and only an estimated 6% have both

D_1 and D_2 receptors (Valjent *et al.*, 2009). This organization of DA receptors on MSNs allows DA release in the NAc to function in a highly dynamic manner.

MSNs are also unique because they exhibit a bistable membrane potential. In the "down state" the resting membrane potential is approximately -77mV, while in the "up state" the resting membrane potential is approximately -54mV (O'Donnell & Grace, 1993; Wilson & Kawaguchi, 1996). Activation of D_1 receptors maintains MSNs in their up state, thus increasing the probability that they will fire an action potential. In contrast, activation of D_2 receptors on MSNs tends to inhibit cell firing (Belle *et al.*, 2013). Thus, DA release in the NAc interacts with postsynaptic MSNs in a complicated manner to best promote appropriate behavior.

Fast-scan cyclic voltammetry provides an ideal technique for measuring rapid chemical signaling in the NAc

Electrochemical techniques combine chemical selectivity with high temporal resolution to measure chemical compounds, including DA, in the brain. These techniques include constant-potential amperometry, high-speed chronoamperometry, and fast-scan cyclic voltammetry (FSCV) (Kawagoe & Wightman, 1994; Michael & Wightman, 1999; Gerhardt & Hoffman, 2001). Among these techniques, FSCV provides the best combination of temporal and chemical sensitivity for measuring rapid changes in extracellular DA (Phillips *et al.*, 2003a).

To evaluate changes in analytes of interest, FSCV measures the current generated by the oxidation of an analyte. This oxidation is caused by a potential waveform applied to a carbon-fiber microelectrode. For analyte identification, current during a voltammetric scan is plotted against the applied potential to yield a cyclic voltammogram (CV). Analytes such as DA oxidize and reduce at predictable potentials, thus their CVs have a characteristic shape that is easy to recognize.

The temporal resolution of FSCV (~100 msec) makes it ideal for recording rapid changes in DA timelocked to behavior in freely moving animals. Additionally, FSCV allows analytes to be easily distinguished from ionic changes (such as pH). These ionic changes can also be separated from other analytes using principal component regression (Heien *et al.*, 2004; Keithley *et al.*, 2010). Therefore, DA and pH signals can be isolated and analyzed separately.

Finally, a particular advantage of FSCV is the ability to probe the kinetics involved in rapid DA signaling in an intact brain circuit. The concentration of DA available in the synapse is determined by two opposing processes: release and uptake. Because of the temporal resolution available with FSCV, these two processes can be distinguished and their relative influences on neurotransmission determined (Wightman *et al.*, 1988; Wightman & Zimmerman, 1990).

Goals of the dissertation

Given the background material outlined above, the primary goals of this work are to examine the functional contribution of rapid DA release in the NAc to goal-directed behaviors for both natural and drug rewards. Studies from this laboratory have repeatedly shown that NAc neurons exhibit differential, nonoverlapping firing patterns during operant responding for natural rewards (food, water, or sucrose) versus cocaine (Carelli *et al.*, 2000; Cardinal, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). Furthermore, following one month of cocaine abstinence, the percentage of NAc core neurons encoding cocaine-seeking behaviors is dramatically increased (Hollander & Carelli, 2005). While rapid DA release and pH shifts in the NAc have been observed on a timescale similar to cell-firing during operant responding for drug and natural rewards (Phillips *et al.*, 2003b; Roitman *et al.*, 2004), it is not known whether this chemical signaling is modulated in a similar manner to NAc phasic cell activity. Therefore, the studies described in this dissertation used FSCV in the NAc to

investigate the role of rapid DA signaling and pH shifts in responding for natural and drug rewards, as well as the influence of cocaine abstinence on DA release and uptake dynamics.

Specific Aims:

1. To examine whether rapid DA release in the NAc core is reinforcer specific during performance of a sucrose/cocaine or sucrose/food multiple schedule. As previously described, the NAc is a key brain area underlying the processing of both natural and drug rewards. Electrophysiological studies in behaving animals have demonstrated that NAc neurons encode information about goal-directed actions for both natural and drug rewards as well as cues associated with those rewards (Carelli & Deadwyler, 1994; Carelli, 2000; Nicola & Deadwyler, 2000; Carelli, 2002; Nicola *et al.*, 2004; Peoples *et al.*, 2004; Roitman *et al.*, 2005; Day *et al.*, 2006; Hollander & Carelli, 2007). Importantly, studies from this laboratory have repeatedly shown that NAc neurons exhibit differential, nonoverlapping firing patterns during operant responding for natural rewards (food, water, or sucrose) versus cocaine (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). In contrast, two natural reinforcers (food versus water) activate largely the same population of neurons in the NAc (Carelli *et al.*, 2000). This pattern holds true even when one of the natural reinforcers is highly palatable sucrose (Roop *et al.*, 2002). These findings indicate that the NAc is composed of distinct neural circuits that process information about natural reinforcers largely separate from information about cocaine.

Additionally, work from this lab using FSCV has shown that rapid DA release is observed in the NAc during goal-directed responding for both cocaine and natural rewards on a timescale similar to NAc phasic cell firing (Phillips *et al.*, 2003b; Roitman *et al.*, 2004). However, it is not known whether rapid DA release is reinforcer specific within discrete

locations in the NAc in a manner similar to the pattern of NAc cell firing observed in our previous studies. Therefore, in this study we will use FSCV to record rapid DA release in the core of the NAc during performance of a sucrose/cocaine multiple schedule. This design will allow us the record DA release in a single NAc location during responding for both reinforcers during the same behavioral session. As a control, we will record rapid DA release in another group of animals as they perform a sucrose/food multiple schedule. This will allow us to compare the pattern of NAc DA release seen during responding for two natural reinforcers to that seen during responding for one natural and one drug reinforcer.

2. To investigate basic pH shifts in the NAc core associated with goal-directed responding for a natural (sucrose) versus drug (cocaine) reinforcer. While FSCV studies examining DA release have provided unprecedented insight into the role of this neurotransmitter in reward processing and addiction, electrochemical recordings with carbon-fiber microelectrodes also allow for the detection of pH with similar (subsecond) temporal resolution (Venton *et al.*, 2003; Takmakov *et al.*, 2010). In fact, fluctuations in pH have been observed relative to electrical stimulation ((Venton *et al.*, 2003; Cheer *et al.*, 2006) as well as reward-related events in awake animals (Ariansen *et al.*, 2012). These shifts in pH are delayed several seconds from the immediate release and reuptake of DA (Venton *et al.*, 2003). Thus, examining changes in pH related to goal-directed actions offers a way to probe dynamic changes in neural activity that occur in combination with changes in DA.

While pH shifts have been observed during behavioral tasks involving either natural or drug rewards (Phillips *et al.*, 2003b; Roitman *et al.*, 2004; Ariansen *et al.*, 2012), a direct comparison in pH dynamics across the two reinforcer conditions has not yet been made. This

study will compare basic shifts in pH in discrete locations in the NAc core and determine if aspects of this signaling differs during each phase of a sucrose/cocaine multiple schedule.

3. To determine whether abstinence from cocaine self-administration alters DA release and uptake dynamics in the NAc core. Neurochemical changes caused by abstinence from cocaine self-administration can be measured in a variety of ways. A particular advantage of FSCV is the ability to probe the kinetics involved in rapid DA signaling in an intact brain circuit. The concentration of DA available in the synapse is determined by two opposing processes: release and uptake. Because of the temporal resolution available with FSCV, these two processes can be distinguished (Wightman *et al.*, 1988; Wightman & Zimmerman, 1990). Furthermore, the actions of drugs such as cocaine on release and uptake can be measured (Jones *et al.*, 1995). Previous work using FSCV in anaesthetized rats has shown that even a short-term withdrawal paradigm (7 consecutive days of cocaine injections followed by I day of withdrawal) can cause a potentiation of DA signaling in the NAc in response to a subsequent cocaine challenge (Addy *et al.*, 2010). This sensitized DA response appears to be primarily a result of an increase in the potency of cocaine's uptake inhibiting actions in pretreated animals (Addy *et al.*, 2010). It is possible that larger changes in DA signaling may be observed following longer periods of abstinence. Therefore, in this study we will expand on these earlier findings by using FSCV to examine rapid DA release in the NAC core of anaesthetized rats that have undergone 14 days of cocaine self-administration followed by 1 or 30 days of abstinence

CHAPTER 2: RAPID DOPAMINE RELEASE ENCODES GOAL-DIRECTED BEHAVIORS FOR BOTH NATURAL AND COCAINE REWARDS WITHIN THE SAME NUCLEUS ACCUMBENS LOCATION

Introduction

Learning about rewards and appropriately directing behavior to obtain them is critical for survival. These processes are subserved by a distributed network of brain nuclei including the NAc and its dopaminergic input. The NAc receives convergent glutamatergic afferents from limbic areas including the prefrontal cortex, hippocampus, and basolateral amygdala (Zahm & Brog, 1992; Brog *et al.*, 1993) and impacts behavior through its projections to motor-related regions such as the ventral pallidum (Zahm, 1999). Further, dopamine is considered to function as a neuromodulator within the NAc, influencing the activity of the glutamatergic afferents onto NAc neurons that ultimately alters output to motor structures (O'Donnell *et al.*, 1999; Nicola *et al.*, 2000). This connectivity supports the classic view of the NAc as a "limbic-motor integrator" that translates motivation into goal-directed actions (Mogenson *et al.*, 1980)

In vivo electrophysiology studies have repeatedly provided evidence that NAc neurons encode goal-directed behaviors for both natural and drug rewards. That is, NAc neurons exhibit phasic changes in activity (excitations and/or inhibitions in firing rate) within seconds before, during, and after operant responses for natural as well as drug rewards (Carelli & Deadwyler, 1994; Chang *et al.*, 1998; Janak *et al.*, 1999; Carelli, 2002; Nicola *et al.*, 2004; Peoples *et al.*, 2004). In order to track the activity of the same NAc neurons across reinforcer conditions, studies from this lab employed multiple schedule designs. Those studies revealed that subsets of

NAc neurons exhibit largely differential, nonoverlapping firing patterns during operant responding for natural rewards (food, water, or sucrose) versus intravenous cocaine (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). In contrast, NAc neurons exhibit similar types of neuronal firing patterns during responding for two natural reinforcers (food versus water) (Carelli *et al.*, 2000). This pattern holds true even when one of the natural reinforcers is highly palatable sucrose (Roop *et al.*, 2002). Collectively, these findings support the contention that the NAc is comprised of discrete, functionally segregated 'microcircuits' that process particular types of reinforcement-related information to influence goal-directed actions (Alexander *et al.*, 1986; Pennartz *et al.*, 1994; Groenewegen *et al.*, 1996; Carelli & Wightman, 2004).

Importantly, studies have also shown that rapid (subsecond) DA release is observed during discrete periods of goal-directed actions for cocaine and natural rewards. Critically, these dynamic changes in NAc DA signaling occur on a timescale similar to NAc phasic cell firing (Phillips *et al.*, 2003b; Roitman *et al.*, 2004). However, it is not known whether rapid DA release is reinforcer specific within discrete locations in the NAc in a manner similar to the pattern of NAc cell firing observed in our previous electrophysiology studies (e.g, Carelli *et* al., 2000). To address this issue, we used fast scan cyclic voltammetry (FSCV) to measure rapid DA release in the NAc core during performance of two different tasks: a sucrose/cocaine or sucrose/food multiple schedule. This design allowed us to compare DA release dynamics in specific locations in the NAc during operant responding for two natural rewards, versus a natural reward and intravenous cocaine.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA; $n = 14$) aged 90-120 days and weighing 260–350 g were used as subjects and individually housed with a 12/12 h light/dark cycle. Body weights were maintained at no < 85% of pre-experimental levels by food restriction (10–15 g of Purina laboratory chow each day). Water was available *ad libitum*. This regimen was in place for the duration of the experiment, except during the post-operative recovery period when food was given *ad libitum*. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgery and Behavioral Training

All training was conducted in custom-made experimental chambers that consisted of a 43 × 43 × 53 cm Plexiglass chamber housed within a commercial sound-attenuated cubicle (Med Associates Inc., St Albans, VT, USA). One side of the chamber was equipped with two retractable levers (Coulbourn Instruments, Allentown, Pennsylvania) 17 cm apart, corresponding cue lights positioned 6 cm above each lever, and a reward receptacle located equidistantly between the levers.

Figure 2.1 shows the experimental timeline for each study. In the first experiment (sucrose/cocaine multiple schedule; Fig 2.1a), rats ($n = 8$) were surgically implanted with an intravenous catheter following established procedures, described in detail previously (Carelli *et al.*, 2000). Following recovery from surgery, rats were first trained to press one lever for sucrose (45 mg pellet) on a fixed-ratio 1 schedule of reinforcement. The start of the sucrose training

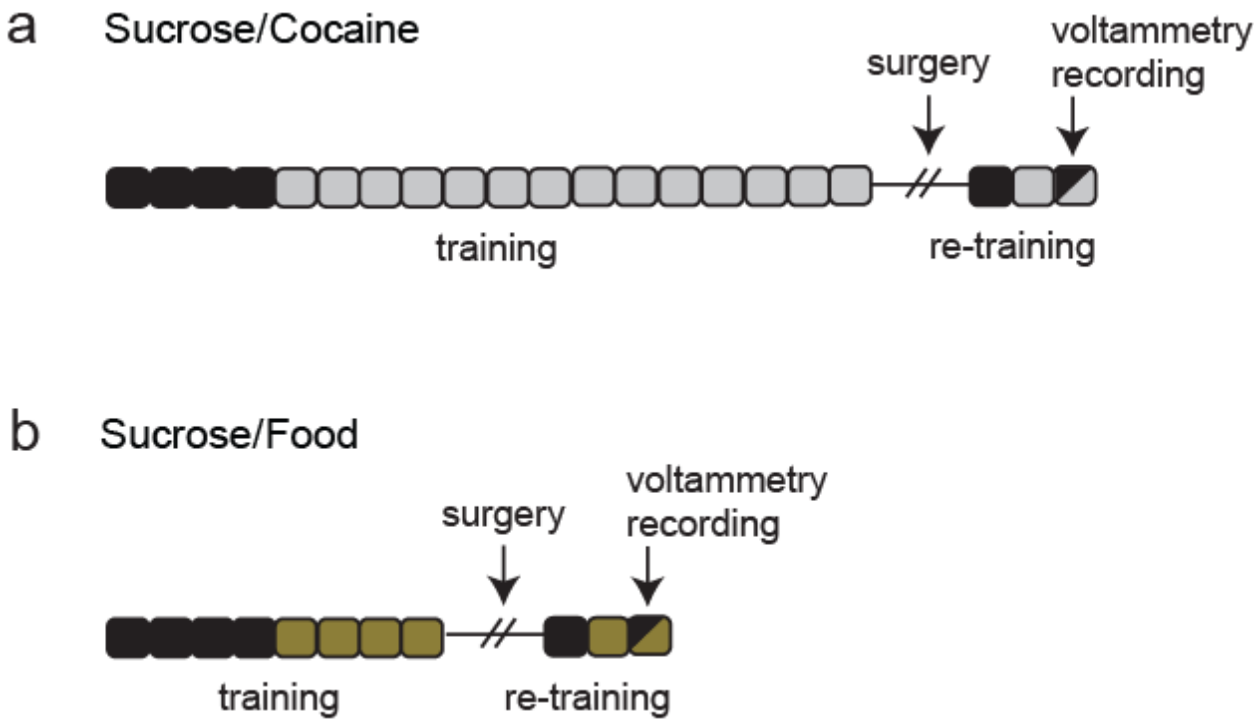

Figure 2.1. Experimental timeline. (a) Timeline for the sucrose/cocaine multiple schedule. Each box represents one day of behavioral training. Black boxes indicate days of sucrose self-administration. Gray boxes indicate days of cocaine self-administration. On the recording day, animals completed a multiple schedule for sucrose and cocaine. (b) Timeline for the sucrose/food multiple schedule. Each box represents one day of behavioral training. Black boxes indicate days of sucrose self-administration. Gold boxes indicate days of sucrose self-administration. On the recording day, animals completed a multiple schedule for sucrose and food.

session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. Lever depression resulted in delivery of a sucrose pellet to the reward receptacle, onset of a tone (65 dB, 2900 Hz, 20 s), and retraction of the lever (20 s). Rats underwent daily 30 min training sessions until they reached criterion (at least 50 presses per session). Rats were then trained to self-administer cocaine on a fixed-ratio 1 schedule of reinforcement during daily 2 h sessions. The start of the self-administration session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. The cocaine-associated lever was spatially distinct from the lever previously used

during sucrose training. Lever depression resulted in intravenous cocaine delivery (0.33 mg/infusion, approximately 1 mg/kg/infusion, 6 s) via a computer-controlled syringe pump, onset of a different tone (65 dB, 800 Hz, 20 s), and retraction of the lever (20 s). The tones and levers (left or right) associated with cocaine vs. sucrose were counterbalanced across animals.

Following 14 days of cocaine self-administration, rats were surgically prepared for voltammetric recording in the NAc core as previously described (Phillips *et al.*, 2003a). Animals were anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture (intramuscular) and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned dorsally to the NAc core (+1.3 mm anterior, -1.3mm lateral from bregma). An Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. The bipolar stimulating electrode (Plastics 1 Inc., Roanoake, VA) was placed dorsally to the VTA (-5.2 mm posterior, -1.0 mm lateral from bregma and -7 mm ventral from brain surface). Stainless steel skull screws and dental cement were used to secure all items. The bipolar stimulating electrode was lowered in 0.2 mm increments until physical responses to electrical stimulation diminished, indicative of proper electrode placement. The stimulating electrode was then fixed with dental cement.

After recovering from surgery, rats were retrained for two consecutive days (one session of sucrose self-administration followed the next day by one session of cocaine self-administration) while tethered to the headstage. This allowed the animals to habituate to the headstage and reestablish normal operant responding. Following retraining, rats underwent voltammetric recording (see below) during a multiple schedule of reinforcement for sucrose and cocaine. Specifically, rats had access to the sucrose-reinforced lever (fixed-ratio 1; 15 min)

followed by a 20 s time-out period (no lever extended; dark chamber) and extension of the second cocaine-reinforced lever (fixed-ratio 1; 2 h). Illumination of a cue light above each lever signaled the phase (sucrose or cocaine) of the multiple schedule. The order of reinforcer availability was varied across animals such that 4 animals self-administered sucrose followed by cocaine while 4 other animals self-administered cocaine followed by sucrose. Importantly, rats only received both reinforcers in the multiple schedule during the test day. Therefore, one reinforcer never came to predict access to the other.

In a second experiment (sucrose/food multiple schedule; Fig 2.1b), another set of rats (*n* = 6) were trained in a similar manner; however, food (45 mg pellet) was substituted for cocaine in the training and during the multiple schedule. Following acquisition of sucrose self-administration, these animals underwent at least two additional days of training on food self-administration. On test day, the rats performed a sucrose/food multiple schedule that consisted of two 15 minute periods of self-administration separated by a brief 20 s time-out. All other aspects of the multiple schedule (reward delivery, tone, lever retraction) were as described above. As in the sucrose/cocaine multiple schedule, the lever and tone associated with each reinforcer was counterbalanced across animals, and the order of reinforcer availability was varied across animals. Specifically, the order of reinforcer availability was varied such that 4 recordings were obtained in which sucrose was self-administered followed by food, and 5 recordings in which food was self-administered followed by sucrose.

Surgery and Behavioral Training

Changes in dopamine (DA) concentration during behavior were assessed using fast-scan cyclic voltammetry as previously described (Roitman *et al.*, 2004; Day *et al.*, 2007). On the day of the experiment, a detachable micromanipulator containing a carbon-fiber electrode (90-110

μm length) was inserted into the guide cannula and lowered into the NAc core. The carbon-fiber and Ag/AgCl reference electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Med-Associates, St. Albans, VT) at the top of the test chamber. Voltammetric recordings were made every 100 msec by applying a triangular waveform (-0.4 to +1.3 V, 400 V/sec). Data were digitized and stored to a computer using software written in LabVIEW (National Instruments, Austin, TX). Dopamine release within the NAc core was electrically evoked by stimulating the VTA (24 biphasic pulses, 60 Hz, 120 μA, 2 msec per phase) to ensure that carbon-fiber electrodes were in close proximity to dopamine release sites. The electrode position was optimized at a location with maximal dopamine release. To create a training set for principal component analysis for the detection of DA and pH changes during the behavioral session, additional stimulations at various parameters were performed (2-24 biphasic pulses, 20-60 Hz, 120 μA, 2 msec/phase). After the session, electrical stimulation was repeated to ensure that the site could still support DA release. A second computer and software system (Med Associates) controlled behavioral events and sent digital outputs for each event to the voltammetry recording computer to be time-stamped along with the electrochemical data. In some cases (two animals that performed the sucrose/food multiple schedule), a second or third recording session was completed on another day in which an electrode was lowered to a new location in the NAc core.

Data Analysis

All lever press events were recorded during performance of the multiple schedule. Number of lever presses as well as inter-response interval (INT) were calculated for each reinforcer during each behavioral session. Number of lever presses and INT were compared for the sucrose/cocaine and sucrose/food multiple schedules with a two-way mixed design ANOVA

(reinforcer type, within subjects factor x reinforcer order, between subjects factor). To eliminate any effect of an uneven number of responses for one reinforcer (sucrose, cocaine or food) over another, lever press responses were randomly selected to allow for an equal number of trials within a recording session. The number of random trials that were selected from each session were equivalent to the number of trials of the reinforcer for which the animal responded less. Thus, if an animal responded 40 times for sucrose and 20 times for cocaine during a sucrose/cocaine multiple schedule, we selected 20 trials each for sucrose and cocaine. This procedure was used for all analyses described below.

DA signals from FSCV were identified as previously described (Roitman *et al.*, 2004). For analyte identification, current during a voltammetric scan is plotted against applied potential to yield a cyclic voltammogram (the chemical signature of the analyte; see examples in Figures 2.2a,b and 2.4a,b insets). Cyclic voltammetric data were analyzed on stimulation trials before and after each experiment, and ±10 s relative to the important behavioral events (lever press). A background signal from 1 voltammetric scan (100 msec time bin) before a stimulation or behavioral trial was subtracted from the remainder of the scans to reveal changes in DA concentration (rather than absolute values).

DA concentration changes were then quantified using principal component regression (Heien *et al.*, 2004; Keithley *et al.*, 2010). Training sets were constructed from representative, background subtracted cyclic voltammograms for DA and pH. These training sets were then used to perform principal component regression on data collected during behavioral sessions. Principal components were selected such that at least 99% of the variance in the training set was accounted for by the model.

Changes in extracellular DA concentration during behavior were assessed by aligning DA concentration traces to lever press events. Data were averaged into 500 msec bins, and analyzed 10 s before to 10 s after lever press. Changes in NAc DA concentration from baseline in response to lever presses were evaluated independently for each reinforcer (sucrose, cocaine, or food) using a one-way repeated measures ANOVA with Newman-Keuls post hoc tests. This analysis compared the baseline bin (-10 to -9.5 s) to each subsequent 500 msec bin.

The pattern of rapid DA release during self-administration of sucrose versus cocaine (or sucrose versus food) during the multiple schedule was compared with a two-way repeated measures ANOVA (reinforcer x time) followed by Newman-Keuls post hoc tests of significant effects. For this analysis, data were averaged into 500 msec bins, and analyzed 10 s before to 10 s after lever press.

Next, we examined *peak* DA concentration elicited by operant responding for sucrose, cocaine, or food. Peak was defined as the highest DA concentration within a 3 s window surrounding a lever press (100 msec bins), and was determined for each sucrose, cocaine, or food trial within a behavioral recording session. These values were then averaged across trials for each animal to obtain the peak DA concentration for each reinforcer within a recording. Average peak DA concentrations across rats were then compared across reinforcer types (sucrose/cocaine or sucrose/food) using paired *t* tests. The influence of reinforcer order and reinforcer type on peak DA concentration was analyzed with a two-way mixed design ANOVA.

The time to reach peak DA concentration was also determined for each reinforcer within a behavioral session. For this analysis, DA concentration across all trials for a particular reinforcer within a recording session were averaged. Again, analysis was restricted to a 3 sec window surrounding the lever press (100 msec bins). The highest DA concentration in this

window was noted and the corresponding time relative to the lever press considered the "time to peak." For this analysis, time zero corresponded to lever press. Thus, negative values represent a peak that occurred prior to a response, while positive values represent a peak that occurred after a response. Time to peak was compared across reinforcer types using paired *t* tests. The influence of reinforcer order and reinforcer type on time to peak was analyzed with a two-way mixed design ANOVA.

For all analyses, the alpha level for significance was 0.05. All statistics were performed with commercially available software (Statistica, Tulsa, Oklahoma; GraphPad Software, La Jolla, CA).

Histology

On completion of each experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively). To mark the placement of electrode tips, a 209 µA current was passed through a stainless steel electrode for 10 s. Brains were removed and postfixed in 10% formalin. After post fixing and freezing, 50 µm coronal brain sections were taken and mounted throughout the rostral–caudal extent of the NAc. The specific position of individual electrodes was assessed by visual examination of successive coronal sections for electrolytic lesions.

Results

Behavioral responding during the multiple schedules

Behavioral data was obtained from 8 recordings (n = 8 rats) during performance of the sucrose/cocaine multiple schedule and from 9 recordings (n = 6 rats) during the sucrose/food multiple schedule. Independent of reinforcer order, during the sucrose portion of the sucrose/cocaine multiple schedule, rats completed an average of 39.75 ± 3.18 lever presses with an average inter-response interval (INT) of 30.14 ± 6.23 s. During the cocaine portion of the multiple schedule, rats completed an average of 23.00 ± 1.75 lever presses with an average INT of 5.40 ± 0.39 min. A two-way mixed design ANOVA with a within-subjects factor of reinforcer type and a between-subject factor of reinforcer order conducted on the number of lever presses revealed a main effect of reinforcer type ($F(1,6) = 37.935$, $p < 0.001$), but no main effect of reinforcer order ($F(1,6) = 0.072$, $p > 0.05$) and no reinforcer type x order interaction ($F(1,6) = 1.023$, $p > 0.05$). Further, a two-way mixed design ANOVA (reinforcer type x reinforcer order) conducted on INT revealed a main effect of reinforcer type ($F(1,6) = 166.023$, $p < 0.0001$), but no main effect of order ($F(1,6) = 0.005$, $p > 0.05$) and no reinforcer type x order interaction ($F(1,6) = 0.474$, $p > 0.05$). Thus, rats pressed more often and faster for sucrose than cocaine, and this pattern held regardless of order of reinforcer presentation in the multiple schedule. These data reflect typical self-administration patterns previously observed in this lab (e.g., Cameron & Carelli, 2012).

During performance of the sucrose portion of the sucrose/food multiple schedule, rats completed an average of 37.56 ± 3.47 lever presses with an average INT of 24.64 ± 1.57 s. During responding for food in the multiple schedule, rats completed an average of 43.22 ± 1.71 lever presses with an average INT of 21.81 ± 0.059 s. A two-way mixed design ANOVA

(reinforcer type x reinforcer order) conducted on number of lever presses revealed no main effect of reinforcer type ($F(1,7) = 2.221$, $p > 0.05$) or order ($F(1,7) = 0.002$, $p > 0.05$), and no reinforcer type x order interaction ($F(1,7) = 4.403$, $p > 0.05$). Further, a two-way mixed design ANOVA (reinforcer type x reinforcer order) conducted on INT revealed no main effect of reinforcer type ($F(1,7) = 2.780$, $p < 0.05$) or order ($F(1,7) = 1.389$, $p > 0.05$), but a significant reinforcer type x order interaction ($F(1,7) = 6.836$, $p > 0.05$). Newman-Keuls post hoc tests on the reinforcer type x order interaction revealed that rats had a slightly longer INT for sucrose when it was presented in phase 2 of the multiple schedule compared to when sucrose was presented in phase 1. Collectively, these data indicate that rats pressed a similar number of times for sucrose and food regardless of order of presentation, but order of presentation did influence the rate of responding for sucrose.

Dopamine release during performance of the sucrose/cocaine multiple schedule

To compare extracellular DA concentration during lever pressing for sucrose pellets versus intravenous infusions of cocaine (i.e., drug self-administration), DA concentration traces were aligned to the execution of lever press responses. Figure 2.2 (a,b) shows example cyclic voltammograms and color representations (colorplots) of a set of background-subtracted cyclic voltammograms and the corresponding DA concentration traces (c,d) for one animal that completed the cocaine/sucrose multiple schedule. In this example, cocaine was the reinforcer in phase 1 followed by sucrose reinforcement in phase 2 of the multiple schedule. In this session, DA release was observed during both phases of the multiple schedule. During cocaine self-administration (phase 1), a one-way repeated-measures ANOVA revealed that DA concentration during lever pressing for cocaine was significantly higher than during baseline ($F(39,1092) = 10.698$, $p < 0.0001$; Fig 2.2c). Newman-Keuls post hoc tests revealed a significant increase in

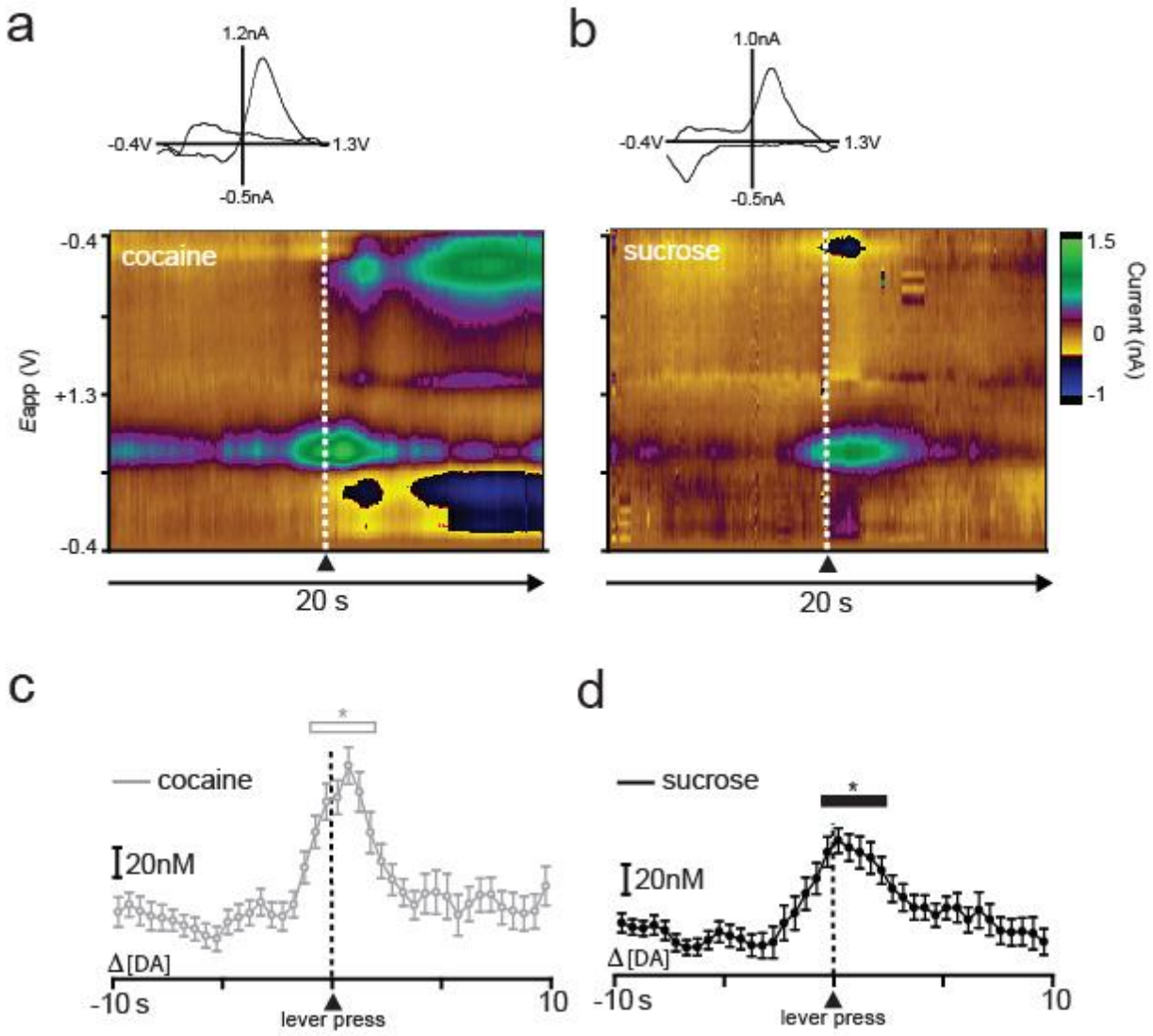

Figure 2.2. Example of dopamine release for one animal during performance of the sucrose/cocaine multiple schedule. This animal self-administered cocaine followed by sucrose. The rat completed 29 responses for cocaine and 48 responses for sucrose. 29 trials for each reinforcer were included in the analysis. Lever press is indicated by dotted line at time zero. (a,b) Two-dimensional color representation of cyclic voltammetric data collected for 20 s around lever pressing for cocaine (a) and lever pressing for sucrose (b). The ordinate is the applied voltage (*E*app) and the abscissa is time (s). Changes in current at the carbon-fiber electrode are indicated in color. Insets: cyclic voltammograms at the time of lever press. (c,d) Differential DA concentrations determined via principal component analysis for cocaine (c) and sucrose (d). Data are plotted in 500 msec bins (mean ± sem). Asterisks above open (c) or closed (d) bars indicate significant increases in DA concentration from baseline, $p < 0.05$.

DA release from baseline beginning 1 s before lever press and ending 2 s after the lever press ($p < 0.05$). Likewise, a one-way repeated measures ANOVA revealed that DA concentration was also increased from baseline during responding for sucrose ($F(39,1092) = 9.501$, $p < 0.0001$; Fig 2.2d). Newman-Keuls post hoc tests revealed significant increases in DA release events that began 0.5 s before the press and ended 2 s after response completion ($p < 0.05$). These findings are consistent with our previous electrochemistry reports showing increased DA concentrations relative to lever pressing behavior when animals responded for either sucrose (Roitman *et al.*, 2004) or cocaine (Phillips *et al.*, 2003b).

This pattern of increased DA release relative to responding for both types of reinforcers in the multiple schedule was observed across all animals. Separate one-way repeated measure ANOVAs were performed to examine when [DA] increased from baseline during responding for cocaine versus sucrose. These statistics revealed that DA concentration increased relative to baseline during both the cocaine ($F(39,273) = 5.152$, $P < 0.0001$) and sucrose ($F(39,273) = 8.864$, $P < 0.0001$) portions of the multiple schedule. Newman-Keuls post hoc tests revealed that for the cocaine portion of the multiple schedule DA concentration increased beginning at lever press, or time 0, and ending 1.5 s after response completion ($p < 0.05$). Likewise, during the sucrose portion of the multiple schedule, Newman-Keuls post hoc tests revealed that DA concentration was significantly elevated beginning 1 s before the response and returned to baseline levels 1.5 s after the lever press ($p < 0.05$).

In order to directly compare changes in DA signaling dynamics across reinforcer type relative to lever press, a two-way repeated measures ANOVA was conducted that examined reinforcer type (sucrose versus cocaine) and time on DA concentration (Fig 2.3). This statistic revealed a main effect of time ($F(39,273) = 10.995$, $p < 0.0001$), but no main effect of reinforcer

type ($F(1,7) = 3.185$, $p > 0.05$) and no reinforcer type x time interaction ($F(39,273) = 0.846$, $p > 0.05$). Thus, DA concentration increased from baseline during responding for both sucrose and cocaine, but there were no significant differences in [DA] between the two types of reinforcers.

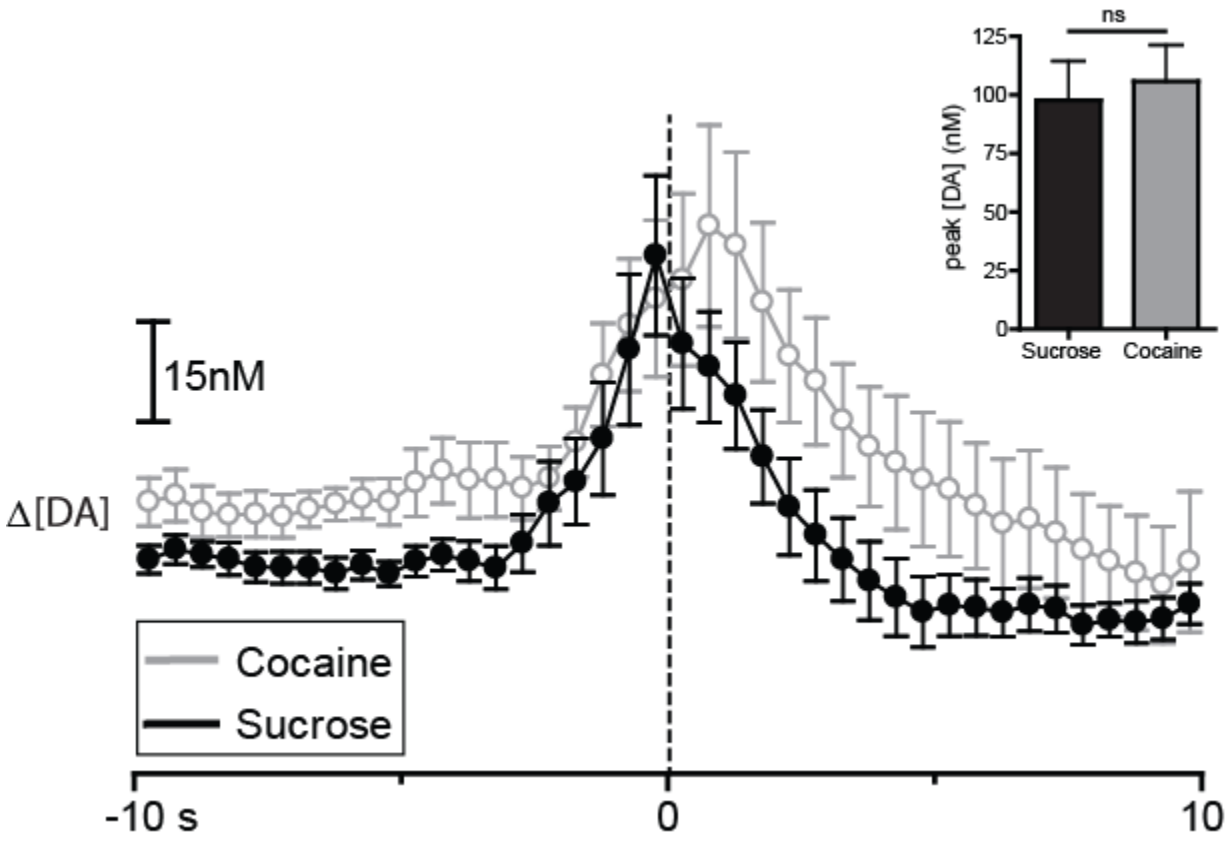

Figure 2.3. Sucrose and cocaine self-administration evoke rapid DA release in the NAc core. DA concentration is averaged into 500 msec bins (mean ± sem) and aligned to lever press (dotted line, time 0 s) for cocaine (gray) and sucrose (black). Data represent all animals ($n = 8$) that completed the sucrose/cocaine multiple schedule. Inset: comparison of peak [DA] within a 3 sec window surrounding lever press for sucrose (black) versus cocaine (gray). Peak [DA] is calculated with 100 msec bins.

Next, we examined if there were significant differences in *peak* [DA] across reinforcer type. Peak [DA] was calculated with 100 msec bins restricted to a 3 s analysis window surrounding lever press. No significant difference was observed in average peak DA concentrations across the two reinforcer types ($t_7 = 0.8347$; $p > 0.05$; Fig 2.3, inset). Unlike our

previous electrophysiology studies that examined NAc cell firing during food/cocaine multiple schedules (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012), rapid DA release does not occur selectively to one component of the sucrose/cocaine multiple schedule, but instead increases to the same extent during responding for both types of reinforcers.

Dopamine release during performance of the sucrose/food multiple schedule

In our prior electrophysiology studies, the same population of NAc neurons was similarly activated during lever press responding on a multiple schedule involving two natural reinforcers (food versus water) (Carelli *et al.*, 2000), even when one of the natural reinforcers was highly palatable sucrose (Roop *et al.*, 2002). Here, we extended that work to determine if similarities in DA release dynamics are observed during performance of a sucrose/food multiple schedule. To compare changes in extracellular DA concentration in each phase of the sucrose/food multiple schedule, DA concentration traces were aligned to the execution of the lever press response for each reinforcer. Figure 2.4 shows example cylic voltammograms, colorplots (a,b) and DA traces (c,d) for one animal that completed the food/sucrose multiple schedule. Note in this session, food was the reinforcer in phase 1 and sucrose was the reinforcer in phase 2. Here, DA release was observed during both portions of the multiple schedule. A one-way repeated-measures ANOVA revealed that lever-pressing for food significantly increased DA concentration compared to baseline ($F(39,1482) = 16.646$, $p < 0.0001$; Fig 2.4c). Newman-Keuls post hoc tests revealed that this increase in DA release was observed 0.5 s before and returned to baseline levels 0.5 s after lever pressing for food ($p < 0.05$). Likewise, a one-way repeated-measures ANOVA revealed that DA concentration increased from baseline from 0.5 s before to 1 s after lever press

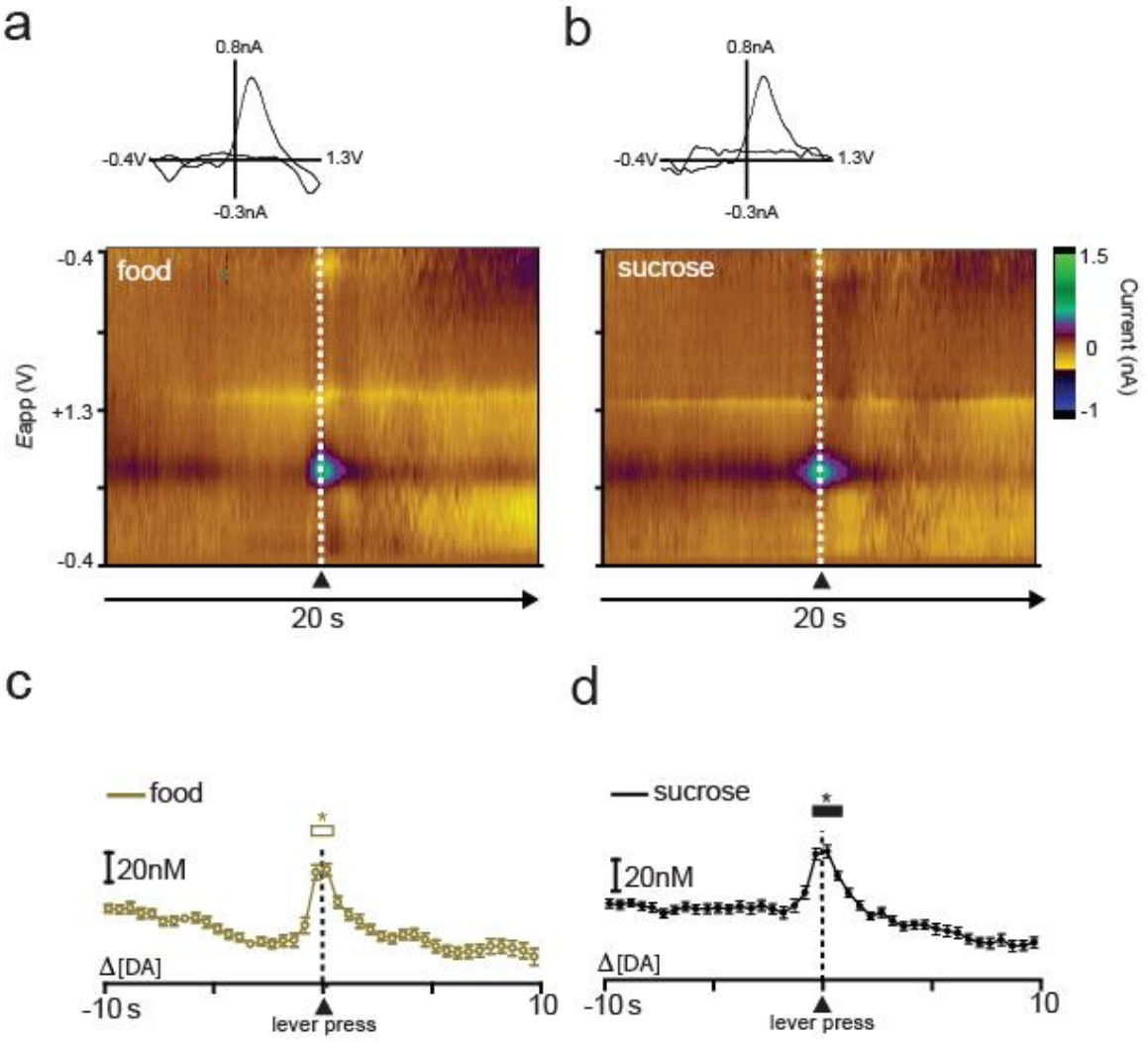

Figure 2.4. Example of dopamine release for one animal during performance of the sucrose/food multiple schedule. This animal self-administered food followed by sucrose. The rat completed 45 responses for food and 40 responses for sucrose. 39 trials for each reinforcer were included in the analysis. Lever press is indicated by dotted line at time zero. (a,b) Two-dimensional color representation of cyclic voltammetric data collected for 20 s around lever pressing for food (a) and lever pressing for sucrose (b). The ordinate is the applied voltage (*E*app) and the abscissa is time (s). Changes in current at the carbon-fiber electrode are indicated in color. Insets: cyclic voltammograms at the time of lever press. (c,d) Differential DA concentrations determined via principal component analysis for food (c) and sucrose (d). Data are plotted in 500 msec bins (mean ± sem). Asterisks above open (c) or closed (d) bars indicate significant increases in DA concentration from baseline, $p < 0.05$.

responding for sucrose ($F(39,1482) = 25.389$, $p < 0.0001$; Newman-Keuls post hoc tests, $p < 0.05$; Fig 2.4d).

This pattern of increased DA release relative to responding for both types of reinforcers was observed in all animals that completed the sucrose/food multiple schedule. Separate one-way repeated measures ANOVAs were performed to examine when [DA] increased from baseline during responding for sucrose versus food. These statistics revealed that DA concentrations increased relative to baseline during both the food ($F(39,312) = 2.159$, $P < 0.001$) and sucrose ($F(39,312) = 5.047$, $P < 0.0001$) portions of the multiple schedule. Newman-Keuls post hoc tests revealed that for the food portion of the multiple schedule DA concentration increased beginning 0.5 s before lever press and ending at lever press, or time 0 ($p < 0.05$). Likewise, during the sucrose portion of the multiple schedule, Newman-Keuls post hoc tests revealed that DA concentration was significantly elevated beginning 0.5 s before the response and returned to baseline levels 0.5 s after the lever press ($p < 0.05$).

To compare DA release dynamics across the two reinforcer conditions (Fig. 2.5), a two-way repeated measures ANOVA (reinforcer type x time) was conducted on DA concentration. There was no main effect of reinforcer type ($F(1,8) = 3.223$, $p > 0.05$); however, there was a main effect of time ($F(39,312) = 3.856$, $p < 0.0001$) and a significant reinforcer type x time interaction ($F(39,312) = 2.552$, $p < 0.0001$). Newman-Keuls post hoc tests on the significant reinforcer type x time interaction revealed that [DA] was higher during responding for sucrose than during responding for food beginning 2 s before lever press and ending 1 s following lever press ($p < 0.05$).

As above, we also examined if there were differences in peak [DA] across the two reinforcer types, where peak [DA] was calculated with 100 msec bins restricted to a 3 s analysis

window surrounding lever press. In this case, our analysis revealed that lever pressing for sucrose increased DA concentrations significantly more than lever pressing for food ($t_8 = 2.759$; $p < 0.05$; Fig 2.5, inset).

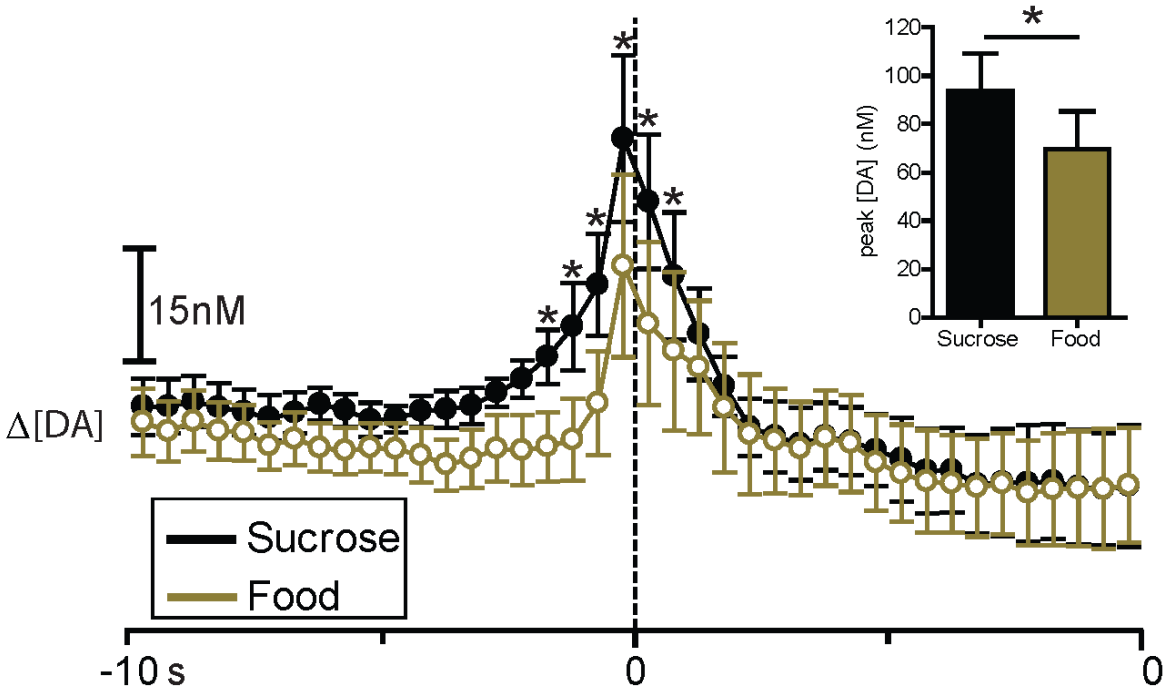

Figure 2.5. Sucrose and food self-administration evoke rapid DA release in the NAc core. DA concentration is averaged into 500 msec bins (mean ± sem) and aligned to lever press (dotted line, time 0s) for food (gold) and sucrose (black). Data represent all animals ($n = 6$) that completed the sucrose/food multiple schedule. Asterisks above closed circles indicate significant differences in DA concentration between the two reinforcer types within the indicated 500 msec bins, $p < 0.05$. Inset: comparison of peak [DA] within a 3sec window surrounding lever press for sucrose (black) versus food (gold). Peak [DA] is calculated with 100 msec bins, $*p < 0.05$.

Reinforcer order influences peak DA concentration during performance of the sucrose/cocaine but not the sucrose/food multiple schedule

The order in which the reinforcer was presented during each phase of the multiple schedule was counterbalanced across animals. Thus, 4 recordings ($n = 4$ rats) were completed in which sucrose was presented in the first phase of the multiple schedule followed by cocaine in

phase 2. In contrast, 4 recordings (n = 4 rats) were completed during sessions where cocaine was available in phase 1, followed by sucrose in phase 2.

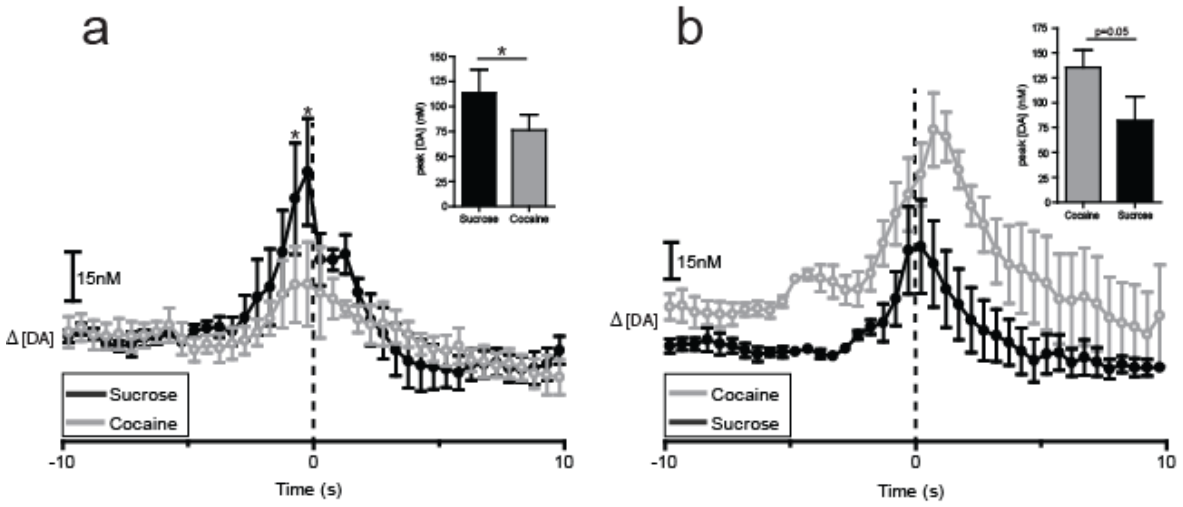

Figure 2.6. DA traces divided by order of sucrose/cocaine multiple schedule. (a) Data from animals (n = 4) that self-administered sucrose followed by cocaine. DA concentrations are averaged into 500 msec bins (mean ± sem) and aligned to lever press (dotted line, time 0s) for sucrose (black) and cocaine (gray). Asterisks above closed circles indicate significant differences in DA concentration between the two reinforcer types within the indicated 500 msec bins, $p < 0.05$. (b) Data from animals (n = 4) that self-administered cocaine followed by sucrose. Insets: comparisons of peak [DA] calculated with 100 msec bins within the 3 s window surrounding lever press, $*p < 0.05$.

We first examined DA release dynamics during the multiple schedule wherein sucrose was presented in phase 1, and cocaine in phase 2. A two-way repeated measures ANOVA (reinforcer type x time) conducted on DA concentration for recordings in which sucrose was self-administered in phase 1 showed no main effect of reinforcer type ($F(1,3) = 3.176$, $p > 0.05$); however, there was a main effect of time ($F(39,117) = 5.603$, $p < 0.0001$) and a significant reinforcer type x time interaction ($F(39,117) = 2.292$, $p > 0.001$; Fig 2.6a). Newman-Keuls post hoc tests on the significant reinforcer type x time interaction revealed that [DA] was higher during responding for sucrose than during responding for cocaine beginning 1 s before lever

press and ending at lever press, or time 0 ($p < 0.05$). Similarly, there were significant differences in peak [DA] concentration across reinforcer conditions. Examination of peak [DA] independent of time epoch showed that when sucrose was self-administered prior to cocaine, peak DA concentrations were higher relative to sucrose-responding compared to cocaine-responding ($t_3 = 4.206$; $p < 0.05$; Fig 2.6a, inset).

A similar analysis was completed to determine [DA] release dynamics during the multiple schedule when reinforcer order was reversed (i.e., when cocaine was given in phase 1 and sucrose in phase 2). A two-way repeated measures ANOVA (reinforcer type x time) conducted on DA concentration for recordings in which cocaine was self-administered in phase 1 showed a main effect of reinforcer type ($F(1,3) = 13.491$, $p > 0.05$) and a main effect of time ($F(39,117) = 7.546$, $p < 0.0001$), but no reinforcer type x time interaction ($F(39,117) = 1.003$, $p > 0.05$; Fig 2.6b). These findings indicate that [DA] increased across time, and this increase was higher during responding for cocaine than during responding for sucrose. Additional analysis was completed to compare peak [DA] across the two reinforcer types. Here, the analysis revealed a trend in higher peak DA concentrations relative to responding for cocaine than during responding for sucrose ($t_3 = 3.097$; $p = 0.0534$; Fig 2.6b, inset).

For the sucrose/food multiple schedule, 4 recordings ($n = 4$ rats) were completed in which sucrose was presented in the first phase of the multiple schedule followed by food in phase 2. In contrast, 5 recordings ($n = 4$ rats) were completed during sessions where food was available in phase 1, followed by sucrose in phase 2. Unlike the sucrose/cocaine multiple schedule, reinforcer order did not appear to influence peak DA concentration in the sucrose/food multiple schedule (Fig. 2.7). Specifically, a two-way repeated measures ANOVA (reinforcer type x time) conducted on DA concentration for recordings in which sucrose was self-

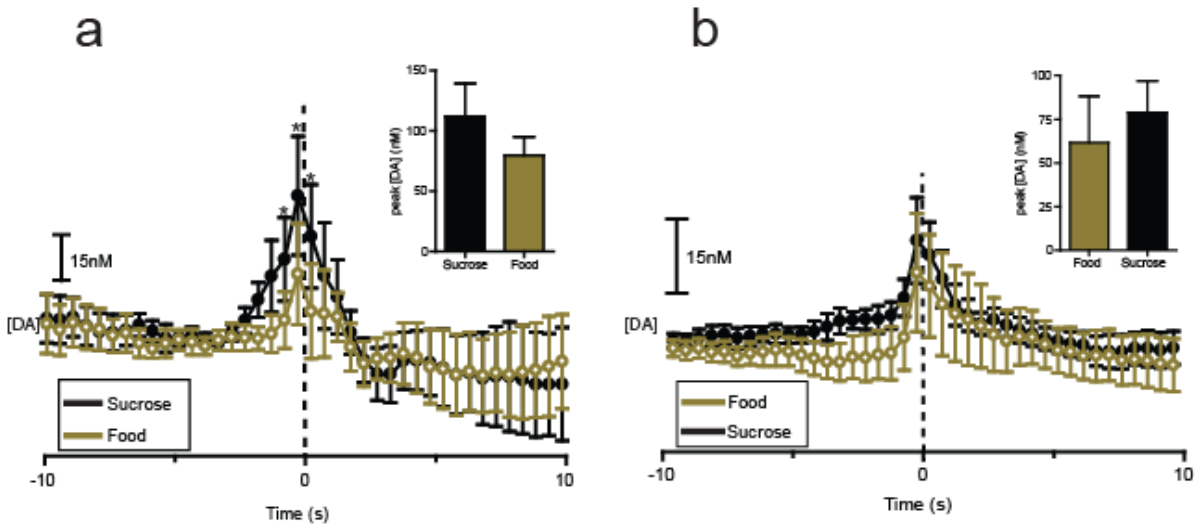

Figure 2.7. DA traces divided by order of sucrose/food multiple schedule. (a) Data from animals (n = 4) that self-administered sucrose followed by food. DA concentrations are averaged into 500 msec bins (mean ± sem) and aligned to lever press (dotted line, time 0s) for sucrose (black) and food (gold). Asterisks above closed circles indicate significant differences in DA concentration between the two reinforcer types within the indicated 500 msec bins, $p < 0.05$. (b) Data from animals (n = 4, 5 recordings) that self-administered food followed by sucrose. Insets: comparisons of peak [DA] calculated with 100 msec bins within the 3 s window surrounding lever press.

administered in phase 1 showed no main effect of reinforcer type ($F(1,3) = 1.359$, $p > 0.05$); however, there was a main effect of time ($F(39,117) = 1.584$, $p < 0.05$) and a significant reinforcer type x time interaction ($F(39,117) = 1.907$, $p < 0.005$; Fig 2.7a). Newman-Keuls post hoc tests on the significant reinforcer type x time interaction revealed that [DA] was higher during responding for sucrose than during responding for food beginning 1 s before lever press and ending 0.5 s following lever press ($p < 0.05$).

A two-way repeated measures ANOVA (reinforcer type x time) conducted on DA concentration for recordings in which food was self-administered in phase 1 showed a main effect of time ($F(39,156) = 3.073$, $p < 0.0001$); however, there was no main effect of reinforcer type ($F(1,4) = 1.834$, $p > 0.05$) and no reinforcer type x time interaction ($F(39,156) = 1.320$, $p >$

0.05; Fig 2.7b). Thus, DA concentration increased from baseline during responding for both sucrose and food, but there were no significant differences in [DA] between the two types of reinforcers.

Average peak DA concentrations were not different between the two reinforcers when sucrose was self-administered in phase 1 ($t_3 = 2.361$; $p > 0.05$; Fig 2.7a, inset) nor when food was self-administered in phase 1 ($t_4 = 1.493$; $p > 0.05$; Fig 2.7b, inset).

Next, we examined the influence of reinforcer order (i.e., self-administered 1st or 2nd) on peak DA concentration during the sucrose/cocaine multiple schedule. Again, peak [DA] was calculated with 100 msec bins restricted to a 3 s analysis window surrounding lever press. A two-way mixed design ANOVA with main effects of reinforcer type (sucrose vs. cocaine; between subjects factor), reinforcer order (1st or 2nd in multiple schedule; within subjects factor), and type x order interaction was performed on peak DA concentration. This statistic revealed a main effect of reinforcer order ($F(1,6) = 21.847$, $p < 0.005$), but no main effect of reinforcer type ($F(1,6) = 0.253$, $p > 0.05$) or interaction ($F(1,6) = 0.705$, $p > 0.05$; Fig 2.8a). Therefore, peak [DA] was significantly higher for the reinforcer self-administered first compared to the reinforcer self-administered second. These findings indicate that a higher average peak DA concentration was observed in phase 1, regardless of the type of reinforcer (sucrose or cocaine).

For the sucrose/food multiple schedule, a similar analysis was completed. Specifically, a two-way mixed design ANOVA with main effects of reinforcer type (sucrose vs. food; within subjects factor), reinforcer order (1st or 2nd in multiple schedule; between subject factor), and type x order interaction was performed on peak DA concentration. This analysis showed a main effect of reinforcer type ($F(1,7) = 7.781$, $p < 0$ .05), but no main effect of reinforcer order ($F(1,7) = 0.682$, $p > 0.05$), and no interaction ($F(1,7) = 0.727$, $p > 0.05$; Fig 2.8b). Therefore, peak [DA]

was higher relative to responding for sucrose than during responding for food independent of order in the multiple schedule.

Figure 2.8. The influence of reinforcer order and reinforcer type on peak DA concentration. (a) Comparison of peak [DA] for the sucrose (black bars) versus cocaine (gray bars) multiple schedule. (b) Comparison of peak [DA] for the sucrose (black bars) versus food (gold bars) multiple schedule. *$p < 0.05$

Time to peak DA concentration during the sucrose/cocaine and sucrose/food multiple schedule

Finally, we examined the influence of both reinforcer type (cocaine, sucrose, or food) and order of reinforcer presentation (self-administered in phase 1 or phase 2) on the time to reach peak DA concentration relative to lever press responding. These data are displayed in Figure 2.9 where negative values indicate time before the reinforced response (time 0) and positive values indicate time after the press. For the sucrose/cocaine multiple schedule, a two-way mixed design ANOVA was completed with main effects of reinforcer type (within subjects factor), reinforcer order (between subject factor) and type x order interaction on peak [DA] concentration. Results indicated no main effect of reinforcer type ($F(1,6) = 4.653$, $p = 0.07$) or reinforcer order ($F(1,6) = 4.539$, $p > 0$ .05), and no interaction ($F(1,6) = 0.987$, $p > 0$ .05; Fig 2.9a). Although not significant, there was a trend for the main effect of reinforcer type such that the time to reach

peak [DA] was later for cocaine compared to sucrose (p = 0.074). These findings indicate that the time to reach peak DA concentration was slightly delayed when animals pressed for intravenous cocaine compared to when they pressed for sucrose pellets, independent of phase.

For the sucrose/food multiple schedule, a two-way mixed design ANOVA was completed with main effects of reinforcer type (within subjects factor), reinforcer order (between subjects factor) and type x order interaction on peak [DA] concentration. Results revealed no main effect of reinforcer type (F(1,7) = 1.028, p > 0 .05), no main effect of reinforcer order (F(1,7) = 2.449, p > 0.05), and no interaction (F(1,7) = 1.622, p > 0.05; Fig 2.9b). Thus, unlike the sucrose/cocaine multiple schedule, there was no difference in time to peak [DA] relative to reinforcer type in the sucrose/food multiple schedule.

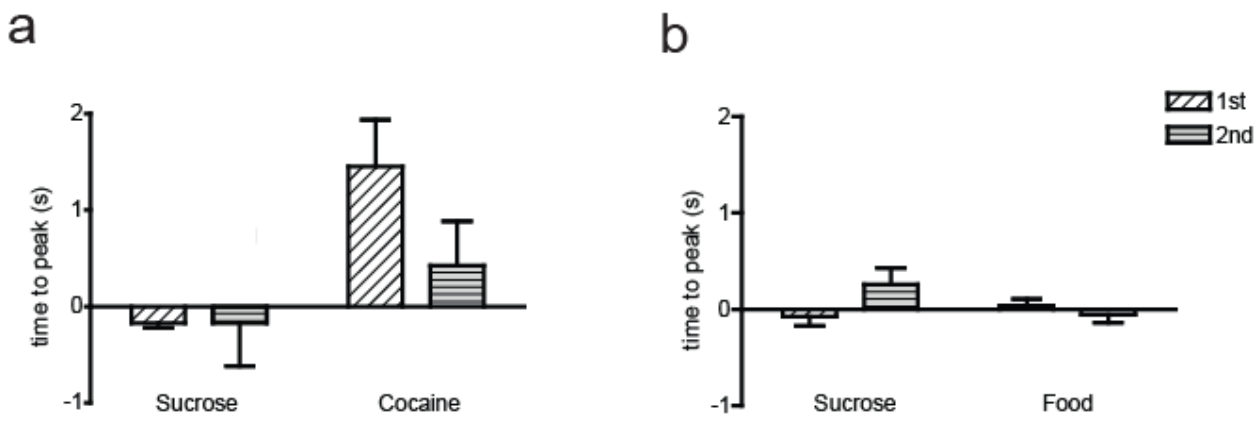

Figure 2.9. The influence of reinforcer type on time to peak. (a) Comparison of time to peak for the sucrose/cocaine multiple schedule. (b) Comparison of time to peak for the sucrose/food multiple schedule. Time 0 corresponds to lever press response. Positive numbers indicate a peak that occurs after lever press, negative numbers indicate a peak that occurs before lever press.

Histology

Histological reconstruction of carbon fiber electrode placements confirmed the location of recording sites in the NAc core (Fig. 2.10). Only data from electrode placements within the borders of the NAc core (Paxinos & Watson, 2007) were included in the analysis.

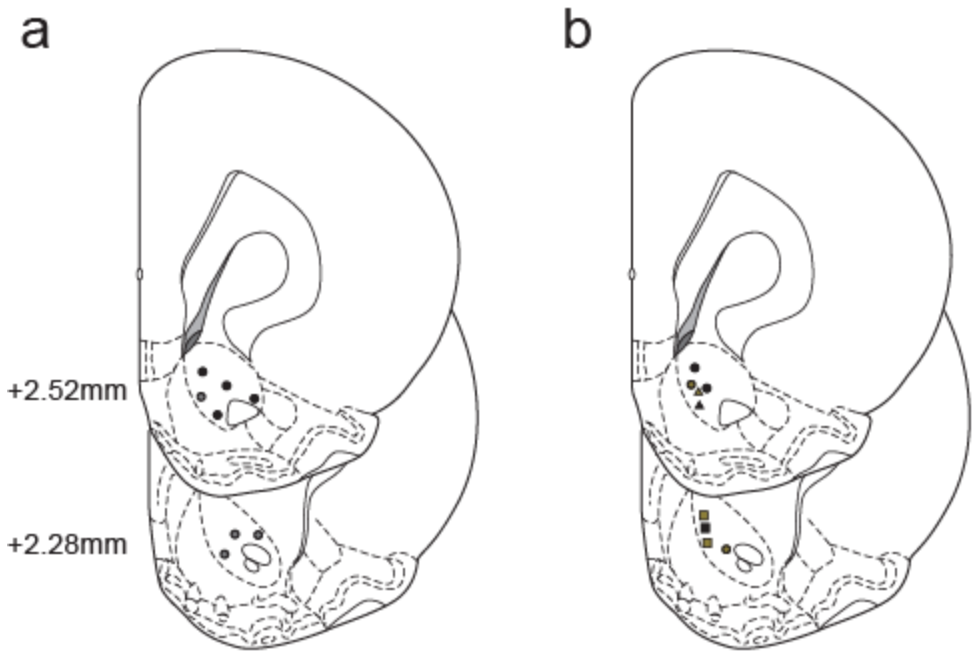

Figure 2.10. Histology. Schematic representation of electrode tip placements in the NAc core. Numbers to the left of coronal sections indicate distance anterior to bregma (Paxinos & Watson, 2007). (a) Electrode placements for the sucrose/cocaine multiple schedule. Black dots indicate recording locations from animals that self-administered sucrose followed by cocaine. Gray dots represent recording locations from animals that self-administered cocaine followed by sucrose. (b) Electrode placements for the sucrose/food multiple schedule. Circles indicate recording locations from 4 separate animals. Triangles indicate 2 recording locations from the same rat over multiple days. Squares indicate 3 recording locations from the same rat over multiple days. Black symbols indicate recording locations in which animals self-administered sucrose followed by food. Gold symbols indicate recording locations in which animals self-administered food followed by sucrose.

Discussion

The present study was completed to determine rapid DA release dynamics in the NAc core during operant responding for a drug (cocaine) versus a natural reward, or two natural rewards (sucrose and food), using multiple schedule designs. Use of the multiple schedules enabled a comparison of DA release events in discrete NAc locations during lever pressing for different rewards within the same behavioral session. During the cocaine/sucrose multiple schedule, similar increases in rapid DA signaling were observed during both phases of the task. Thus, unlike the selective cell firing of NAc neurons related to reinforcer type observed in previous studies (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012), rapid DA release does not 'turn on' or 'turn off' across each phase of the cocaine/sucrose multiple schedule. This finding indicates that rapid DA release is not reinforcer specific during situations involving goal-directed actions for cocaine versus a natural reward. However, more subtle differences were observed in peak DA concentration across the drug versus natural reward conditions related to the order of reinforcer presentation, the type of reinforcer, and the time to reach peak [DA]. Likewise, similar DA release dynamics were observed during operant responding for two natural rewards, sucrose and food, also with subtle differences in peak [DA] release across reinforcer conditions. The primary findings of the present study are discussed in detail below.

Rapid DA release is not reinforcer specific during goal-directed behavior for cocaine versus a natural reward

As stated above, electrophysiology studies from this laboratory have repeatedly shown that separate populations of NAc neurons selectively encode goal-directed actions for a natural reinforcer (food, water, or sucrose) versus cocaine (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). We have also

observed phasic DA release in the NAc on a timescale similar to phasic cell firing in separate studies that examined cocaine versus natural rewards (Phillips *et al.*, 2003b; Roitman *et al.*, 2004). Given these two lines of research, we hypothesized that rapid DA release in discrete NAc locations might also show differential activity relative to goal-directed actions for cocaine versus sucrose. As such, we predicted that we would measure increases in DA release during one phase of the cocaine/sucrose multiple schedule, but not during the other phase.

However, unlike previous electrophysiology studies, we did not observe reinforcer specificity in DA release dynamics during lever pressing for a natural reinforcer (sucrose) versus intravenous cocaine. That is, we observed rapid DA release during both phases of the sucrose/cocaine multiple schedule. Further, not only did we observe similar DA release to both reinforcers in our task, but peak [DA] was not different. That is, collapsed across all animals, peak sucrose-evoked [DA] was almost identical to peak cocaine-evoked [DA]. These findings may provide important insight into the functional organization of the NAc (see below).

Despite these findings, there were more subtle differences in peak DA concentration during the sucrose/cocaine multiple schedule. For example, we observed differences in rapid DA release as a function of reinforcer order in our task. Specifically, when cocaine was self-administered in phase 1, peak [DA] was higher relative to cocaine-responding than during sucrose-responding in phase 2, and vice versa for the opposite order of presentation. Although we designed the experiment to minimize reward comparison (i.e., 'contrast effects'), the subtle differences may be attributed to this phenomenon (Grigson & Twining, 2002; Grigson & Hajnal, 2007; Wheeler *et al.*, 2008; Wheeler *et al.*, 2011). Regardless, these findings are inconsistent with our previous electrophysiology studies that found no effect of the order of reinforcer presentation on NAc phasic cell firing during our multiple schedule design (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012).

Another more subtle difference between DA release dynamics across the phases of the sucrose/cocaine multiple schedule was the time to reach peak [DA]. Prior work using FSCV indicated that DA concentrations began to increase just prior to operant responding for cocaine (Phillips *et al.*, 2003b) and sucrose (Roitman *et al.*, 2004), and this increase in DA peaked near the execution of the lever press response. While similar in their temporal pattern, the actual time to reach peak [DA] was never compared between the two different types of reinforcers since those measurements were completed in different animals. However, here we observed that the time to reach peak [DA] was delayed during cocaine-responding compared to responding for sucrose. While the delayed peak for cocaine did not reach statistical significance, this is likely a result of the relatively lower temporal resolution (100 msec) of FSCV compared to other techniques such as electrophysiology. Nonetheless, a clear difference emerged in time to peak between cocaine and the natural reinforcers food and sucrose. This difference could be related to the interoceptive cues associated with cocaine and food/sucrose (i.e., an intravenous drug infusion over 6 s versus the immediate delivery of a pellet to a foodcup). Since these features are intrinsic to the reinforcer and do not change with the internal state of the animal, the time to peak findings suggest that DA may be encoding some *absolute* properties of a reinforcer in addition to *relative* properties such as hedonic value.

Rapid DA release is not reinforcer specific during goal-directed behavior for two natural rewards

Similarly, we did not observe reinforcer specificity during performance of the sucrose/food multiple schedule, as DA release was recorded relative to lever pressing behavior during both phases of the task. However, given previous findings from *in vivo* electrophysiology showing that two natural reinforcers activate largely the same population of NAc neurons (Carelli *et al.*, 2000; Roop *et al.*, 2002), this later finding was not unexpected. Specifically, our

earlier electrophysiology work found that the majority of phasic NAc neurons (68%) showed similar types of patterned discharges across two natural reinforcers (food versus water; Carelli *et al.*, 2000). This overlapping pattern of phasic activity was maintained even when one of the natural reinforcers was of greater hedonic value. Specifically, when animals performed a multiple schedule for water and highly palatable sucrose, the majority of NAc neurons (65%) were similarly activated (Roop *et al.*, 2002).

As described above, we also observed more subtle differences in peak DA concentration during to sucrose/food multiple schedule. Unlike the sucrose/cocaine multiple schedule in which peak sucrose-evoked [DA] was almost identical to peak cocaine-evoked [DA], peak sucrose-evoked [DA] was significantly higher than peak food-evoked [DA] in the sucrose/food multiple schedule. Importantly, this difference persisted regardless of reinforcer order. These findings are somewhat surprising in light of our prior electrophysiology studies. For example, NAc neurons showed similar patterns of activity relative to water and sucrose reinforcement even though sucrose was presumably of higher hedonic value (Roop *et al.*, 2002). However, other studies suggest that hedonic value can influence the percentage of NAc neurons that display phasic activity. Specifically, electrophysiology recording during a sucrose/cocaine multiple schedule (Cameron & Carelli, 2012) found a higher percentage of overlapping neurons (21%) compared to during a multiple schedule for cocaine and water (8% overlapping) or cocaine and food (7% overlapping) (Carelli *et al.*, 2000). Further, there was a greater percentage of neurons that were selective for sucrose-related information (70%) compared with neurons that were selective for water-related information (48%) in the earlier study. Ultimately, the NAc is functionally complex and the percentage of NAc neurons activated by any one reinforcer could be influenced

by several interdependent factors including hedonics, reinforcer type (e.g. sweet tastant vs. food/water), and the inclusion of a drug reinforcer in the multiple schedule task.

Studies that specifically examined DA (rather than NAc cell firing) suggest that the value of a reward is encoded by rapid DA release in the NAc, even when this value is subjective (Day *et al.*, 2010; Sugam *et al.*, 2012). Therefore, the differences (or lack thereof) in peak [DA] relative to sucrose-, food-, or cocaine-responding in the present study could reflect the subjective value of the particular reinforcer being self-administered. For example, sucrose-predictive cues evoke greater DA release in the NAc core than saccharin-predictive cues, and this difference in DA signaling is correlated with a behavioral preference for sucrose over saccharin ((McCutcheon *et al.*, 2012). The authors suggest that rats' preference for sucrose may be driven by several factors, including nutrition and taste; regardless, NAc DA clearly tracks this preference. While we did not directly test rats' preference for sucrose versus food in the present study, decades of research have shown that rats find sucrose more palatable than food (for a recent review, see (Berridge *et al.*, 2010). Therefore, it seems likely that the greater DA release observed relative to sucrose self-administration compared to food self-administration in this study reflects a neural correlate of subjective hedonic value.

Finally, the time to reach peak [DA] was not different between sucrose-responding and food-responding. Thus, we observed a dissociation between the level of peak [DA] and the time to reach this peak between our two multiple schedule conditions. In the sucrose/cocaine multiple schedule, peak DA concentrations were not different between the two reinforcers, but the time to reach the peak was delayed for cocaine. In contrast, peak [DA] was higher relative to sucrose-responding than to food-responding, but there were no differences in the time to reach peak DA

concentration. Again, rapid DA release may function to encode both absolute and relative properties of an individual reinforcer.

Insights into the role of rapid DA signaling in the functional organization of the NAc

It is often hypothesized that drugs of abuse exert their effects by 'tapping into' a brain reward system that has evolved to process natural reinforcers, causing aberrant reward processing and, ultimately, addiction (Wise, 1997). However, electrophysiology studies from this laboratory have put this idea into question, as natural and drug reinforcers are encoded by largely separate populations of NAc neurons (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012). Further, two natural reinforcers are encoded by largely overlapping populations of NAc neurons (Carelli *et al.*, 2000; Roop *et al.*, 2002).

The anatomical organization of the NAc shows that this structure receives convergent synaptic inputs from a variety of cortical (e.g., PFC) and subcortical (e.g., BLA and hippocampus) structures, and in turn, sends efferent projections to motor areas, thereby supporting its role as a limbic-motor integrator (Mogenson *et al.*, 1980). However, it is unlikely that the NAc as a whole sends a single integrated output to its target structures in order to initiate behavior. Theories of basal ganglia function suggest that the NAc is embedded in a larger system that is organized into several structurally and functionally discrete circuits that are essentially parallel in nature (Alexander *et al.*, 1986; Alexander & Crutcher, 1990). Further, Pennartz *et al.* (1994) proposed that the NAc is composed of a collection of functionally heterogeneous 'neuronal ensembles' that are characterized by distinct afferent/efferent projections. Within this framework, DA acts as a neuromodulator, differentially influencing the activity of glutamatergic

afferents (O'Donnell *et al.*, 1999; Nicola *et al.*, 2000) rather than exerting global actions across all NAc neurons.

In further support of the role of DA as a neuromodulator, work combining FSCV with simultaneous electrophysiological recordings from the same electrode reveal that DA release occurs primarily at NAc locations with phasic cell activity and little to no DA release is observed at sites with nonphasic cell activity (Owesson-White *et al.*, 2009; Cacciapaglia *et al.*, 2011). While these findings might initially suggest that rapid DA release directly influences phasic cell firing, pharmacological manipulations show that this is not always the case. For example, reducing phasic DA release in the NAc has no effect on phasic inhibition of NAc neurons (Cheer *et al.*, 2005; Cacciapaglia *et al.*, 2011), but does reduce phasic excitation of NAc neurons (Cacciapaglia *et al.*, 2011). Further, antagonism of D1-type DA receptors selectively reduces excitatory phasic activity of NAc neurons without altering DA release (Cheer *et al.*, 2005). Together these findings suggest that DA release plays a clear role in excitatory NAc cell firing, but is perhaps not functionally linked to inhibitory activity. Thus, rapid DA release may not directly influence global NAc phasic activity, but make certain neurons more attuned to glutamatergic afferent inputs from brain regions such as the prefrontal cortex, basolateral amygdala and hippocampus.

Within distinct NAc microcircuits, rapid DA release likely modulates NAc cell activity based on a variety of factors such as DA receptor sub-type, phasic activity (excitatory versus inhibitory), reward type (sucrose versus food versus cocaine), or ongoing behavior. In the present study, we report rapid DA release to both sucrose and cocaine; therefore, rapid DA release must differentially modulate distinct subsets of NAc neurons such that subsequent phasic activity is predominantly nonoverlapping to the two reinforcer types (Cameron & Carelli, 2012).

Conclusions

Overall, the results of this study support the idea that the brain processes drug rewards in a different way than it does natural rewards. We observed a dissociation between the influence of reinforcer order and reinforcer type on rapid DA signaling. Reinforcer order mattered when rats compared cocaine to sucrose, but the type of reinforcer mattered when rats compared sucrose to food. While intimately linked, rapid DA release in the NAc differs in important ways from phasic cell firing. Here we demonstrated that operant responding for sucrose and cocaine evoke similar peak DA concentrations even when earlier work clearly showed that these reinforcers are encoded by separate population of NAc neurons. The exact relationship between DA release and phasic cell firing during self-administration of a natural versus drug reward remains to be established. Future studies could couple iontophoresis with FSCV and electrophysiology to determine the causal relationship between DA signaling and cell firing in the NAc (Belle *et al.*, 2013).

CHAPTER 3: EXTRACELLULAR pH INCREASES ARE GREATER RELATIVE TO RESPONDING FOR COCAINE THAN RESPONDING FOR THE NATURAL REINFORCER SUCROSE

Introduction

The mesolimbic dopamine (DA) system, particularly the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is a critical component of the brain reward circuit and has been implicated in processing information about both natural and drug rewards (Robinson & Berridge, 2000; Kelley, 2004). Indeed, electrochemical techniques such as fast-scan cyclic voltammetry (FSCV) have repeatedly shown that rapid DA release is observed in the NAc during operant responding for cocaine and natural rewards (Phillips *et al.*, 2003b; Roitman *et al.*, 2004). Importantly, this system also plays a key role in drug addiction, as it is a crucial substrate involved in discrete aspects of the addiction cycle (Koob & Nestler, 1997; Kalivas & McFarland, 2003; Kalivas & Volkow, 2005; Carlezon & Thomas, 2009).

While FSCV studies examining DA release have provided unprecedented insight into the role of this neurotransmitter in reward processing and addiction, electrochemical recordings with carbon-fiber microelectrodes also allow for the detection of pH with similar (subsecond) temporal resolution (Venton *et al.*, 2003; Takmakov *et al.*, 2010). In fact, fluctuations in pH have been observed relative to electrical stimulation (Venton *et al.*, 2003; Cheer *et al.*, 2006) as well as reward-related events in awake animals (Ariansen *et al.*, 2012). These shifts in pH are delayed several seconds from the immediate release and reuptake of DA (Venton *et al.*, 2003). Thus,

examining changes in pH related to goal-directed actions offers a way to probe dynamic changes in neural activity that occur in combination with changes in DA.

Importantly, pH fluctuations are intimately coupled with changes in brain oxygen through blood flow and metabolism. Increases in neural activity lead to a transient increase in blood flow, resulting in increased oxygen, clearance of carbon dioxide, and alkaline (basic) shifts in pH (Chesler & Kaila, 1992; Chesler, 2003; Venton *et al.*, 2003). It is this relationship between neural activation, blood flow, and oxygen that provides the basis for blood oxygen level-dependent (BOLD) fMRI (Raichle, 1998). Therefore, a detailed examination of pH shifts in rats during the performance of reward-related behaviors could help to bridge the gap in the study of similar behaviors in humans revealed with fMRI.

Finally, while pH shifts have been observed during behavioral tasks involving either natural or drug rewards (Phillips *et al.*, 2003b; Roitman *et al.*, 2004; Ariansen *et al.*, 2012), a direct comparison in pH dynamics across the two reinforcer conditions has not yet been made. Notably, cocaine exerts direct pharmacological effects on brain oxygen pressure and blood flow (Yonetani *et al.*, 1994) independent of its more transient effects on DA release associated with operant responding and reward-related cues. While rewards such as sucrose do not exert pharmacological actions, changes in pH have also been observed during tasks involving natural rewards (Roitman *et al.*, 2004; Ariansen *et al.*, 2012). Here, we used the same data set obtained for Chapter 2, to compare basic shifts in pH in discrete locations in the NAc core and determine if aspects of this signaling differs during each phase of the sucrose/cocaine multiple schedule. Our findings revealed that although increases in pH were observed under both reinforcer conditions, the dynamics of this signaling was significantly different when animals responded for intravenous cocaine versus the natural reward, sucrose.

Materials and Methods

Animals

The same animals used in Chapter 2 were used here. Briefly, male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA; $n = 8$) aged 90-120 days and weighing 260–350 g were used as subjects and individually housed with a 12/12 h light/dark cycle. Body weights were maintained at no < 85% of pre-experimental levels by food restriction (10–15 g of Purina laboratory chow each day). Water was available *ad libitum*. This regimen was in place for the duration of the experiment, except during the post-operative recovery period when food was given *ad libitum*. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgery and Behavioral Training

Surgical procedures and behavioral training were as described for the sucrose/cocaine multiple schedule in Chapter 2. Refer to Figure 2.1a for the experimental timeline of this study. Briefly, rats were surgically implanted with an intravenous catheter following established procedures, described in detail previously (Carelli *et al.*, 2000). Following recovery from surgery, rats were first trained to press one lever for sucrose (45 mg pellet) on a fixed-ratio 1 schedule of reinforcement. The start of the sucrose training session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. Lever depression resulted in delivery of a sucrose pellet to the reward receptacle, onset of a tone (65 dB, 2900 Hz, 20 s), and retraction of the lever (20 s). Rats underwent daily 30 min training sessions until they reached criterion (at least 50 presses per session). Rats were then trained to

self-administer cocaine on a fixed-ratio 1 schedule of reinforcement during daily 2 h sessions. The start of the self-administration session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. The cocaine-associated lever was spatially distinct from the lever previously used during sucrose training. Lever depression resulted in intravenous cocaine delivery (0.33 mg/infusion, approximately 1 mg/kg/infusion, 6 s) via a computer-controlled syringe pump, onset of a different tone (65 dB, 800 Hz, 20 s), and retraction of the lever (20 s). The tones and levers (left or right) associated with cocaine vs. sucrose were counterbalanced across animals.

Following 14 days of cocaine self-administration, rats were surgically prepared for voltammetric recording in the NAc core as previously described (Phillips *et al.*, 2003a). Animals were anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture (intramuscular) and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned dorsally to the NAc core (+1.3 mm anterior, -1.3mm lateral from bregma). An Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. The bipolar stimulating electrode (Plastics 1 Inc., Roanoake, VA) was placed dorsally to the VTA (-5.2 mm posterior, -1.0 mm lateral from bregma and -7 mm ventral from brain surface). Stainless steel skull screws and dental cement were used to secure all items. The bipolar stimulating electrode was lowered in 0.2 mm increments until physical responses to electrical stimulation diminished, indicative of proper electrode placement. The stimulating electrode was then fixed with dental cement.

After recovering from surgery, rats were retrained for two consecutive days (one session of sucrose self-administration followed the next day by one session of cocaine self-administration) while tethered to the headstage. This allowed the animals to habituate to the

headstage and reestablish normal operant responding. Following retraining, rats underwent voltammetric recording (see below) during a multiple schedule of reinforcement for sucrose and cocaine. Specifically, rats had access to the sucrose-reinforced lever (fixed-ratio 1; 15 min) followed by a 20 s time-out period (no lever extended; dark chamber) and extension of the second cocaine-reinforced lever (fixed-ratio 1; 2 h). Illumination of a cue light above each lever signaled the phase (sucrose or cocaine) of the multiple schedule. The order of reinforcer availability was varied across animals such that 4 animals self-administered sucrose followed by cocaine while 4 other animals self-administered cocaine followed by sucrose. Importantly, rats only received both reinforcers in the multiple schedule during the test day. Therefore, one reinforcer never came to predict access to the other.

Fast Scan Cyclic Voltammetry

Basic pH shifts (as well as changes in DA concentration described in Chapter 2) during behavior were assessed using fast-scan cyclic voltammetry as previously described (Roitman *et al.*, 2004; Day *et al.*, 2007). On the day of the experiment, a detachable micromanipulator containing a carbon-fiber electrode (90-110 μm length) was inserted into the guide cannula and lowered into the NAc core. The carbon-fiber and Ag/AgCl reference electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Med-Associates, St. Albans, VT) at the top of the test chamber. Voltammetric recordings were made every 100 msec by applying a triangular waveform (-0.4 to +1.3 V, 400 V/sec). Data were digitized and stored to a computer using software written in LabVIEW (National Instruments, Austin, TX). Dopamine release within the NAc core was electrically evoked by stimulating the VTA (24 biphasic pulses, 60 Hz, 120 μA, 2 msec per phase) to ensure that carbon-fiber electrodes were in close proximity to DA release sites. The electrode position was optimized at a location with maximal dopamine

release. To create a training set for principal component analysis for the detection of DA and pH changes during the behavioral session, additional stimulations at various parameters were performed (2-24 biphasic pulses, 20-60 Hz, 120 μA, 2 msec/phase). After the session, electrical stimulation was repeated to ensure that the site could still support DA release. A second computer and software system (Med Associates) controlled behavioral events and sent digital outputs for each event to the voltammetry recording computer to be time-stamped along with the electrochemical data.

Data Analysis

All lever press events were recorded during performance of the multiple schedule and behavioral data was analyzed as described in Chapter 2. Briefly, the number of lever presses as well as inter-response interval (INT) were calculated for each reinforcer during each behavioral session. Number of lever presses and INT were compared for the sucrose/cocaine multiple schedule with a two-way mixed design ANOVA (reinforcer type, within subjects factor x reinforcer order, between subjects factor). To eliminate any effect of an uneven number of responses for one reinforcer (sucrose or cocaine) over another, lever press responses were randomly selected to allow for an equal number of trials within a recording session. The number of random trials that were selected from each session were equivalent to the number of trials of the reinforcer for which the animal responded less. Thus, if an animal responded 40 times for sucrose and 20 times for cocaine during a sucrose/cocaine multiple schedule, we selected 20 trials each for sucrose and cocaine. This procedure was used for all analyses described below.

pH signals from FSCV were identified as previously described (Roitman *et al.*, 2004). For analyte identification, current during a voltammetric scan is plotted against applied potential to yield a cyclic voltammogram (the chemical signature of the analyte; see examples in Figure

3.1a,b insets). Cyclic voltammetric data were analyzed on stimulation trials before and after each experiment, and ±10 s relative to the important behavioral events (lever press). A background signal from 1 voltammetric scan (100 msec time bin) before a stimulation or behavioral trial was subtracted from the remainder of the scans to reveal relative changes in pH (rather than absolute values).

pH shifts were then quantified using principal component regression (Heien *et al.*, 2004; Keithley *et al.*, 2010). Training sets were constructed from representative, background subtracted cyclic voltammograms for DA and pH. These training sets were then used to perform principal component regression on data collected during behavioral sessions. Principal components were selected such that at least 99% of the variance in the training set was accounted for by the model.

Basic shifts in pH during behavior were assessed by aligning pH traces to lever press events. Data were averaged into 500 msec bins, and analyzed 10 s before to 10 s after lever press. Shifts in NAc pH from baseline in response to lever presses were evaluated independently for each reinforcer (sucrose or cocaine) using a one-way repeated measures ANOVA with Newman-Keuls post hoc tests. This analysis compared the baseline bin (-10 to -9.5 s) to each subsequent 500 msec bin.

The pattern of pH shifts during self-administration of sucrose versus cocaine during the multiple schedule was compared with a two-way repeated measures ANOVA (reinforcer x time) followed by Newman-Keuls post hoc tests of significant effects. For this analysis, data were averaged into 500 msec bins, and analyzed 10 s before to 10 s after lever press. To examine the influence of reinforcer order in the multiple schedule, a three-way mixed model ANOVA was performed (reinforcer type and time as within subjects factors, reinforcer order as between subjects factor).

Next, we examined the *peak* pH shift elicited by operant responding for sucrose or cocaine. This analysis was performed separately for two different 3 s time windows (surrounding lever press, or -1.5 to 1.5 s; the end of the 20 s analysis window, or 7-10 s). Peak was defined as the highest pH shift within the 3 s window (100 msec bins), and was determined for each sucrose and cocaine trial within a behavioral recording session. These values were then averaged across trials for each animal to obtain the peak pH shift for each reinforcer within a recording. Average peak pH shifts across rats were then compared across reinforcer types (sucrose versus cocaine) using paired *t* tests. The influence of reinforcer order and reinforcer type on peak pH shift was analyzed with a two-way mixed design ANOVA.

To examine the relationship between peak pH and peak [DA] (acquired from the data set in Chapter 2), regression analyses were completed that correlated the peak pH with the peak [DA] elicited on each behavioral trial. To examine the relationship between basic shifts in pH (or changes in [DA]) across time in the behavioral session, linear regression analyses were completed that correlated peak pH or peak [DA] with time of lever press. This analysis was performed for both the sucrose and cocaine portions of the multiple schedule.

For all analyses, the alpha level for significance was 0.05. All statistics were performed with commercially available software (Statistica, Tulsa, Oklahoma; GraphPad Software, La Jolla, CA).

Histology

Since the same animals were used here as in Chapter 2, histological verification of electrode placement is as described in Chapter 2.

Results

Behavioral responding during the multiple schedule

See Chapter 2 for a full description of the behavioral response patterns during the sucrose/cocaine multiple schedule.

Basic pH shifts during performance of the sucrose/cocaine multiple schedule

To compare basic shifts in pH during lever pressing for sucrose pellets versus intravenous infusions of cocaine (i.e., drug self-administration), pH traces were aligned to the execution of lever press responses. Figure 2.1 (a,b) shows example cyclic voltammograms and color representations (colorplots) of a set of background-subtracted cyclic voltammograms and the corresponding pH traces (c,d) for one animal that completed the cocaine/sucrose multiple schedule. In this example, cocaine was the reinforcer in phase 1 followed by sucrose reinforcement in phase 2 of the multiple schedule. In this session, shifts in pH were observed during both phases of the multiple schedule. During cocaine self-administration (phase 1), a one-way repeated-measures ANOVA revealed that pH was significantly higher following lever pressing for cocaine compared to baseline ($F(39,1092) = 55.450$, $p < 0.0001$; Fig 2.1c). Newman-Keuls post hoc tests revealed a significant increase in pH from baseline beginning at lever press, or time 0, that continued up to 10 s following lever press ($p < 0.05$). Likewise, a one-way repeated measures ANOVA revealed that pH was changed over time relative to responding for sucrose ($F(39,1092) = 3.829$, $p < 0.0001$; Fig 2.1d). However, Newman-Keuls post hoc tests did not reveal any significant increases in pH at any one 500 msec time epoch relative to baseline.

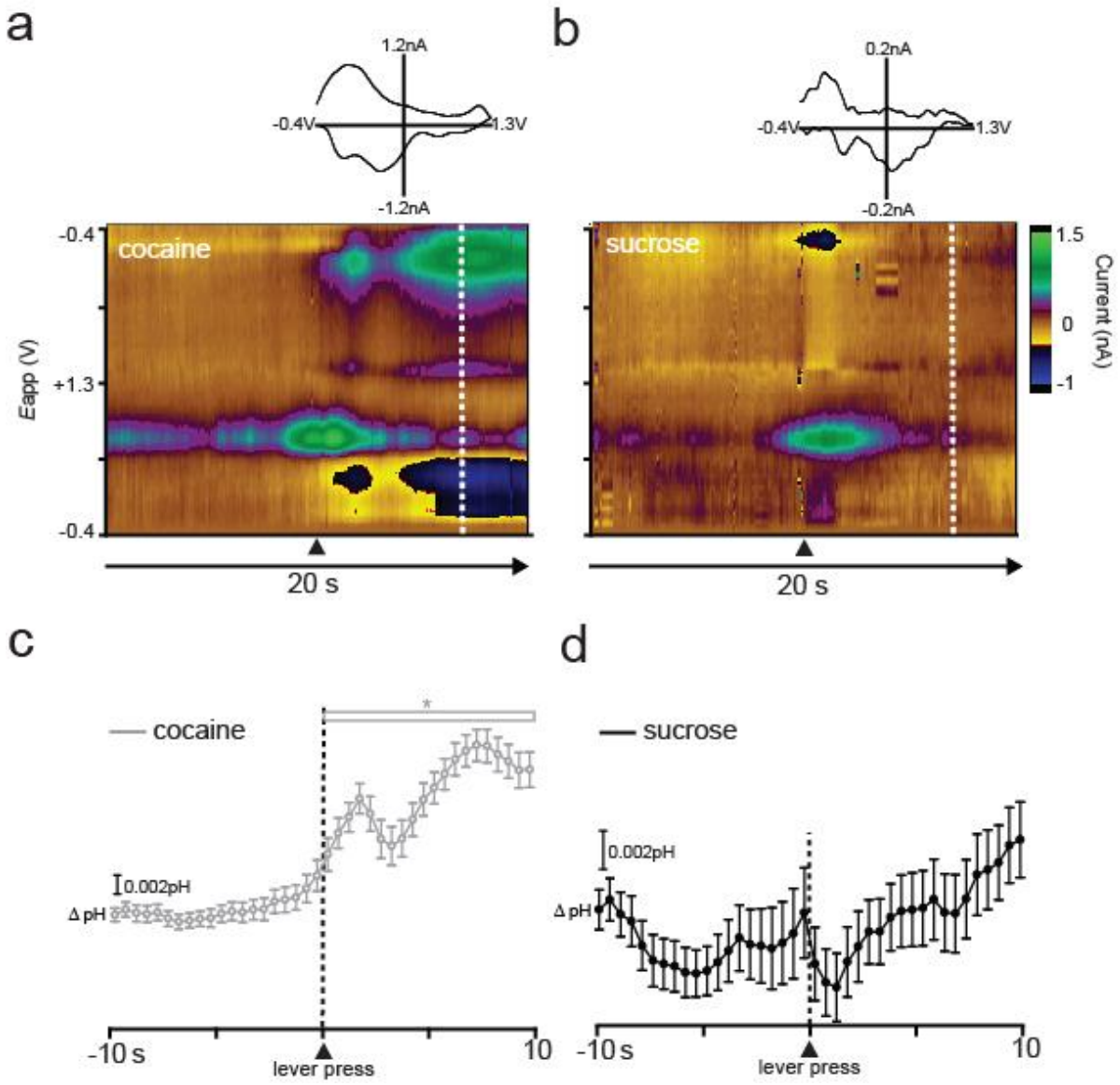

Figure 3.1. Example of basic pH shifts for one animal during performance of the sucrose/cocaine multiple schedule. This animal self-administered cocaine followed by sucrose. The rat completed 29 responses for cocaine and 48 responses for sucrose. 29 trials for each reinforcer were included in the analysis. Lever press is indicated by dotted line at time zero. (a,b) Two-dimensional color representation of cyclic voltammetric data collected for 20 s around lever pressing for cocaine (a) and lever pressing for sucrose (b). The ordinate is the applied voltage (*E*app) and the abscissa is time (s). Changes in current at the carbon-fiber electrode are indicated in color. Insets: cyclic voltammograms (CVs) indicating basic shifts in pH following lever press (CVs were averaged at 7s following lever press, indicated by the white dotted line). (c,d) Basic pH shifts determined via principal component analysis for cocaine (c) and sucrose (d). Data are plotted in 500 msec bins (mean ± sem). Asterisk above open bar (c) indicates significant increases in pH from baseline, $p < 0.05$.

This pattern of increased basic pH shifts following operant responding in the cocaine/sucrose multiple schedule was observed across all animals (Fig 3.2a). Separate one-way repeated measures ANOVAs were performed to examine changes in pH relative to baseline during responding for cocaine versus sucrose. These statistics revealed that pH levels were changed over time relative to responding for both cocaine ($F(39,273) = 10.336$, $P < 0.0001$) and sucrose ($F(39,273) = 3.1943$, $P < 0.0001$). Newman-Keuls post hoc tests revealed that for the cocaine portion of the multiple schedule pH increased beginning at lever press (time 0). This increase in pH was sustained for at least 10 s following lever press ($p < 0.05$). However, during the sucrose portion of the multiple schedule, Newman-Keuls post hoc tests did not reveal any significant increases in pH at any one 500 msec time epoch relative to baseline.

In order to directly compare changes in pH across reinforcer type relative to lever press, a two-way repeated measures ANOVA was conducted that examined reinforcer type (sucrose versus cocaine) and time on pH (Fig 3.2a). This statistic revealed a main effect of reinforcer type ($F(1,7) = 20.131$, $p < 0.005$) and a main effect time ($F(39,273) = 12.367$, $p > 0.0001$), but no reinforcer type x time interaction ($F(39,273) = 1.325$, $p > 0.05$). Thus, pH increased over time during responding for both cocaine and sucrose, but this increase was higher during cocaine-responding.

Next, we examined the influence of reinforcer order on pH. As noted previously, the order in which a reinforcer was presented during each phase of the multiple schedule was counterbalanced across animals. Thus, 4 recordings ($n = 4$ rats) were completed in which sucrose was presented in the first phase of the multiple schedule followed by cocaine in phase 2. In contrast, 4 recordings ($n = 4$ rats) were completed during sessions where cocaine was available in phase 1, followed by sucrose in phase 2. A three-way mixed model ANOVA (reinforcer type and

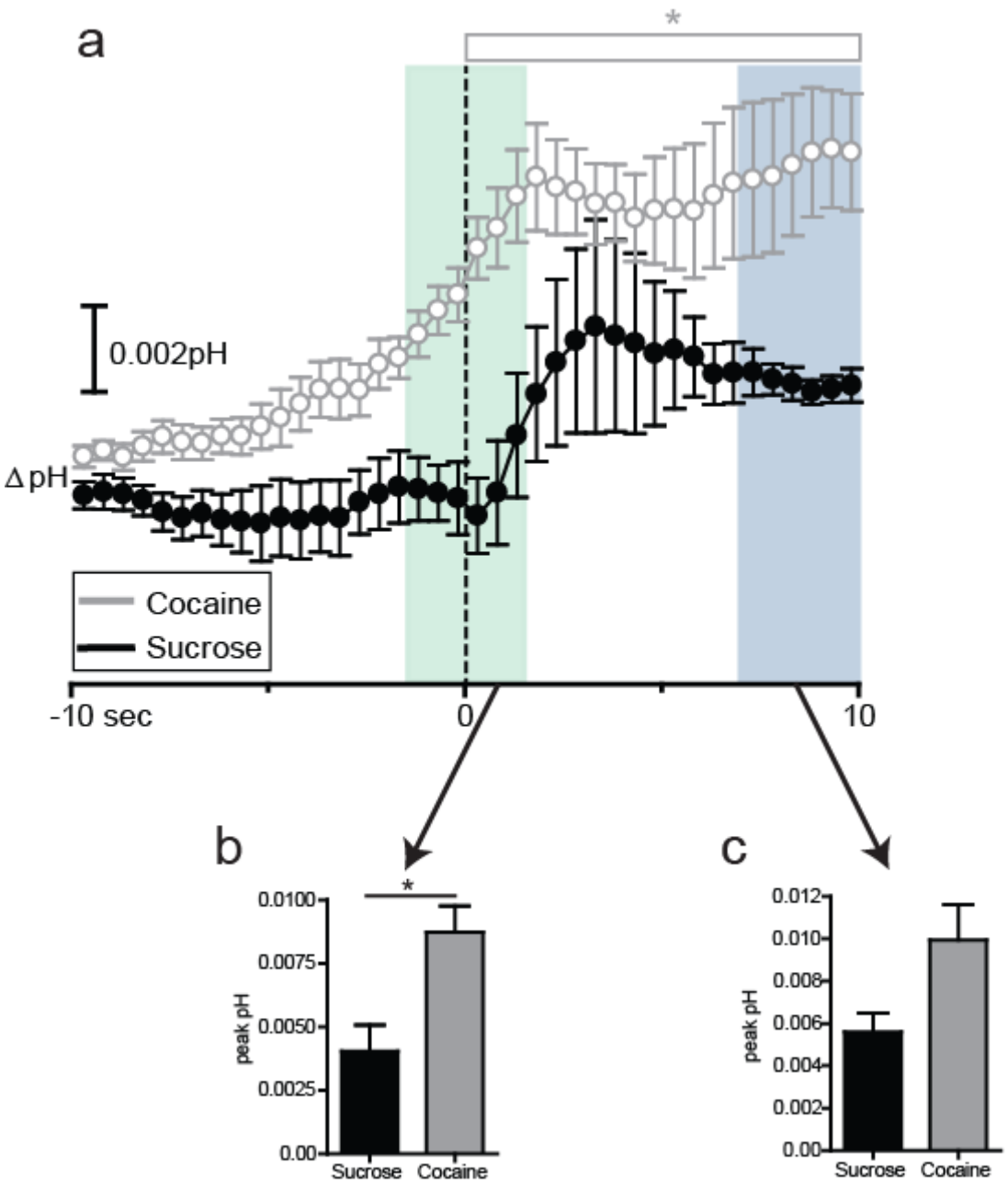

Figure 3.2. Changes in pH in the NAc core during sucrose and cocaine self-administration. (a) pH is averaged into 500 msec bins (mean ± sem) and aligned to lever press (dotted line, time 0 s) for cocaine (gray) and sucrose (black). Data represent all animals (n = 8) that completed the sucrose/cocaine multiple schedule. (b) Comparison of peak pH within a 3 sec window surrounding lever press (indicated by green column) for sucrose (black) versus cocaine (gray). Peak pH is calculated with 100 msec bins, *$p < 0.05$. (c) Comparison of peak pH within a 3 sec window from time 7-10 s (indicated by blue column) for sucrose (black) versus cocaine (gray).

time as within subjects factors, reinforcer order as the between subjects factor) revealed main effects of reinforcer type ($F(1,6) = 23.081$, $P < 0.005$) and time ($F(39,234) = 13.140$, $P < 0.0001$), but no main effect of reinforcer order ($F(1,6) = 1.858$, $P > 0.05$) and no reinforcer type x time x order interaction ($F(39,234) = 0.272$, $P > 0.05$). Thus, the *pattern* of higher pH relative to cocaine- compared to sucrose-responding reported above held regardless of the order of reinforcer presentation in the multiple schedule.

Peak pH during performance of the sucrose/cocaine multiple schedule is influenced by the type of reinforcer, but not the order of reinforcer presentation

Next, we examined if there were significant differences in *peak* pH shifts across reinforcer type. Peak pH was calculated within 100 msec bins restricted to a 3 s analysis window surrounding lever press (-1.5 s to 1.5 s) or at the end of the 20 s analysis window (7 s to 10 s; see Fig 3.2a). We chose these two analysis windows because there appeared to be an initial increase in pH directly following a lever press response as well as a more sustained increase lasting at least until the end of the entire 20 s analysis window. In the 3 s window surrounding lever press, peak pH was higher relative to cocaine-reinforced responding compared to sucrose-reinforced responding ($t_7 = 3.288$; $p < 0.05$; Fig 3.2b). No significant difference was observed in pH across the two reinforcer types at time 7-10 s ($t_7 = 1.989$; $p > 0.05$; Fig 3.2c). However, there was a trend for pH to be higher relative to cocaine than to sucrose ($p = 0.087$).

Because we observed a significant difference in pH within the -1.5-1.5 s analysis window, we used this timepoint to further examine the influence of both reinforcer type (sucrose versus cocaine) and reinforcer order (1st or 2nd in the multiple schedule) on peak pH. A two-way mixed design ANOVA with main effects of reinforcer type (sucrose vs. cocaine; within subjects factor), reinforcer order (1st or 2nd in multiple schedule; between subjects factor), and type x order interaction was performed on peak pH. This statistic revealed a main effect of reinforcer

type ($F(1,6) = 9.317$, $p < 0.05$), but no main effect of reinforcer order ($F(1,6) = 2.354$, $p > 0.05$) and no interaction ($F(1,6) = 0.031$, $p > 0.05$; Fig 3.3). Therefore, peak pH was higher relative to responding for cocaine than during responding for sucrose independent of order in the multiple schedule.

Figure 3.3. The influence of reinforcer type and reinforcer order on peak pH. (a) Comparison of peak pH for sucrose and cocaine when self-administered first (white bars) versus second (gray bars) in the multiple schedule, *$p < 0.05$

Peak pH is correlated with peak [DA] during cocaine self-administration, but not during sucrose self-administration

Another important issue addressed here is if peak pH was correlated to peak [DA] obtained in Chapter 2. To examine this relationship across individual behavioral trials, linear regression analyses were completed that correlated peak pH versus peak [DA]. This analysis was done separately for each phase of the multiple schedule (i.e. cocaine and sucrose self-administration). A significant positive linear regression was observed between peak pH and peak [DA] during cocaine self-administration ($F(1,153) = 10.01$, $p < 0.01$, $r^2 = 0.06$; Fig. 3.4a).

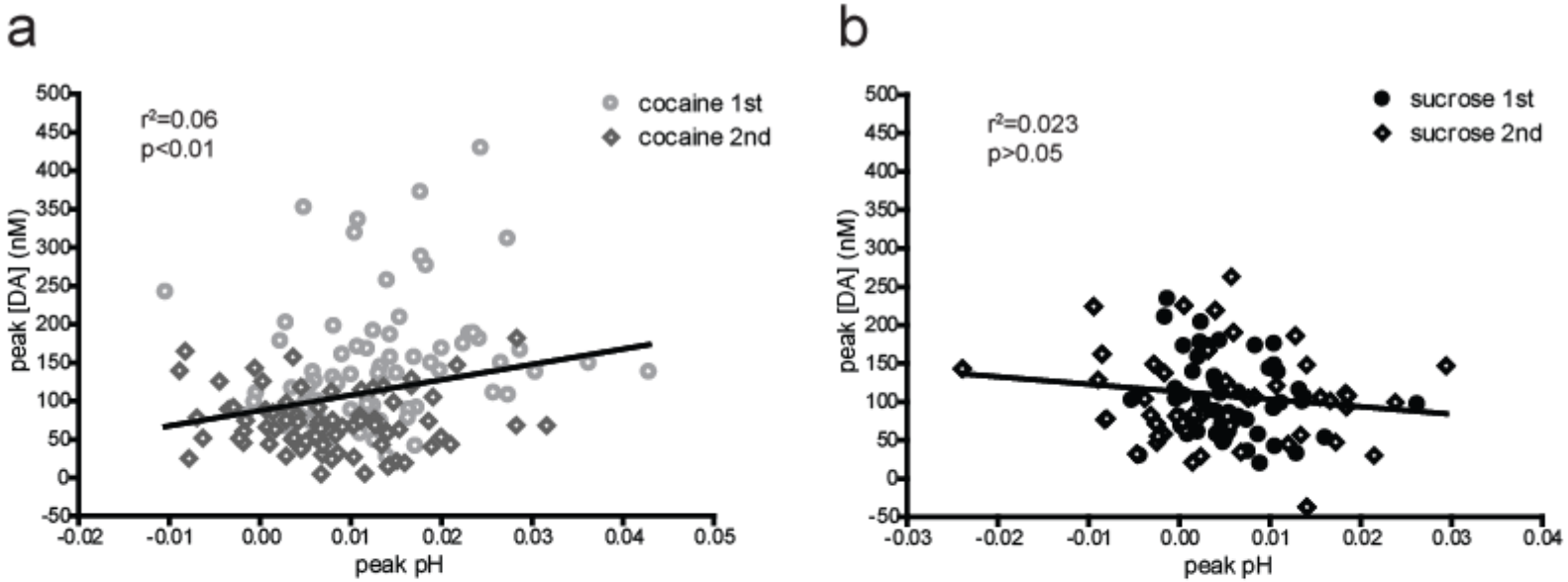

Figure 3.4. Linear regression analyses correlating peak pH with peak [DA] during cocaine or sucrose self-administration session. (a) A significant positive linear regression was observed between peak pH and peak [DA] during cocaine self-administration. Circles indicate data points from animals that self-administered cocaine in phase 1 of the multiple schedule. Diamonds indicate data points from animals that self-administered cocaine in phase 2 of the multiple schedule. (b) No linear regression was observed between peak pH peak [DA] during sucrose self-administration. Circles indicate data points from animals that self-administered sucrose in phase 1 of the multiple schedule. Diamonds indicate data points from animals that self-administered sucrose in phase 2 of the multiple schedule.

Therefore, higher peak [DA] was associated with a greater subsequent increase in peak pH. In contrast, no significant linear regression was observed between peak pH and peak [DA] during sucrose self-administration ($F(1,121) = 2.816$, $p > 0.05$, $r^2 = 0.023$; Fig. 3.4b).

Peak pH decreases over the course of cocaine self-administration, but not sucrose self-administration

To examine the relationship between basic shifts in pH across time in behavioral sessions, linear regression analyses were completed that correlated peak pH versus the time of lever press execution for all cocaine and sucrose trials. This analysis was performed using peak pH values obtained from the 7-10 s time period after lever press. This analysis window was chosen because sustained changes in pH were observed at this later time point for both reinforcers (see Fig. 3.2a). During cocaine self-administration, a significant negative linear regression was observed between peak pH and time in the self-administration session ($F(1,158) = 6.267$, $p < 0.05$, $r^2 = 0.038$; Fig. 3.5a). Thus, the peak pH observed on any one cocaine trial relative to the lever press decreased over the course of the 2 h self-administration session. In contrast, no significant linear regression was observed between peak pH and time in the sucrose self-administration phase ($F(1,157) = 0.002$, $p > 0.05$, $r^2 = 0.000$; Fig. 3.5b).

Interestingly, these changes in peak pH appeared to parallel changes in peak DA concentration over the course of the session. A trend toward a positive linear regression was observed between peak [DA] and time in the cocaine self-administration session ($F(1,158) = 3.865$, $p = 0.051$, $r^2 = 0.024$; Fig. 3.5c). Therefore, we observed a relationship with time in the cocaine self-administration for both peak pH and peak [DA]. Importantly, this relationship went in opposite directions depending on the analyte examined (a negative correlation for peak pH and a positive correlation for peak [DA]). Like the above peak pH analysis during sucrose self-

administration, no significant linear regression was observed between peak [DA] and time in the sucrose self-administration session ($F(1,153) = 0.179$, $p > 0.05$, $r^2 = 0.001$; Fig. 3.5d).

Histology

Histological reconstruction of carbon fiber electrode placements confirmed the location of recording sites in the NAc core (see Fig. 2.10a from Chapter 2).

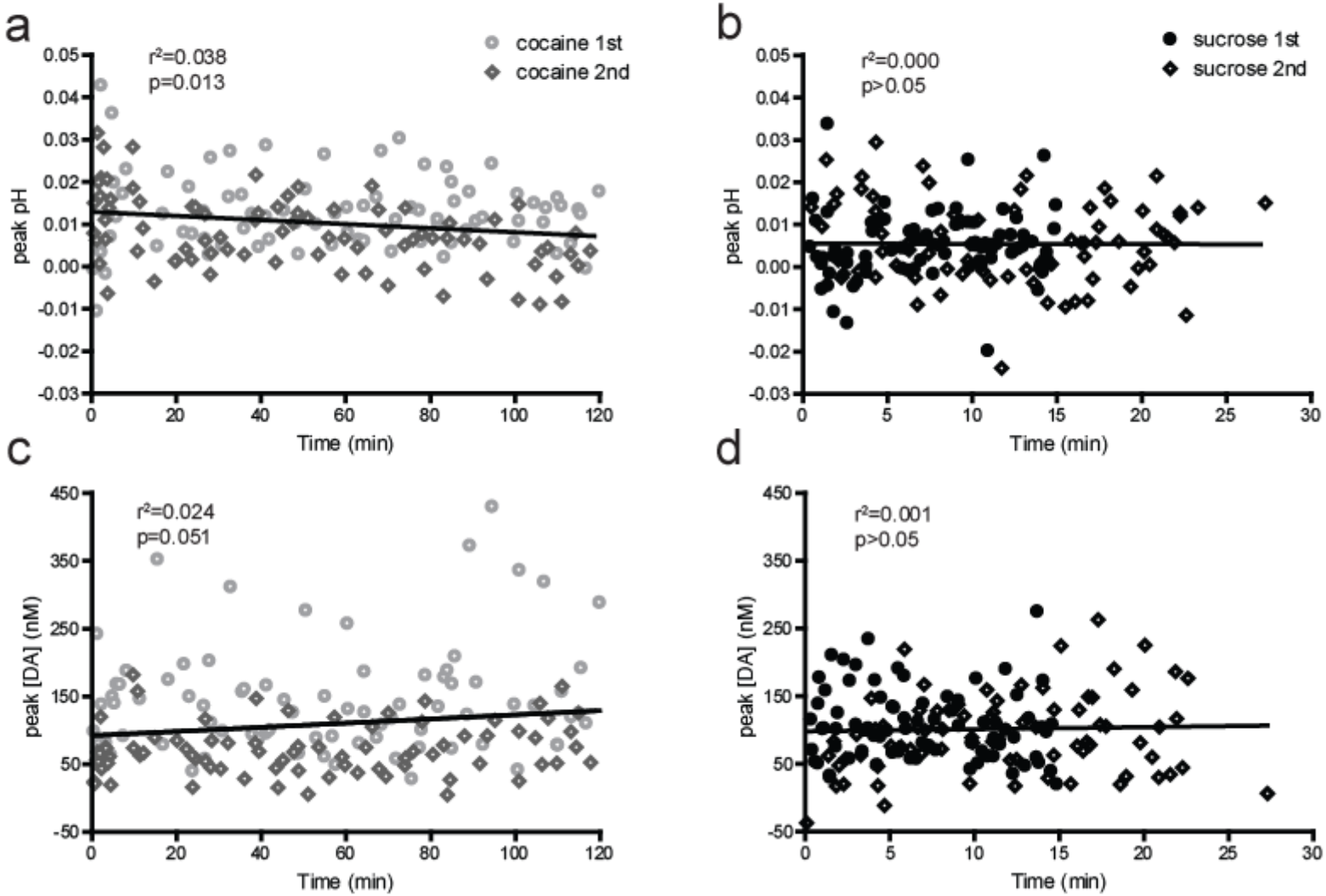

Figure 3.5. Linear regression analyses correlating peak pH or peak [DA] with time in cocaine or sucrose self-administration session. (a) A significant negative linear regression was observed between peak pH and time in the cocaine self-administration session. (b) No linear regression was observed between peak pH and time in the sucrose self-administration session. (c) A trend toward a significant positive linear regression was observed between peak [DA] and time in the cocaine self-administration session. (d) No linear regression was observed between peak [DA] and time in the sucrose self-administration session.

Discussion

The present study was completed to directly compare basic shifts in pH relative to operant responding for a natural reward (sucrose) versus intravenous cocaine. We observed increases in pH time locked to goal-directed behavior for both reinforcers. These increases began within seconds of the reinforced lever press response and were sustained for at least 10 s following reward delivery. The main finding of this study was that while pH increased over time during responding for both cocaine and sucrose, this increase was higher relative to lever press responding for cocaine. We also found that peak pH was correlated with peak DA concentration during cocaine-reinforced trials, but not during sucrose-reinforced trials. Finally, peak pH decreased over the course of cocaine self-administration, but not over the sucrose self-administration phase. These findings highlight important differences in the dynamics of pH signaling in the NAc during reward directed behaviors, considered in more detail below.

Basic pH shifts were higher relative to cocaine-responding than to sucrose-responding

pH changes have previously been observed in rats during intercranial self-stimulation (Kilpatrick *et al.,* 2000), following naturally occurring DA transients (Wightman & Robinson, 2002), and during responding for both natural (Roitman *et al.*, 2004) and drug (Phillips *et al.*, 2003b) rewards. Therefore, we replicated earlier findings demonstrating basic pH shifts timelocked to operant responding for sucrose and cocaine. As observed in these earlier studies, our findings showed that the shifts in pH followed the initial increase in DA that was associated with the lever press response. Importantly, the multiple schedule design used in the present study allowed for a direct comparison between the pH shifts associated with cocaine- versus sucrose-responding in a single NAc core location. These findings are the first direct evidence that pH

shifts associated with lever pressing for intravenous cocaine are higher than those associated with responding for the natural reinforcer sucrose.

Interestingly, unlike our findings with peak DA concentrations (see chapter 2), we found no effect of order of reinforcer presentation in peak pH shifts. Specifically, peak pH was higher relative to cocaine-responding whether cocaine was presented in phase 1 or phase 2 of the multiple schedule. This suggests that alterations in pH may be encoding certain absolute pharmacological properties of the reinforcer, such as interoceptive or metabolic changes associated with intravenous delivery of cocaine, rather than any relative hedonic properties that DA appeared to be encoding in the multiple schedule.

Despite these differences between pH and DA, the peak concentrations of the two analytes were correlated during cocaine self-administration (but not during sucrose self-administration). Specifically, on any one cocaine behavioral trial, a higher peak [DA] was associated with a greater subsequent increase in peak pH. This finding provides further evidence that pH shifts are associated with downstream changes in neural activity that are intimately linked with known changes in DA associated with operant responding for rewards. The lack of a correlation between peak pH and peak [DA] during sucrose self-administration could be related to the smaller pH shifts observed relative to sucrose-responding in the present study.

Finally, we found that the peak pH observed on each cocaine trial decreased over the course of the 2 h self-administration session. This change was paralleled by an increase in peak [DA] over the course of cocaine self-administration. Given previous findings showing that cocaine increases extracellular DA (Nicolaysen *et al.*, 1988) while simultaneously decreasing striatal blood flow and cortical oxygen pressure (Yonetani *et al.*, 1994), these findings are not surprising. pH shifts are linked to changes in extracellular oxygen associated with neural activity

(Venton *et al.*, 2003); therefore, a decrease in blood flow and oxygen caused by the pharmacological effects of cocaine would account for the decrease in peak pH over time that we observed here.

Implications for the detection of DA using electrochemical techniques

The findings described here and ealier in Chapter 2 highlight the importance of identifying and isolating different chemical signals from one another during behavior. For example, the basic shifts in pH observed following operant responding for cocaine and sucrose could be interpreted as a decrease in [DA] below baseline levels if they were not isolated and subtracted from the DA signal. In fact, earlier studies concluded that there was a decrease in DA following operant responding for food (Kiyatkin & Gratton, 1994; Richardson & Gratton, 1996), which was likely a reflection of changes in pH, not [DA].

Importantly, FSCV allows for the isolation of DA and pH signals using principal component regression (Heien *et al.*, 2004; Keithley *et al.*, 2010). Cyclic voltammograms (CVs) provide a chemical signature of an analyte by plotting current against applied potential during a voltammetric scan. Characteristic CVs for both DA and basic pH have been well-established (for example, Roitman *et al*., 2004; Venton *et al*., 2003). By collecting individual training sets from stimulated DA release in each experimental animal, we are able to construct representative, background subtracted CVs for DA and pH. These representative CVs can then be used to perform principal component regression on data collected during behavioral sessions in each animal. DA and pH signals can then be isolated and analyzed separately, as was done in the present study. Without this careful analysis of different electrochemical signals, it is difficult to make conclusions about the relative contributions of DA and pH during behavior.

Relationship of pH to neural activity, brain blood flow, and oxygen

Brain activity depends on blood flow to maintain a necessary supply of energy. One important function of increased blood flow is to deliver oxygen. Thus, a local increase in neural activity necessitates an increase in cerebral blood flow to that area. Ultimately, extracellular concentrations of oxygen are a balance between that provided by increased blood flow and that consumed by metabolism. Neural activity results in a transient increase in oxygen because oxygen delivery rates (through increases in blood flow) temporarily overshoot the increases in metabolism and oxygen use by neural activity (Fox & Raichle, 1986). These oxygen changes are coupled to changes in pH because they depend on the same processes. Local increases in oxygen clear CO_2 which causes a basic shift in pH (Urbanics *et al.*, 1978). This process is balanced by increases in metabolism which increase CO_2 and cause acidic shifts in pH (Chesler & Kaila, 1992; Chesler, 2003).

The temporary increase in oxygen after neural activation is the basis for blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (Raichle, 1998). Thus, examination of oxygen and pH changes in rats is an important step in bridging the gap between animal and human studies of brain activity. Importantly, previous work using FSCV has shown that basic pH shifts are intimately coupled to increases in brain oxygen that accompany increases in local neural activity (Venton *et al.*, 2003), thus validating the utility of measuring pH as a proxy for changes in blood flow and metabolism that underlie fMRI.

As described above, both basic and acidic changes in pH occur as a result of decreases or increases in CO_2 related to blood oxygenation and metabolism. While we did not observe any acidic pH shifts in the present study, FSCV work in primates has demonstrated both acidic and basic pH shifts in the striatum in response to reward delivery (Ariansen *et al.*, 2012). This

primate study observed two patterns of acidic pH changes following cue presentation: an acidic pH increase following a basic pH shift and an *initial* acidic pH shift. Again, acidic pH shifts are a result of increases in CO_2 that occur with increased metabolic activity. The acidic pH shift following the basic shift likely resulted from increases in metabolism exhausting the transient increases in oxygen. The initial acidic shift is hypothesized to be a result of a fast increase in oxygen consumption coinciding with beginning of neural activation (Kim *et al.*, 2000; Thompson *et al.*, 2003). Another interesting finding of the study by Ariansen and colleagues was that changes in pH overshadowed any observable changes in [DA]. Therefore, here we observed large reward-related changes in [DA] followed by basic shifts in pH. In primates, few changes in DA concentration were reported compared to pH shifts; and these shifts were both basic and acidic. Differences in the vasculature of rodent versus primate brains could account for the differences in these studies.

Conclusions

Though previous work consistently showed shifts in pH following increases in reward-related increases in [DA], these pH changes were never compared between different types of rewards from the same anatomic location. Overall, this study provides the first direct evidence that pH shifts associated with lever pressing for intravenous cocaine are different than those associated with responding for the natural reinforcer sucrose. These findings have important implications for analyzing reward-evoked DA using FSCV, highlighting the critical importance of using proper methods to isolate and separate these analytes. Finally, examination of pH changes in rodents can provide important insights into BOLD fMRI studies in humans by highlighting differences in the signals obtained with FSCV versus fMRI.

CHAPTER 4: ONE MONTH OF COCAINE ABSTINENCE POTENTIATES RAPID DOPAMINE SIGNALING IN THE NUCLEUS ACCUMBENS CORE

Introduction

In humans, cocaine addiction is characterized by excessive drug use followed by intermittent periods of abstinence and resumption of drug taking, or relapse (Gawin, 1991). This high propensity of addicts to relapse is often caused by increased craving for the drug that typically occurs following prolonged abstinence (Gawin & Kleber, 1986). In support, research with animal models has consistently shown that drug-seeking behavior progressively increases with longer periods of abstinence from cocaine self-administration, a phenomenon termed 'incubation of craving' (Tran-Nguyen *et al.*, 1998; Grimm *et al.*, 2001; Lu *et al.*, 2004).

Many studies have demonstrated that these motivational changes in behavior are correlated with neuroadaptations in brain areas important for reward processing, including the nucleus accumbens (NAc) (Robinson *et al.*, 2001; Lu *et al.*, 2003; Conrad *et al.*, 2008; Pickens *et al.*, 2011). Work from this lab using *in vivo* electrophysiology has shown that the percentage of NAc neurons that encode goal-directed behaviors for cocaine is dramatically increased following 30 days of cocaine abstinence (Hollander & Carelli, 2005; Hollander & Carelli, 2007). Further, NAc neural activity is also profoundly enhanced to cocaine cues following 30 days, compared to 1 day, of cocaine abstinence (Hollander & Carelli, 2007). These findings reflect a unique form of cellular plasticity related to drug-seeking behavior and cocaine-associated cues that develops following prolonged abstinence from cocaine.

The NAc is situated within the mesolimbic dopamine (DA) system and receives a dense dopaminergic projection from the ventral tegmental area (VTA). A common feature of many abused substances, including cocaine, is to increase mesolimbic DA (Di Chiara & Imperato, 1988); therefore, the DA projection from the VTA to NAc has long been implicated in drug addiction (Wise, 2004; Kalivas & Volkow, 2005). While we have demonstrated an increase in NAc core cell firing during cocaine-seeking and relative to cocaine cues following abstinence noted above, less is known about changes in NAc DA release dynamics under similar situations. Previous work using fast-scan cyclic voltammetry (FSCV) in anaesthetized rats has shown that even a short-term withdrawal paradigm (7 consecutive days of cocaine injections followed by 1 day of withdrawal) can cause a potentiation of DA signaling in the NAc in response to a subsequent cocaine challenge (Addy *et al.*, 2010). Given the well established cellular (Robinson *et al.*, 2001; Ferrario *et al.*, 2005; Hollander & Carelli, 2005; Hollander & Carelli, 2007), molecular (Toda *et al.*, 2002; Kalivas *et al.*, 2003; Lu *et al.*, 2003; Xi *et al.*, 2003; Conrad *et al.*, 2008) and behavioral (Tran-Nguyen *et al.*, 1998; Grimm *et al.*, 2001; Lu *et al.*, 2004) changes associated with prolonged abstinence (30 days), it is possible that larger changes in DA signaling may be observed following these longer periods of cocaine abstinence, and as a result of self-administered versus experimenter-administered drug.

Therefore, in this study we used FSCV to examine rapid DA release in the NAc of rats that had undergone 14 days of cocaine self-administration followed by 1 or 30 days of cocaine abstinence. After abstinence, all rats underwent a single extinction session (lever pressing had no programmed consequence). Rats were then deeply anesthetized and several measures of DA release dynamics were obtained in the NAc core before and following a single cocaine injection.

Using this approach, we reveal unique alterations in dopamine signaling linked to changes in drug-seeking behavior that are a result of prolonged cocaine abstinence.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA; $n = 14$) aged 90-120 days and weighing 260–350 g were used as subjects and individually housed with a 12/12 h light/dark cycle. Body weights were maintained at no < 85% of pre-experimental levels by food restriction (10–15 g of Purina laboratory chow each day). Water was available *ad libitum*. This regimen was in place for the duration of the experiment, except during the post-operative recovery period when food was given *ad libitum*. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Behavioral Training

All training was conducted in custom-made experimental chambers that consisted of a 43 × 43 × 53 cm Plexiglass chamber housed within a commercial sound-attenuated cubicle (Med Associates Inc., St Albans, VT, USA). One side of the chamber was equipped with two retractable levers (Coulbourn Instruments, Allentown, Pennsylvania) 17 cm apart, corresponding cue lights positioned 6 cm above each lever, and a reward receptacle located equidistantly between the levers.

Rats were surgically implanted with an intravenous catheter following established procedures, described in detail previously (Carelli *et al.*, 2000). Following recovery from surgery, rats were initially trained to self-administer food reinforcement (45 mg pellet; FR1) to

establish lever pressing behavior. Following acquisition of lever pressing (at least 50 presses during a 30 min session), rats were trained to self-administer cocaine in daily 2 hour sessions. The start of the self-administration session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. A response on the lever resulted in intravenous cocaine delivery (0.33 mg/infusion, approximately 1 mg/kg/infusion, 6 s) via a computer-controlled syringe pump, termination of the cue light and simultaneous onset of a compound tone (65 dB, 2900 Hz) /houselight cue (20 s), and retraction of the lever (20 s).

Rats were then divided into two groups (Day 1, n = 7; Day 30, n = 7). Day 1 rats underwent a single extinction session conducted 24 hours after the last day of cocaine self-administration. Day 30 rats were placed in abstinence for 30 days during which time they remained in their home cages without access to cocaine. Following this abstinence period these rats also underwent a single extinction session. The extinction session took place in the same behavioral chamber where rats self-administered cocaine. During extinction, the cue light above the cocaine-associated lever was illuminated and the lever was extended into the chamber; however, lever depression had no programmed consequence. The extinction session ended when an animal made no lever press response for 30 min. Immediately following each extinction session, rats were deeply anaesthetized with urethane and prepared for voltammetric recording (see below).

Surgery and Electrochemistry

Following completion of the extinction session, rats were surgically prepared for voltammetric recording in the NAc core as previously described (Addy *et al.*, 2010). Animals were deeply anesthetized with urethane (1.2g/kg, i.p.) and placed in a stereotaxic frame. An Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left

forebrain and held in place with a stainless steel skull screw. A bipolar stimulating electrode (Plastics 1 Inc., Roanoake, VA) was placed dorsally into the VTA (-5.2 mm posterior, -1.0 mm lateral from bregma, and -8 mm ventral from brain surface). During the experiment, carbon-fiber microelectrodes (90-110 μm length) were placed into the NAc core (+1.3 mm anterior, -1.3mm lateral from bregma, -6.5 to -7.5 mm ventral from brain surface).

Changes in DA concentration during electrical stimulation of the VTA were assessed using FSCV as previously described (Roitman *et al.*, 2004; Day *et al.*, 2007; Addy *et al.*, 2010). The carbon-fiber and Ag/AgCl reference electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Med-Associates, St. Albans, VT) at the top of the test chamber. Voltammetric recordings were made every 100 msec by applying a triangular waveform (-0.4 to +1.3 V, 400 V/sec). Data were digitized and stored to a computer using software written in LabVIEW (National Instruments, Austin, TX).

For each individual animal, a fresh carbon-fiber microelectrode was initially inserted 6 mm into the brain above the region of interest, and the triangular waveform was applied at a frequency of 60 Hz for 20 min. This conditioning phase increases the sensitivity of the electrode as a result of oxidative etching of the carbon-fiber surface that occurs with 60-Hz application of the −0.4 to 1.3 V waveform (Takmakov *et al.*, 2010). Waveform application was then changed to 10 Hz, and the electrode was lowered into the NAc core. Stimulating electrode placement was optimized along the dorsal-ventral axis by the presence of a slight whisker twitch in response to stimulation of the VTA (24 biphasic pulses, 60 Hz, 300 μA, 2 msec per phase). Microelectrode depth was optimized based on depth and the presence of a characteristic cyclic voltammogram for DA. Electrical stimulation was applied to the VTA and consisted of 24 biphasic pulses (300 μA), 2 ms each phase, applied at frequencies of 10, 20, 40, and 60 Hz with train lengths of 2.4,

1.2, 0.6, and 0.4 s, respectively. These stimulation parameters were repeated 5 times each in the predrug state. Rats then received an acute injection of cocaine (20 mg/kg, i.p.) followed by 60-Hz stimulations of the VTA performed every 5 min until the cocaine effect on rapid DA reached an asymptote. VTA stimulations were then repeated at 10, 20, 40, and 60 Hz to evaluate the cocaine effect at multiple stimulation frequencies.

Data Analysis

DA signals from FSCV were identified as previously described (Roitman *et al.*, 2004). For analyte identification, current during a voltammetric scan is plotted against applied potential to yield a cyclic voltammogram (the chemical signature of the analyte). Cyclic voltammetric data were analyzed on stimulation trials. A background signal from 1 voltammetric scan (100 msec time bin) before a stimulation was subtracted from the remainder of the scans to reveal changes in DA concentration (rather than absolute values).

DA is oxidized at 0.6 V on the positive scan of the voltage; therefore current was examined at this potential to reveal changes in DA current versus time during the trial (I vs. t). After the experiment, changes in current were converted to changes in DA concentration by post calibration. The carbon-fiber microelectrode was removed from the brain and placed into a flow-injection analysis system in which known DA concentrations, ranging from 100 nM to 3 μM, were presented at the electrode surface. The calibration curve was generated using 12 different electrodes and gave a linear response across dopamine concentrations with an r^2 value of 0.95 (data not shown). The equation generated from the linear response was used to determine electrode response of 27.03nA/μM. This calibration was used to determine the peak evoked concentration of DA observed in the brain, $[DA]_{max}$, after stimulation of the VTA.

$[DA]_{max}$ was compared with a three-way mixed design ANOVA (stimulation frequency x drug condition x abstinence condition) followed by Tukey HSD post hoc tests of significant effects. The percent of pre-drug baseline was calculated for each stimulation parameter by dividing $[DA]_{max}$ achieved post-drug injection by $[DA]_{max}$ achieved pre-drug injection. Percent of pre-drug baseline was then analyzed with a two-way mixed design ANOVA (stimulation frequency x abstinence condition) followed by Tukey HSD post hoc tests of significant effects.

We then examined release and uptake parameters at the 10Hz stimulation frequency (see Fig. 4.1). Release rate was calculated by fitting lines to the rising phase of DA release for each animal. Specifically, a linear regression was calculated using all data points (100 msec bins) from the beginning of stimulation to the peak evoked concentration, $[DA]_{max}$. The slope of this line was considered the release rate. Clearance half-rate ($t_{1/2}$) was calculated in Clampfit (Molecular Devices, LLC, USA) as the decay time between 100% and 50% evoked concentration for each animal. $t_{1/2}$ is the time required for [DA] to fall from its peak ($[DA]_{max}$) to half of this concentration and provides an accurate measure of DA uptake (Yorgason *et al.*, 2011). Net overflow was calculated in Clampfit using the area under the curve (AUC) for 10 s following the stimulation event for each animal. This provided another measure of DA release. Release rate, clearance half-rate, and AUC were compared with a two-way mixed design ANOVA (drug condition x abstinence condition).

All lever press events were recorded during performance of cocaine self-administration and the extinction session. A two-way mixed design ANOVA (self-administration day x abstinence condition) was performed on number of lever presses to compare cocaine self-administration history between Day 1 and Day 30 animals. The average number of cocaine-reinforced lever presses was compared between Day 1 and Day 30 animals with an unpaired *t*-

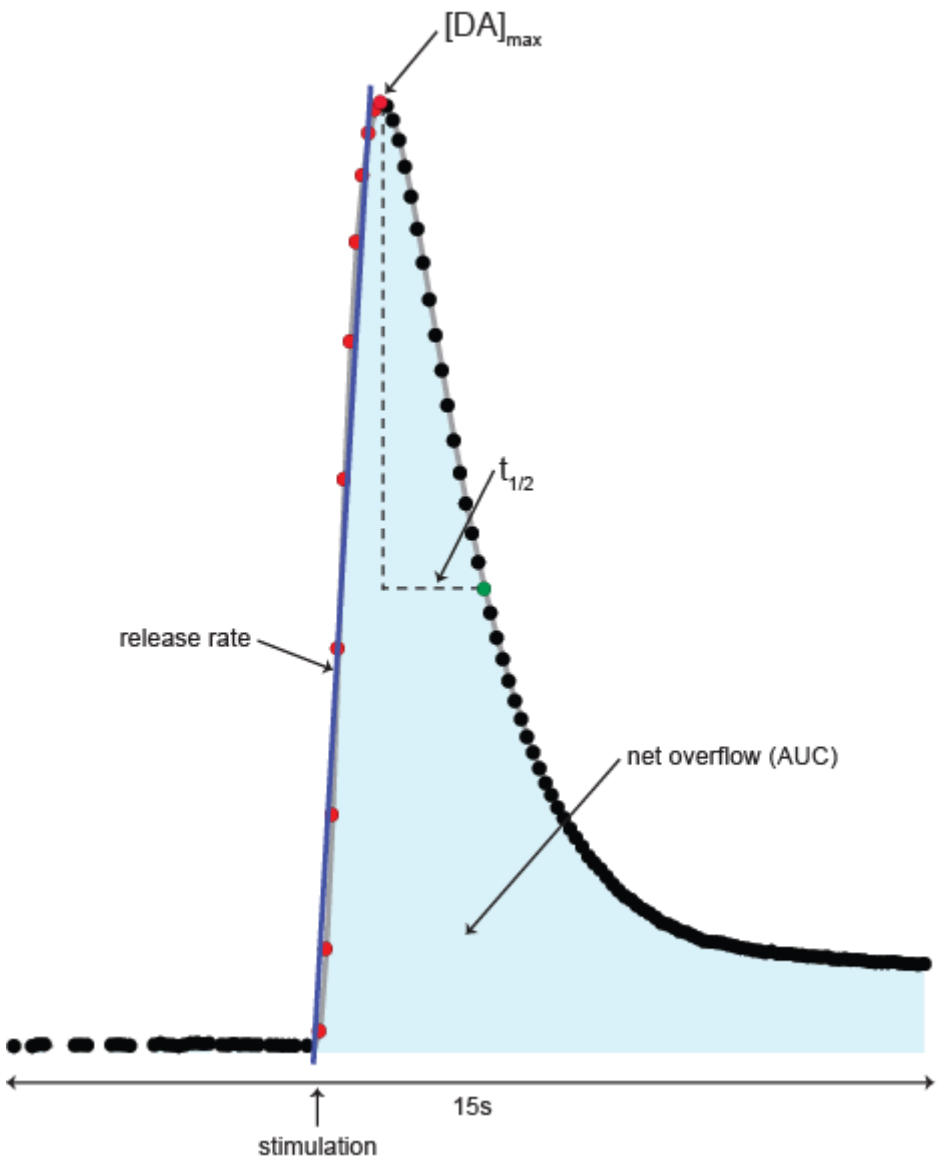

Figure 4.1. DA release and uptake parameters. Circles represent current collected every 100 msec during a representative stimulation file (VTA stimulation occurred at 5 s, indicated by arrow at this timepoint). Current was then converted to DA concentration (in µM) by post calibration of carbon fiber microelectrodes. **$[DA]_{max}$** represents the peak evoked concentration of DA (in µM). **Release rate** was calculated by fitting a line to the rising phase of DA release. A linear regression (blue line) was calculated using all data points from the beginning of stimulation to the peak evoked concentration (red circles). The slope of this line (in µM/s) was taken as release rate. **$t_{1/2}$** (represented by horizontal dotted line) was the time (in s) required for [DA] to fall from its peak ($[DA]_{max}$) to half of this concentration (indicated by green circle). **Net overflow** (light blue shaded area) was calculated as the area under the curve from stimulation to 10 s following stimulation (in µm · s).

test. The number of extinction presses and the amount of time spent in the extinction session were compared between Day 1 and Day 30 animals with unpaired *t*-tests. To examine the relationship between extinction behavior and changes in stimulated DA, linear regression analyses were completed that correlated the percent of pre-drug baseline versus the amount of time spent in the extinction session or the number of extinction presses completed in the first 10 min of the extinction session.

For all analyses, the alpha level for significance was 0.05. All statistics were performed with commercially available software (Statistica, Tulsa, Oklahoma; GraphPad Software, La Jolla, CA).

Histology

To mark the placement of electrode tips, a 209 µA current was passed for 10 s through a stainless steel electrode lowered to the location of the carbon-fiber microelectrode. Brains were removed and postfixed in 10% formalin. After post fixing and freezing, 50 µm coronal brain sections were taken and mounted throughout the rostral–caudal extent of the NAc. The specific position of individual electrodes was assessed by visual examination of successive coronal sections for electrolytic lesions.

Results

Behavioral responding during cocaine self-administration and extinction

Behavioral data was obtained from 7 rats that underwent 1 day of abstinence (D1 group) and 7 rats that underwent 30 days of abstinence (D30 group). There were no differences in cocaine self-administration in animals destined for the D1 or D30 groups. A two-way mixed design ANOVA with a within-subjects factor of day of cocaine self-administration training and a between-subjects factor of group conducted on the number of cocaine-reinforced lever presses revealed no main effect of group ($F(1,12) = 1.29$, $p > 0.05$), no main effect of day of self-administration ($F(9,108) = 1.336$, $p > 0.05$) and no group x day interaction ($F(9,108) = 1.375$, $p > 0.05$; Fig. 4.2a). This statistic was performed on data from the last 10 days of cocaine self-administration when all animals had achieved stable responding. Further, there were no differences in the average number of daily presses between groups over the entire 14 days of cocaine self-administration ($t_{12} = 0.7812$; $p > 0.05$; Fig. 4.2a, inset). Because animals self-administered under an FR1 schedule of reinforcement, this also indicates that there were no differences in the total number of drug infusions between animals destined for the D1 and D30 groups.

Similar to previous studies (Grimm *et al.*, 2001; Hollander & Carelli, 2005; Hollander & Carelli, 2007), extinction responding revealed an 'incubation' effect in D30 animals compared to D1 animals. That is, D30 animals lever pressed significantly more times during extinction than D1 animals ($t_{12} = 2.700$; $p < 0.05$; Fig. 4.2b). There was also a trend for D30 animals to spend more time in the extinction session compared to D1 animals ($t_{12} = 2.110$; $p = 0.057$; Fig. 4.2c).

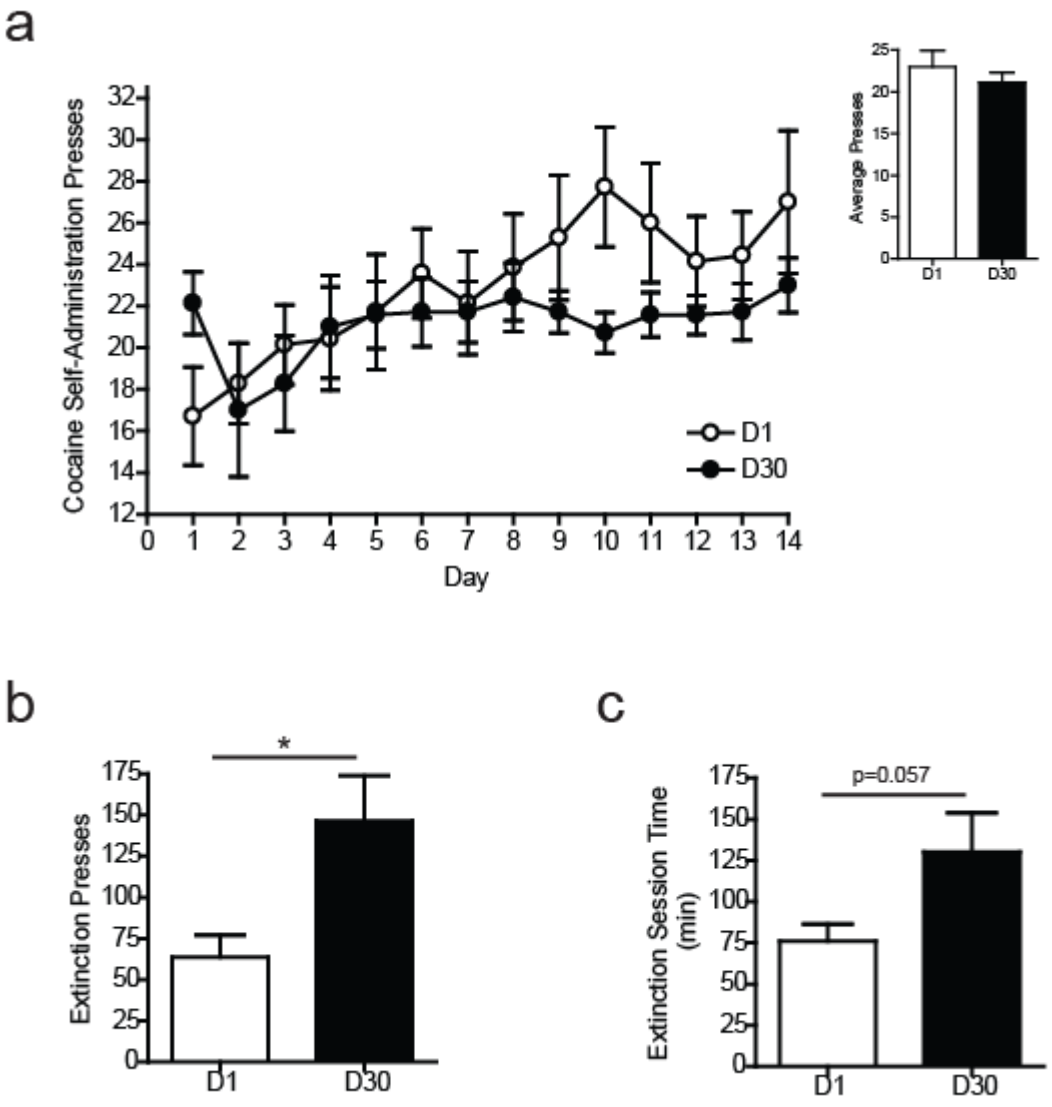

Figure 4.2. Behavioral responding during cocaine self-administration and extinction. (a) Mean ± sem presses during 14 days of cocaine self-administration training for animals destined for D1 (open circles) or D30 (closed circles) group. Inset: comparison of mean ± sem daily presses for animals destined for D1 (open bar) or D30 (closed bar) group. (b) Comparison of mean ± sem presses during extinction for animals that underwent 1 day of abstinence (open bar) versus animals that underwent 30 days of abstinence (closed bar), $*p < 0.05$. (c) Comparison of mean ± sem time spent in extinction for animals that underwent 1 day of abstinence (open bar) versus animals that underwent 30 days of abstinence (closed bar).

$[DA]_{max}$ is potentiated following an acute cocaine injection, and this potentiation is greater following 30 days of abstinence

To evaluate the effects of cocaine abstinence on rapid dopamine dynamics, we first examined $[DA]_{max,}$ the peak evoked concentration of DA, in rats that underwent 1 day versus 30 days of abstinence. Baseline FSCV recordings were first performed before experimenter-delivered drug administration, then following an injection of cocaine (20mg/kg, i.p.). In each condition (pre- and post-drug), we measured DA responses at 4 different electrical stimulation parameters (60, 40, 20 and 10Hz). $[DA]_{max}$ was compared with a three-way mixed design ANOVA (stimulation frequency as within-subjects factor x drug condition as within-subjects factor x abstinence condition as between-subjects factor). This analysis revealed no main effect of abstinence condition ($F(1,12) = 2.820$, $p > 0.05$), but a main effect of drug condition ($F(1,12) = 38.261$, $p < 0.0001$), and a main effect of stimulation frequency ($F(3,36) = 31.348$, $p < 0.0001$). There was no abstinence x drug x stimulation interaction ($F(3,36) = 0.683$, $p > 0.05$). Tukey HSD post hoc tests revealed that $[DA]_{max}$ was higher following cocaine injection at each stimulation parameter ($p < 0.05$; Fig. 4.3). This pattern held for both D1 (Fig. 4.3a) and D30 (Fig. 4.3b) animals.

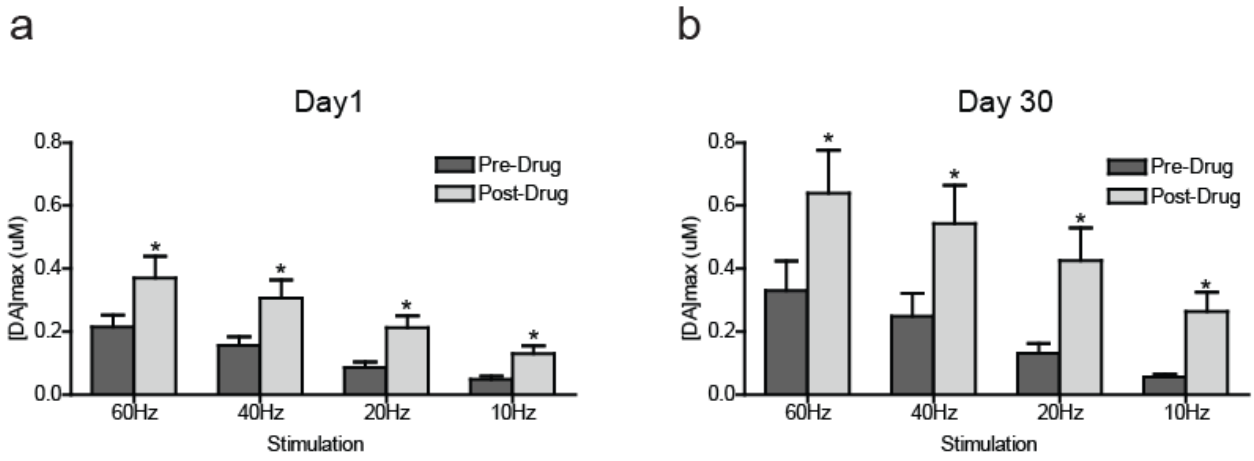

Figure 4.3. Electrically evoked DA release in the NAc core. (a) Peak evoked concentration of DA ($[DA]_{max}$) at 60, 40, 20, and 10Hz stimulation frequencies in animals that underwent 1 day of abstinence. (b) Peak evoked concentration of DA ($[DA]_{max}$) at 60, 40, 20, and 10Hz stimulation frequencies in animals that underwent 30 days of abstinence. Dark gray bars represent $[DA]_{max}$ before cocaine injection while light gray bars represent $[DA]_{max}$ following cocaine injection. Data shown are mean ± sem. Tukey HSD post hoc tests: $*p < 0.05$, post-drug compared to pre-drug within each stimulation frequency.

The ANOVA also showed a significant abstinence x drug interaction ($F(1,12) = 5.163$, $p < 0.05$). Collapsed across all stimulation parameters, the post-drug increase in $[DA]_{max}$ was significant in D30, but not D1, animals (Tukey HSD, $p < 0.05$; Fig. 4.4). Thus, while an injection of cocaine increased $[DA]_{max}$, this increase was potentiated in animals that underwent 30 days of abstinence compared to animals that underwent only 1 day of abstinence.

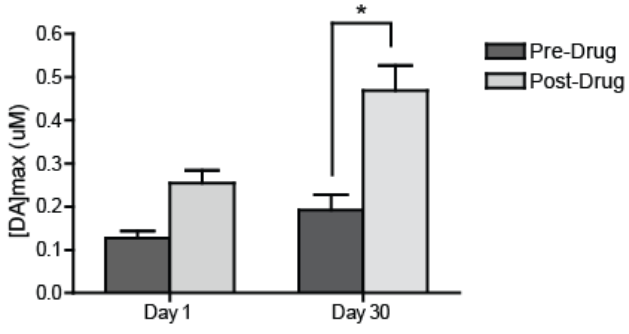

Figure 4.4. $[DA]_{max}$ is potentiated following 30 days of cocaine abstinence. Peak evoked concentration of DA ($[DA]_{max}$) collapsed across all stimulation frequencies for animals that underwent 1 day of abstinence (left) versus 30 days of abstinence (right). Dark gray bars represent $[DA]_{max}$ before cocaine injection while light gray bars represent $[DA]_{max}$ following cocaine injection. Data shown are mean ±sem. Tukey HSD post hoc tests: $*p < 0.05$, post-drug compared to pre-drug.

We also examined changes in $[DA]_{max}$ following an injection of cocaine by calculating the percent of pre-drug baseline (Fig. 4.5). 100% of pre-drug baseline would indicate no change in $[DA]_{max}$ in the post-drug (cocaine on board) compared to pre-drug (no cocaine) condition. Any percent higher than 100% would indicate an increase in $[DA]_{max}$ in the post-drug compared to pre-drug condition. This measure was then analyzed with a two-way mixed design ANOVA (stimulation frequency as within-subjects factor x abstinence condition as between subjects factor). This statistic revealed no main effect of abstinence condition ($F(1,12) = 2.177$, $p > 0.05$), but a main effect of stimulation frequency ($F(3,36) = 13.800$, $p < 0.0001$) and a significant abstinence x stimulation interaction ($F(3,36) = 3.827$, $p < 0.05$). Tukey HSD post hoc tests on the significant abstinence x stimulation interaction showed that, in D30 animals, the % of pre-drug baseline was higher for the 10Hz stimulation compared to the 60Hz or 40Hz stimulations. Thus, the potentiated increase in $[DA]_{max}$ in D30 animals was driven primarily by the lower 10Hz stimulation frequency.

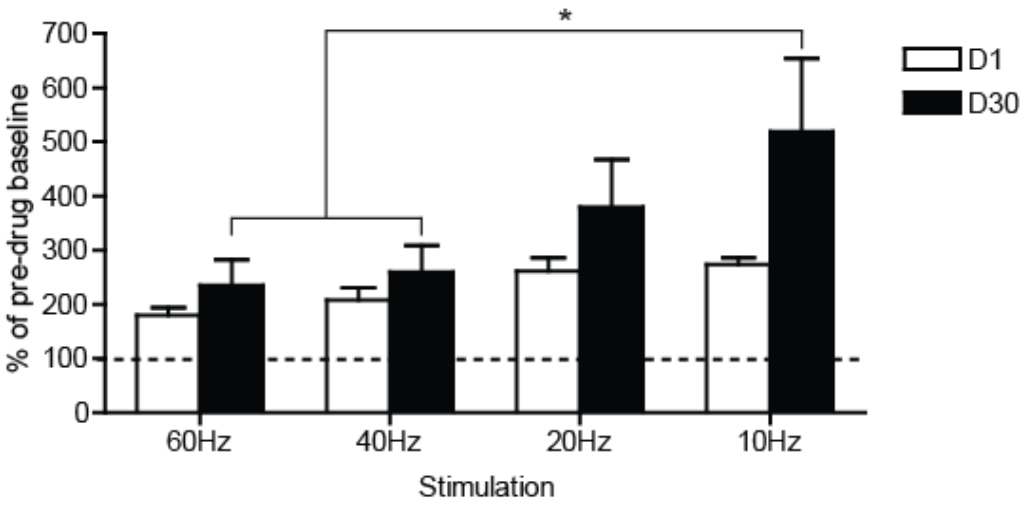

Figure 4.5. $[DA]_{max}$ percent of pre-drug baseline. 100% of pre-drug baseline (indicated by dotted line) represents no change in $[DA]_{max}$ in the post-drug (cocaine on board) compared to pre-drug (no cocaine) condition. Percentages higher than 100% represent an increase in $[DA]_{max}$ in the post-drug compared to pre-drug condition. Data shown are mean ± sem for D1 (open bars) and D30 (closed bars) animals. Tukey HSD post hoc tests: *$p < 0.05$, D30 10Hz compared to D30 60 and 40Hz.

Potentiated $[DA]_{max}$ following 30 days of cocaine abstinence appears related to facilitation of release

To determine the roles of release and uptake in the observed changes in $[DA]_{max}$, we analyzed rate of release, clearance half-life ($t_{1/2}$), and net overflow in DA concentrations evoked by the 10Hz stimulation parameter (Fig. 4.6). We chose this frequency since our above analysis showed that the potentiated increase in $[DA]_{max}$ in D30 animals was driven primarily by the 10Hz stimulation frequency. Figures 4.6a & b show average concentration traces of DA evoked by 10 Hz stimulation of the VTA for D1 and D30 animals. The first parameter in our analysis, release rate, was calculated as the slope of a line fit to the rising phase of DA release. Release rate was compared with a two-way mixed design ANOVA (drug condition x abstinence condition; Fig. 4.6c). This statistic revealed a main effect of drug ($F(1,12) = 21.007$, $p < 0.001$) , but no main effect of abstinence ($F(1,12) = 2.963$, $p > 0.05$), indicating an increase in release rate in both groups following injection of cocaine. However, the drug x abstinence interaction just missed statistical significance ($F(1,12) = 4.111$, $p = 0.06$). Tukey HSD post hoc tests on the drug x abstinence interaction showed a trend toward greater release rate following cocaine injection in D30 animals compared to D1 animals ($p = 0.07$). Together, these results suggest that release rate was potentiated following 30 days of abstinence. Next, we examined net overflow, defined as the area under the curve (AUC) for 10 s following the stimulation event. A similar pattern to release rate was observed for net overflow (Fig. 4.6d). Specifically, there was a significant main effect of drug ($F(1,12) = 28.220$, $p < 0.0001$), but no main effect of abstinence ($F(1,12) = 1.268$, $p > 0.05$), and a trend toward a significant drug x abstinence interaction ($F(1,12) = 4.157$, $p = 0.06$). Finally, clearance half-life ($t_{1/2}$) defined as the decay time between 100% and 50% evoked DA concentration, was not altered by abstinence condition (Fig. 4.6e). Specifically, a two-way

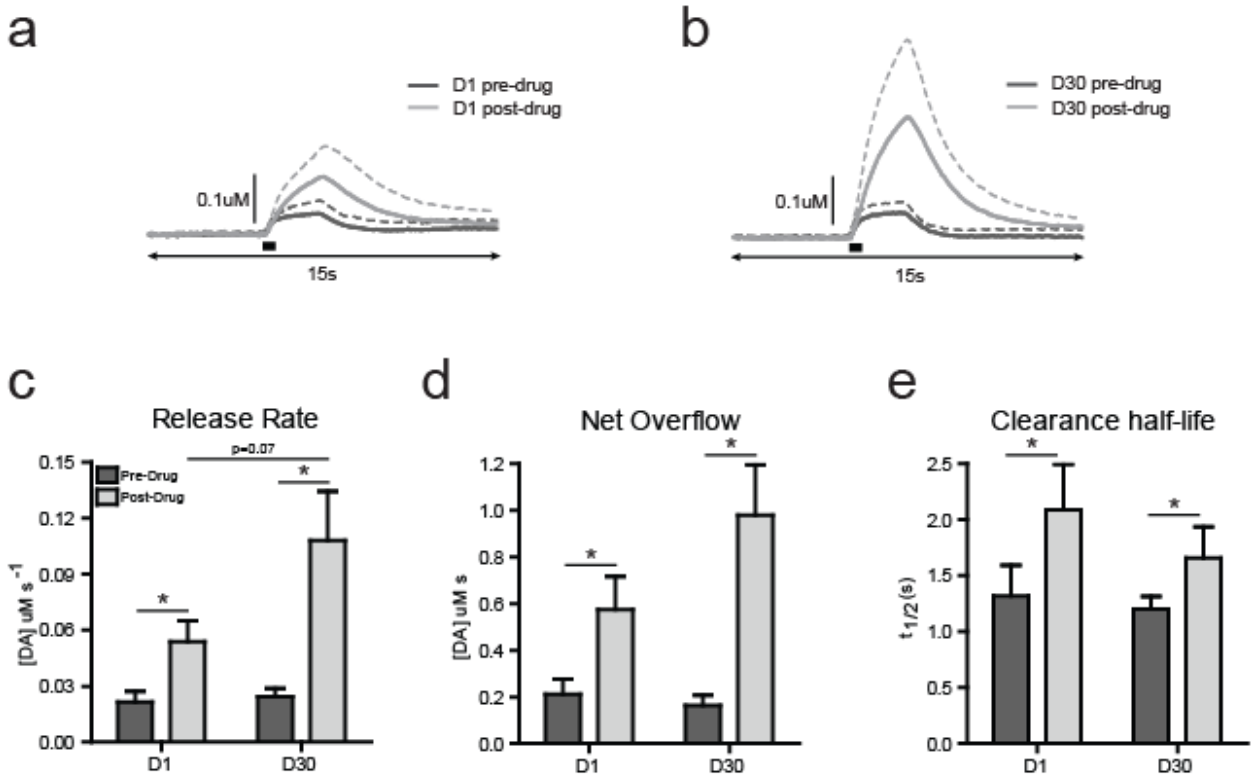

Figure 4.6. Analysis of release and uptake parameters for the 10Hz stimulation frequency. (a) Average concentration traces + sem of DA evoked by 10 Hz stimulation of the VTA in D1 animals (n = 7) before (dark gray line) and after (light gray line) injection of cocaine. (b) Average concentration traces + sem of DA evoked by 10 Hz stimulation of the VTA in D30 (n = 7) animals before (dark gray line) and after (light gray line) injection of cocaine. (c) Mean rate of change in [DA] during the rising phase of release evoked by 10Hz, 24 pulse stimulation. (d) Mean net overflow (area under curve) following 10Hz, 24 pulse stimulation. (e) $t_{1/2}$ of evoked DA following 10Hz, 24 pulse stimulation. Data shown are mean ± sem. Tukey HSD post hoc tests: *$p < 0.05$.

mixed design ANOVA (drug condition x abstinence condition) showed no main effect of abstinence ($F(1,12) = 0.653$, $p > 0.05$) and no abstinence x drug interaction ($F(1,12) = 0.541$, $p > 0.05$), but a significant main effect of drug ($F(1,12) = 7.15$, $p < 0.05$). Thus, consistent with its actions as a DA reuptake blocker, cocaine increased the clearance time of DA; however, this effect was not increased further by 30 days of cocaine abstinence.

Taken together, these data revealed a trend in release dynamics between D1 and D30 animals with prolonged abstinence increasing DA release. Somewhat surprisingly, we observed

no differences in clearance half-life between abstinence conditions. Therefore, it appears that the potentiated $[DA]_{max}$ observed in D30 animals may be due mainly to a facilitation of release rather than a potentiation of uptake inhibition by 30 days of abstinence.

Correlation between extinction behavior and changes in stimulated DA responses

To examine the relationship between extinction behavior and changes in stimulated DA, linear regression analyses were completed that correlated the $[DA]_{max}$ percent of pre-drug baseline versus the number of extinction presses. In D1 animals, no relationship was observed between the $[DA]_{max}$ percent of pre-drug baseline and extinction presses ($F(1,26) = 0.7575$, $p < 0.05$, $r^2 = 0.028$; Fig. 4.7a). D30 animals, a significant negative linear regression was observed between the $[DA]_{max}$ percent of pre-drug baseline and number of extinction presses ($F(1,26) = 4.838$, $p < 0.05$, $r^2 = 0.157$; Fig. 4.7b). Thus, animals with the most extinction presses had less potentiation in $[DA]_{max}$ following 30 days of abstinence.

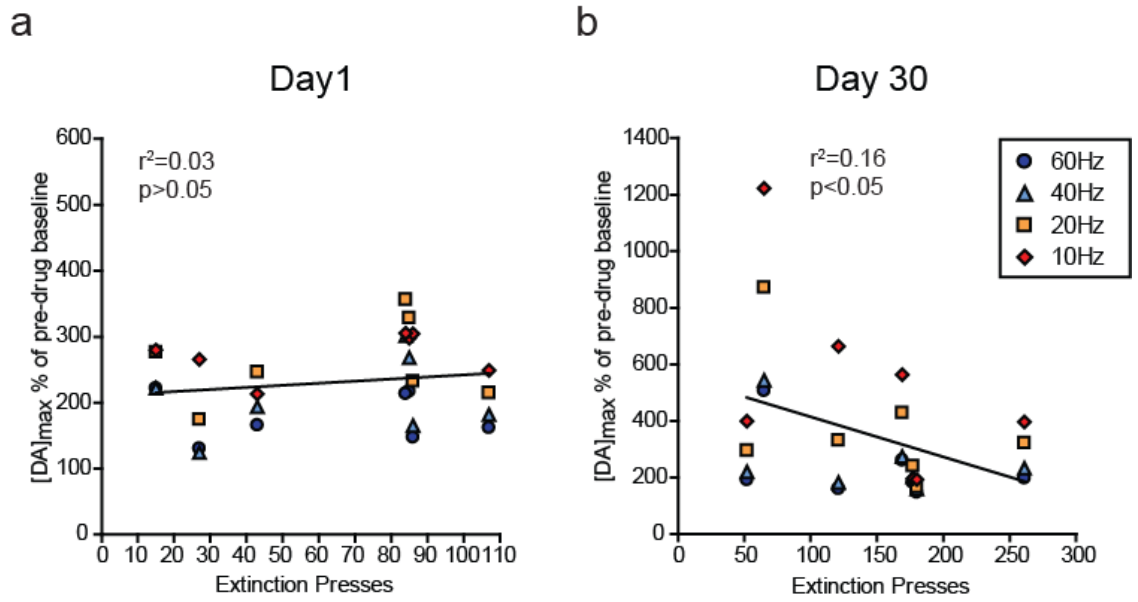

Figure 4.7. Linear regression analyses correlating $[DA]_{max}$ percent of pre-drug baseline with number of extinction presses. (a) No linear regression was observed between $[DA]_{max}$ percent of pre-drug baseline and extinction presses in D1 animals. (b) A significant negative linear regression was observed between $[DA]_{max}$ percent of pre-drug baseline and extinction presses in D30 animals.

Histology

Histological reconstruction of carbon fiber electrode placements confirmed the location of recording sites in the NAc core (Fig. 4.8). Only data from electrode placements within the borders of the NAc core, as depicted in the atlas of Paxinos & Watson (2007), were included in the analysis.

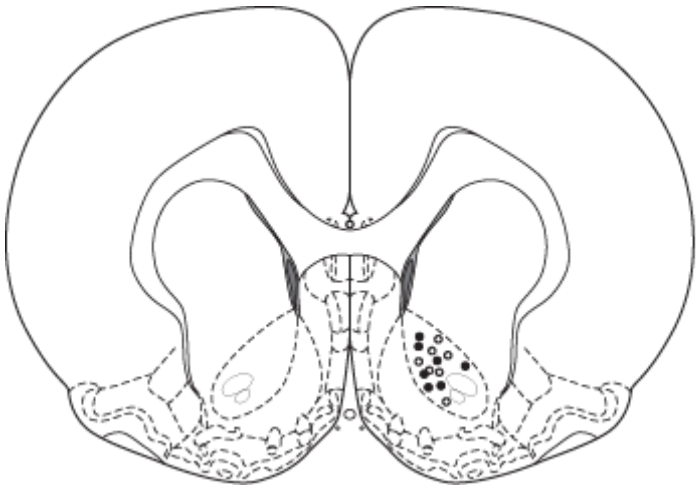

Figure 4.8. Histology. Schematic representation of electrode tip placements in the NAc core. Open circles represent recording sites from D1 animals. Closed circles represent recording sites from D30 animals.

Discussion

In this study, we used FSCV to examine rapid DA release in the NAc core of rats that had undergone 14 days of cocaine self-administration followed by 1 or 30 days of cocaine abstinence. We found that a month of cocaine abstinence potentiated the peak concentration of electrically evoked DA in the NAc following an acute injection of cocaine. Furthermore, this potentiation was largely driven by the lower 10Hz stimulation frequency. Examination of release and uptake parameters revealed that the potentiated $[DA]_{max}$ observed in D30 animals was due mainly to a facilitation of release rather than a potentiation of uptake inhibition. Finally, in D30 (but not D1 animals) we observed a correlation between electrically-evoked DA release and behavioral responding during extinction. The implications of these findings are discussed in below.

Increased $[DA]_{max}$ following a month of abstinence is due to a sensitized response to cocaine

A major finding of the present study is that the increase in $[DA]_{max}$ following an acute injection of cocaine was potentiated in animals that had undergone 30 days of cocaine abstinence compared to animals that underwent 1 day of abstinence. This finding could be a result of tolerance to the reuptake inhibiting effects of cocaine in D1 animals. In other words, in D1 rats repeated cocaine exposure during self-administration may result in less reuptake inhibition and, therefore, less extrasynaptic DA. As such, the potentiated evoked DA in response to a cocaine injection in D30 animals may represent a recovery of these effects over time. In contrast, the observed increase in $[DA]_{max}$ in D30 animals could be a result of a sensitized response to cocaine following a month of abstinence.

Previous studies have reported both increases and decreases in cocaine-induced levels of evoked DA concentration in the NAc following cocaine experience that may be related to

amount of cocaine, and route of administration. For example, Calipari and colleagues (2014) reported that extended access cocaine self-administration (1.5 mg.kg/inj; 6 h or 40 inj; 5 days) resulted in reduced electrically-evoked DA release in the NAc core measured by slice voltammetry, as well as a reduction in cocaine-induced DA overflow measured by microdialysis. Likewise, high-intake, 24 h access to cocaine self-administration for 10 days decreased extracellular DA concentrations and electrically stimulated DA release in the NAc core (Mateo *et al.*, 2005). This self-administration paradigm also attenuated DA release following an acute administration of cocaine (Mateo *et al.*, 2005). In contrast, 7 days of experimenter-administered cocaine injections resulted in a *potentiated* rapid DA response in the NAc core after an acute cocaine challenge (Addy *et al.*, 2010).

The different results of these studies highlight the importance of the cocaine administration paradigm used (experimenter-administered versus self-administration, extended versus short access, etc.). In fact, a direct comparison between several different cocaine self-administration paradigms showed that the temporal pattern of cocaine intake determined the subsequent effect on stimulated DA release in the NAc (Calipari *et al.*, 2013). Both short access (2 h, 14 days) and long access (6 h, 14 days) paradigms produced no changes in stimulated DA release compared to controls; however, intermittent access (6 h, 5 min access periods alternating with 25 min timeout periods, 14 days) resulted in increased stimulated DA release. The self-administration paradigm used in the present study is almost identical to the short access paradigm used by Calipari *et al.* (2013), thus it seems likely that the pattern of DA release observed in our D1 animals represents 'control' levels of electrically evoked DA. Therefore, the observed increase in $[DA]_{max}$ in D30 animals appears to be a result of a sensitized response to cocaine following a month of abstinence.

In further support of this interpretation, Hooks *et al*. (1994) reported a similar pattern in extracellular DA (as measured by microdialysis) following cocaine-self administration and 1 or 21 days of withdrawal. Specifically, there was no difference between rats that self-administered cocaine and yoked saline controls in the observed increase in extracellular dopamine following an acute cocaine challenge given after 24 h of withdrawal. However, when the cocaine challenge was given after 21 days of withdrawal, extracellular DA in the ventral striatum was significantly augmented in rats that self-administered cocaine compared to controls.

Previous work using cocaine similar self-administration paradigms similar to the one used in the present study show that 14 days of cocaine self-administration does not alter cocaine-evoked DA overflow in the NAc following 24 h of withdrawal (Hooks, 1994; Calipari *et al.*, 2013). Therefore, the increased $[DA]_{max}$ we observed in the present work after a month of cocaine abstinence is most likely a result of a sensitized response to cocaine.

The role of DA release and uptake dynamics following cocaine abstinence

We next determined the relative contributions of DA release and uptake in the potentiated cocaine-evoked $[DA]_{max}$ observed in D30 animals. It appeared that the potentiated $[DA]_{max}$ observed in D30 animals was due mainly to a facilitation of release rather than a potentiation of uptake inhibition. This is in contrast to several other studies which reported a potentiation in uptake inhibition by cocaine following a history of drug exposure (Addy *et al.*, 2010; Calipari *et al.*, 2013). A critical difference between these studies and the present work is the inclusion of a prolonged (30 day) abstinence period. One of the only other studies to examine DA release/uptake parameters following cocaine self-administration and abstinence found no differences between cocaine- and saline-treated rats in the levels of DA uptake, DA transporter (DAT) V_{max}, and K_m values, following 3 weeks of abstinence (Ramamoorthy *et al.*, 2010). Thus,

it appears that prolonged abstinence (at least 1 month) produces fundamentally different changes in DA signaling, primarily increasing cocaine-evoked DA release without differentially affecting uptake inhibition.

Several mechanisms could account for the abstinence-induced increases in DA release observed in the present study. One possibility is abstinence-induced neuroadaptations in the VTA that could potentiate presynaptic DA release. A single cocaine injection has been shown to enhance LTP at excitatory synapses onto DA neurons in the VTA (Ungless *et al.*, 2001). While this potentiation does not last beyond 5 days of withdrawal, others have shown that self-administration of cocaine, rather than experimenter-administered injections, produces a persistent potentiation of VTA excitatory synapses that persists after 3 months of abstinence (Chen *et al*., 2008). Furthermore, longer-lasting changes have been observed in corticotrophin-releasing factor (CRF)-mediated stress-induced DA release from the VTA (Wang *et al.*, 2005). A month of cocaine abstinence also increases brain-derived neurotrophic factor (BDNF) levels in the VTA, and BDNF injections into the VTA potentiate incubation of cocaine craving (Lu *et al.*, 2004). The exact mechanism of this potentiation in drug-seeking behavior remains to be elucidated as application of BDNF to the VTA does not enhance DA neuron synaptic strength by itself, but does facilitate the induction of *in vitro* LTP (Pu *et al.*, 2006).

A recent study by Bocklisch and colleagues (2013) also suggests that post-synaptic alterations in NAc neurons can alter DA release from the VTA. A subset of D_1-expressing MSNs in the NAc project to GABA neurons of VTA, and these neurons are capable of driving disinhibition of VTA DA neurons. Further, exposure to cocaine evokes synaptic potentiation in these MSNs, further increasing disinhibition of VTA neurons and increasing DA release to the NAc (Bocklisch *et al.*, 2013).

A greater potentiation in DA release following abstinence is correlated with a reduction in extinction responding

In D30 animals, we observed an intriguing correlation between DA release and behavioral responding. Specifically, animals that had a greater potentiation in $[DA]_{max}$ (calculated as the percent increase from pre-drug baseline after an injection of cocaine) made fewer extinction presses. We observed no correlation between the $[DA]_{max}$ percent of pre-drug baseline and extinction responding in D1 animals. The lack of a correlation in D1 animals suggests that NAc DA release does not exert a consistent influence on drug-seeking behavior before and after a short (24 hour) abstinence period. Instead, we propose that DA release in the NAc interacts with other abstinence-induced neuroadaptations such that a correlation between DA release and drug-seeking behavior emerges following a month of cocaine abstinence. Furthermore, DA release likely exerts a compensatory effect on these other neuroadaptations since a greater potentiation in $[DA]_{max}$ was correlated with less drug-seeking behavior.

Based on the above findings, a likely target for DA's actions following abstinence may be the enduring neuroplasticity in glutamate signaling from the PFC to the NAc (Kalivas, 2009). Cocaine self-administration followed by withdrawal leads to a disruption in glutamate homeostasis, specifically in the afferent PFC projection to the NAc. Reduced cystine-glutamate exchange causes a decrease in extrasynaptic glutamate levels (Baker *et al.*, 2003). Extrasynaptic glutamate acts on presynaptic metabatropic glutamate receptors 2 and 3 (mGluR2/3) to inhibit synaptic release of glutamate (Xi *et al.*, 2002). Thus, reduced extrasynaptic glutamate releases the inhibitory effect of mGluR2/3, leading to an increase in synaptic glutamate release when PFC-NAc projections are activated during drug-seeking behavior (McFarland *et al.*, 2003).

Importantly, DA does not have direct excitatory or inhibitory actions on NAc MSNs, but instead functions to modulate incoming glutamatergic activity. Specifically, NAc DA signaling

dampens the effect of glutamatergic input from the PFC, and this inhibitory action appears to be mediated through D_2 receptors (Brady & O'Donnell, 2004; Goto & Grace, 2005). Taken together with disrupted glutamate homeostasis following cocaine withdrawal, this pattern of DA signaling offers a potential model for the correlation between DA release and drug-seeking behavior observed in this study (Fig. 4.9).

In our proposed model, D1 animals (24 hour abstinence) have normal levels of extrasynaptic glutamate, and thus normal inhibition of synaptic glutamatergic signaling through presynaptic mGlu2/3 receptors. These animals also have baseline levels of DA signaling. Therefore, their behavior represents "normal" or "adaptive" extinction of drug-seeking behavior. In contrast, we propose that D30 animals (one month of cocaine abstinence) have both potentiated PFC-NAc glutamatergic signaling and potentiated VTA-NAc dopaminergic signaling in response to drug-paired stimuli. These two neurotransmitters exert opposing actions on behavior, with glutamate potentiating drug-seeking and dopamine blunting this effect. Therefore, more DA signaling leads to less extinction responding. It is important to note that overall, D30 animals pressed more during extinction than D1 animals, likely due to potentiated PFC-NAc glutamate signaling. However, within the D30 group, individual variability in extinction pressing between animals could be attributed to individual differences in DA signaling because a greater potentiation in $[DA]_{max}$ was correlated with a reduction in drug-seeking behavior. In support of this hypothesis, a single cocaine injection (which increase NAc DA levels) completely abolishes the behavioral incubation effect following a month of abstinence (Lu *et al.*, 2004). Future studies that directly manipulate NAc DA levels (in combination with antagonism of D_1 or D_2 receptors)

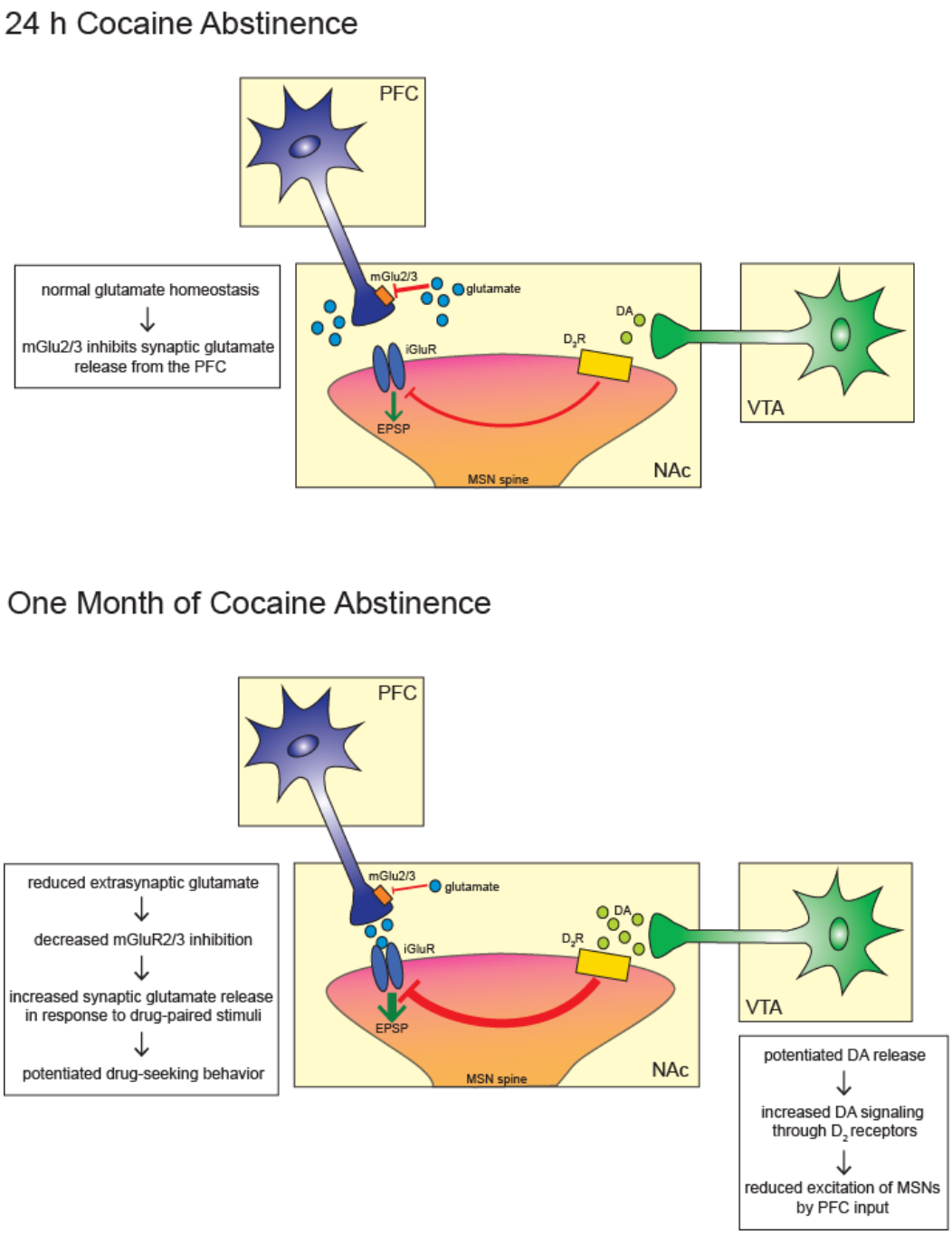

Figure 4.9. Proposed model for the interaction between glutamatergic and dopaminergic signaling in the NAc. Following one month of cocaine abstinence, both PFC-NAc glutamatergic signaling and VTA-NAc dopaminergic signaling are potentiated. See text for more details. Green lines represent excitatory neurotransmission. Red lines indicate inhibitory modulation. Line thickness represents relative signaling strength. PFC: prefrontal cortex; VTA: ventral tegmental area; NAc: nucleus accumbens; MSN: medium spiny neuron; DA: dopamine; mGlu2/3: metabotropic glutamate receptor type 2/3; iGluR: ionotropic glutamate receptor; D_2R: D_2-like dopamine receptor; EPSP: excitatory postsynaptic potential

during extinction are necessary to confirm whether this model accurately explains the findings of the present study.

Conclusions

Overall, the results of this study further support the abundance of literature showing that cocaine abstinence induces neuroplasticity in brain areas implicated in drug reward and relapse. Here we demonstrated that a month of abstinence results in a potentiated response to cocaine, resulting in greater electrically evoked DA concentrations in the NAc core, a brain region critical for drug reward. This increase in extracellular DA was primarily due to an increase in release rather than uptake inhibition, demonstrating an important difference between abstinence-induced neuroadaptations in DA signaling and those caused by drug exposure itself. Finally, we showed that a greater potentiation in DA release following abstinence was correlated with a reduction in extinction responding. We hypothesize that this relationship between DA signaling and behavioral responding could be due to an interaction between the mesolimbic DA system and persistent neuroplasticity in glutamate signaling from the PFC to the NAc induced by cocaine withdrawal. Further studies are necessary to explore this possibility, but if proven accurate this model suggests that increases in NAc DA signaling could prove a suitable target for therapeutic interventions to prevent relapse following cocaine abstinence.

CHAPTER 5: GENERAL DISCUSSION

Summary of experiments

The studies described in the previous chapters were designed to investigate the role of rapid DA signaling and pH shifts on learning about natural and drug rewards, as well as the influence of cocaine abstinence on DA release and uptake dynamics. Taken together, the results indicate that DA signaling in the NAc is highly dynamic and can be influenced by many factors, including the type of reinforcer (natural or drug) being self-administered or the pattern of drug exposure (1 day versus 30 days of abstinence). Furthermore, rapid DA signaling does not interact with NAc cell firing in a simple manner, but instead differentially modulates neuronal activity depending on many factors including reward type, specific afferent-efferent projections, and the presence of different receptor subtypes. A brief summary of each experiment is presented below.

Rapid DA release during responding for a natural versus drug reward or two natural rewards

The study described in chapter two examined rapid DA release dynamics in the NAc core during operant responding for a drug (cocaine) versus a natural reward, or two natural rewards (sucrose and food), using multiple schedule designs. DA release events in discrete NAc locations were compared across the reinforcer conditions within the same behavioral session. During the cocaine/sucrose multiple schedule, similar increases in rapid DA signaling were observed during both phases of the task. Thus, unlike the selective cell firing of NAc neurons related to reinforcer type observed in previous studies (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Carelli & Wondolowski, 2006; Cameron & Carelli, 2012), rapid DA release does not 'turn

on' or 'turn off' across each phase of the cocaine/sucrose multiple schedule. This finding indicates that rapid DA release is not reinforcer specific during situations involving goal-directed actions for cocaine versus a natural reward. However, more subtle differences were observed in peak DA concentration across the drug versus natural reward conditions related to the order of reinforcer presentation, the type of reinforcer, and the time to reach peak [DA]. Specifically, when cocaine was self-administered in phase 1, peak [DA] was higher relative to cocaine-responding than during sucrose-responding in phase 2, and vice versa for the opposite order of presentation. The time to reach peak [DA] was delayed during cocaine-responding compared to responding for sucrose. Similar DA release dynamics were observed during operant responding for two natural rewards, sucrose and food; however, we did not observe any effects of reinforcer order or time to reach peak [DA]. Overall, the results of this study support the idea that the mesolimbic dopamine system processes drug rewards in a different way than it does natural rewards.

pH shifts measured by FSCV during responding for sucrose versus cocaine

The findings presented in chapter three directly compared basic shifts in pH relative to operant responding for a natural reward (sucrose) versus intravenous cocaine. We observed increases in pH time locked to goal-directed behavior for both reinforcers; however, these increases were higher relative to lever press responding for cocaine than for sucrose. We also found that peak pH was correlated with peak DA concentration during cocaine-reinforced trials, but not during sucrose-reinforced trials. Finally, peak pH decreased over the course of cocaine self-administration, but not over the sucrose self-administration phase. These findings highlight important differences in the dynamics of pH signaling in the NAc during reward-directed behaviors. Further, these findings have important implications for analyzing reward-evoked DA

using FSCV, highlighting the critical importance of using proper methods to isolate and separate these analytes. Examination of pH changes in rodents can also provide important insights into BOLD fMRI studies in humans given the well-established link between pH and blood oxygenation.

Rapid DA signaling in the NAc following 30 days of cocaine abstinence

The study described in chapter four used FSCV to examine rapid DA release in the NAc core of rats that had undergone 14 days of cocaine self-administration followed by 1 or 30 days of cocaine abstinence. We found that a month of cocaine abstinence potentiated the peak concentration of electrically evoked DA in the NAc following an acute injection of cocaine. Furthermore, this potentiation was largely driven by the lower 10Hz stimulation frequency. Examination of release and uptake parameters revealed that the potentiated $[DA]_{max}$ observed in D30 animals was due mainly to a facilitation of release rather than a potentiation of uptake inhibition. Finally, in D30 (but not D1 animals) we observed a correlation between evoked DA release and behavioral responding during extinction, such that greater potentiation in DA signaling led to a decrease in extinction responding.

General discussion and relevance of findings

Although the unique implications of each study are discussed individually following each original data chapter, these findings also have further implications for how rapid DA signaling in the NAc interacts with phasic neural activity to influence subsequent behavior. This interaction is modulated by many factors, but the findings of this dissertation provide unique insights into the role of reward type (natural versus drug) and cocaine abstinence. Therefore, these topics are discussed below.

The relationship between mesolimbic DA release and NAc cell firing during responding for natural versus drug rewards

The studies described in this dissertation were guided by previous electrophysiology work from this lab (Carelli *et al.*, 2000; Carelli, 2002; Carelli & Wondolowski, 2003; Hollander & Carelli, 2005; Carelli & Wondolowski, 2006; Hollander & Carelli, 2007; Cameron & Carelli, 2012). We took this approach because rapid DA signaling and NAc cell firing are functionally linked. Phasic DA release exerts its effects postsynaptically by altering cellular activity in NAc MSNs (O'Donnell *et al.*, 1999; Nicola *et al.*, 2000; O'Donnell, 2003). Thus, we hypothesized that rapid DA release might mirror the phasic firing patterns of NAc neurons during goal-directed behaviors in our multiple schedule design. However, the study described in chapter two demonstrated that this is not always the case. Unlike previous electrophysiology studies, we did not observe reinforcer specificity in DA release dynamics during lever pressing for a natural reinforcer (sucrose) versus intravenous cocaine. In fact, we observed rapid DA release during both phases of the sucrose/cocaine multiple schedule. Ultimately these findings support the role of DA as a neuromodulator. That is, phasic DA release does not simply inhibit or excite NAc MSNs, but instead modulates the ability of afferent projections to elicit cell firing (O'Donnell *et al.*, 1999; Nicola *et al.*, 2000; O'Donnell, 2003).

In further support of the role of DA as a neuromodulator, work combining FSCV with simultaneous electrophysiological recordings from the same electrode reveal that DA release occurs primarily at NAc locations with phasic cell activity and little to no DA release is observed at sites with nonphasic cell activity (Owesson-White *et al.*, 2009; Cacciapaglia *et al.*, 2011). While these findings might initially suggest that rapid DA release directly influences phasic cell firing, pharmacological manipulations show that this is not always the case. For example, reducing phasic DA release in the NAc has no effect on phasic inhibition of NAc neurons (Cheer

et al., 2005; Cacciapaglia *et al.*, 2011), but does reduce phasic excitation of NAc neurons (Cacciapaglia *et al.*, 2011). Further, antagonism of D1-type DA receptors selectively reduces excitatory phasic activity of NAc neurons without altering DA release (Cheer *et al.*, 2005). Together these findings suggest that phasic (subsecond) DA plays a clear role in excitatory NAc cell firing, but is perhaps not functionally linked to inhibitory activity. Thus, rapid DA release may not directly influence global NAc phasic activity, but make certain neurons more attuned to glutamatergic afferent inputs from brain regions such as the prefrontal cortex, basolateral amygdala and hippocampus. Within distinct NAc microcircuits, rapid DA release likely modulates NAc cell activity based on a variety of factors such as DA receptor sub-type, phasic activity (excitatory versus inhibitory), reward type (sucrose versus food versus cocaine), or ongoing behavior.

Integrating rapid DA signaling, NAc cell-firing, reward type, and cocaine abstinence

Chapter two of this dissertation demonstrated that rapid DA signaling is not reinforcer specific during responding for sucrose versus cocaine, while chapter four found that NAc DA signaling is potentiated following 30 days of cocaine abstinence. However, this leaves open the important question of the interplay between these two components (reward type and abstinence). While we did not examine the effects of a month of cocaine abstinence on rapid DA signaling during performance of a sucrose/cocaine multiple schedule in this dissertation, we previously examined the interaction between abstinence and responding for a natural versus drug reward on NAc cell firing using *in vivo* electrophysiology (Cameron & Carelli, 2012). Given the model proposed in chapter four describing the relationship between disrupted glutamate homeostasis and DA signaling following cocaine withdrawal, we can hypothesize a possible role for DA signaling in responding for a natural versus drug reward following cocaine abstinence.

In our electrophysiology study (Cameron & Carelli, 2012) we found that, prior to abstinence, encoding was consistent with earlier work (Carelli *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003). Specifically, a subset of NAc neurons encoded goal-directed behaviors for both intravenous cocaine and sucrose; however, the majority of neurons were selective for either sucrose- or cocaine-related information. After abstinence the majority of NAc cells continued to display differential, nonoverlapping patterns of phasic activity relative to cocaine-reinforced vs. sucrose-reinforced responding. However importantly, there was a significant *increase* in the overall percentage of cells that displayed phasic activity relative to cocaine-related information and a significant *decrease* in the percentage of cells that displayed phasic activity relative to sucrose-related information.

Although others have documented an incubation of sucrose craving (Lu *et al.*, 2004; Grimm *et al.*, 2005), we have not observed this effect (either behaviorally or in terms of an increase in NAc cell firing) in our previous work (Jones *et al.*, 2008; Cameron & Carelli, 2012). This is in contrast to our work examining incubation of cocaine craving, in which we observed a dramatic increase in the percentage of NAc neurons that encode goal-directed behaviors for cocaine as well as a concomitant increase in drug-seeking behaviors following a month of abstinence (Hollander & Carelli, 2005; Hollander & Carelli, 2007). Furthermore, evidence suggests that abstinence-induced neuroadaptations in PFC-NAc glutamate signaling are specific to cocaine-related relapse (McFarland *et al.*, 2003; Kalivas *et al.*, 2005; Kalivas & O'Brien, 2007). Taken together, these findings suggest that a month of cocaine abstinence would differentially affect the sucrose-selective versus cocaine-selective populations of NAc neurons recorded in our earlier electrophysiology study, leading to an increase in the percentage of the

former population but a decrease in the percentage of the latter population of cells (Cameron & Carelli, 2012).

Specifically, glutamate homeostasis would be disrupted in neurons that selectively encode cocaine-related information, but unaltered in cells selective for sucrose-related information. Overall, a month of cocaine abstinence leads to a potentiation in VTA-NAc DA signaling (shown in chapter four). The data presented in chapter two suggests that rapid DA signaling in the NAc is not reinforcer specific for sucrose versus cocaine, although as noted, we did not examine rapid DA signaling in our sucrose/cocaine multiple schedule following prolonged (1 month) abstinence. Regardless, I developed a model of the role of glutamate and dopamine signaling on NAc reinforcer selective cell firing under 'normal' conditions (i.e., baseline,) versus how neuroadaptations in this chemical signaling may alter NAc phasic activity following extended (1 month) cocaine abstinence, diagramed in Fig. 5.1.

In this model, D1 represents 'baseline' activity in PFC-NAc glutamate signaling, VTA-NAc DA signaling, and the influence of this combined chemical signaling on NAc cell firing that encodes sucrose (left) versus cocaine (right). While glutamate signaling from the PFC increases NAc cell firing, DA signaling serves primarily to dampen the excitatory input from the PFC to the NAc (Brady & Odonnell, 2004; Goto & Grace, 2005; also see Fig. 4.9). Therefore, these two processes tend to act in opposition to one another and help orchestrate the activation of selective populations of NAc neurons to encode sucrose versus cocaine directed actions. Indeed, as noted above, our prior electrophysiology work (Cameron & Carelli, 2012) showed that separate populations of NAc neurons selectively encode sucrose versus cocaine-related information (i.e., sucrose-selective cells show phasic activity relative to responding for sucrose but not cocaine,

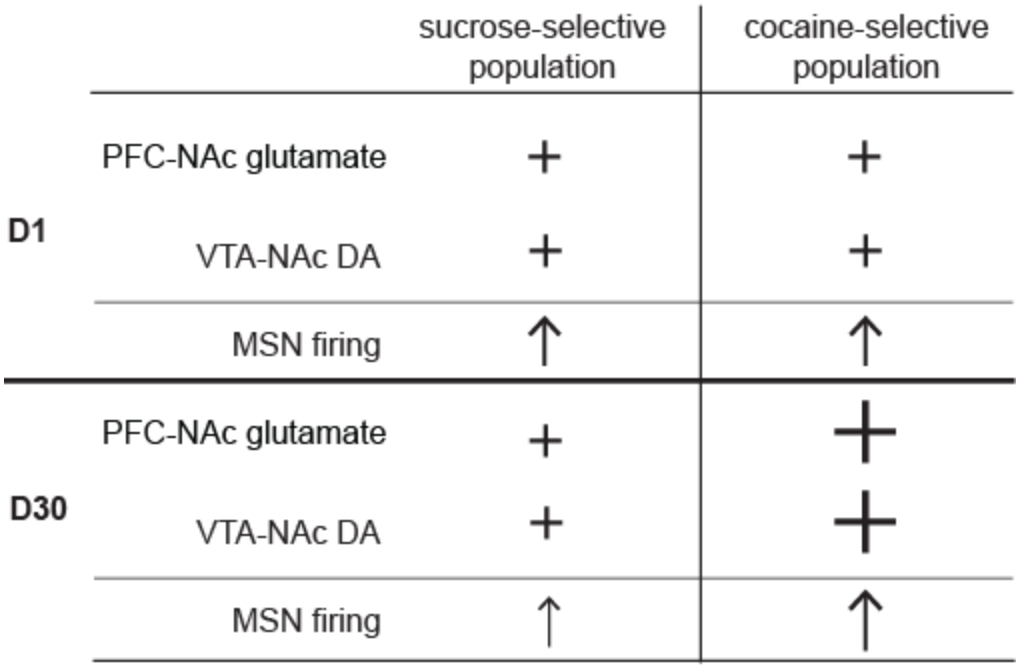

Figure 5.1. Glutamate and DA signaling in sucrose-selective versus cocaine-selective populations of NAc medium spiny neurons, a hypothesis. Pluses indicate relative levels of glutamate and/or DA signaling on MSN phasic activity. Arrows indicate phasic activity of NAc MSNs. The size of pluses/arrows indicates increases or decrease from baseline (D1).

and cocaine-selective cells show phasic activity relative to responding for cocaine but not sucrose). This selective phasic activity is indicated by the arrows representing MSN firing on D1 under the columns for sucrose versus cocaine selective population (prior to extended abstinence, Fig 5.1 top).

Following a month of cocaine abstinence (D30), I propose no change in PFC-NAc glutamate signaling actions on neurons that selectively process information about sucrose (i.e, the sucrose-selective population). This proposition arises from the lack of neuroadapations in NAc glutamate signaling dynamics under sucrose reinforcement conditions (McFarland *et al.*,

2003; Kalivas *et al.*, 2005; Kalivas & O'Brien, 2007). In contrast, derived from work by Kalivas and colleagues, I postulate there would be a significant increase in PFC-NAc glutamate signaling specific to cocaine-related information (Kalivas, 2009). Based on my findings from chapter 4 showing an increase in $[DA]_{max}$ following a month of cocaine abstinence, I further postulate that there would be a potentiation, or increase, in DA signaling in the NAc under this condition, that will likely not occur to the same degree for cells that encode sucrose-related information. Collectively then, while the influence of glutamate and DA signaling remains similar across abstinence conditions on sucrose-selective neurons, I propose that cocaine-selective neurons would be more strongly influenced by an overall increase in both glutamate and DA signaling, leading to an overall increase in the percentage of cocaine-selective NAc neurons (indicated by the larger up arrow for D30, cocaine-selective cells in Fig. 5.1). In support, our lab has shown that one month of cocaine abstinence increases the percentage of NAc neurons that encode goal-directed behaviors for cocaine as well as the amount of drug-seeking behavior itself (Hollander & Carelli, 2005; Hollander & Carelli, 2007).

Although it is not known what leads to the increase in cocaine selective neurons following abstinence, it is interesting to speculate possible causes. One possibility is that the changes in glutamate and DA signaling following a month of cocaine abstinence may cause previously sucrose-selective neurons to 'shift' and become cocaine-selective cells. Alternatively, it is also possible that the increase in cocaine-selective neurons is a result of a new population of cells not previously activated before abstinence being recruited after abstinence, perhaps related to the neuroadaptions revealed in this dissertation and postulated in the model shown in Fig. 5.1. Regardless of the mechanism, our studies show that unique neuroadapations occur in the NAc

following repeated cocaine experience and prolonged abstinence that may relate to the addictive nature of this drug.

Future directions

The results discussed in the previous chapters generated many new questions about the role of rapid NAc DA signaling in goal-directed behaviors for natural versus drug rewards. Additionally, the interaction between cocaine abstinence and responding for natural or drug remains is a largely unexplored topic. The following is a brief discussion of future experiments that would further expand our knowledge of the role of rapid DA signaling in these processes.

Simultaneous recording of NAc cell-firing and rapid DA release during performance of a sucrose/cocaine multiple schedule

As described in chapter two, we showed a dissociation between the pattern of NAc cell-firing and rapid DA release during performance of a sucrose/cocaine multiple schedule. Unlike our prior electrophysiology work (Carelli *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003), rapid DA release in the NAc core was not reinforcer specific during self-administration of a natural reinforcer (sucrose) versus intravenous cocaine. This leaves open the question of how rapid DA signaling acts postsynaptically to drive the differential, nonoverlapping pattern of phasic activity typically observed in the NAc.

The development of the combined FSCV and electrophysiology technique (Cheer *et al.*, 2005; Owesson-White *et al.*, 2009; Cacciapaglia *et al.*, 2011) offers the opportunity to explore the relationship between NAc cell firing and rapid DA release within the same discrete location during the performance of behavior. Using this technique, phasic activity and rapid DA release could be recorded simultaneously while rats performed a multiple schedule for sucrose and cocaine. Furthermore, iontophresis can be coupled with combined FSCV/electrophysiology to evaluate the effects of controlled local application of dopaminergic agonists and antagonists

(Belle *et al*., 2013). This technique could be employed to identify differential responses to DA in cells that show phasic activity to sucrose versus cells that show phasic activity to cocaine.

Establishing a causal role for rapid DA signaling in preventing the incubation of drug-seeking following cocaine abstinence

In chapter four we reported that, following a month of abstinence, animals that had a greater potentiation in $[DA]_{max}$ (calculated as the percent increase from pre-drug baseline after an injection of cocaine) made fewer extinction presses. We hypothesized that DA acts through D_2 receptors located on the spines of MSNs to dampen the effects of increased PFC-NAc glutamatergic signaling, thus decreasing drug-seeking behavior. However, this hypothesis remains to be tested. Optogenetic techniques offer one possibility for establishing a causal link between NAc DA signaling following cocaine abstinence and drug-seeking behavior. Using optogenetic stimulation paired with TH::Cre$^{(+/-)}$ rats, we could specifically stimulate DA release in the VTA-NAc pathway. If an increase in DA signaling is causally linked to drug-seeking behavior following cocaine abstinence, animals that receive stimulation should show less drug-seeking behavior during an extinction session than littermate controls. Finally, this manipulation could be paired with pharmacological inactivation of D_1 or D_2 receptors to isolate their role in relapse to drug-seeking following abstinence.

Concluding Remarks

The findings of this dissertation add to a substantial body of literature demonstrating that dopamine signaling within the NAc is critical for processing information related to rewarding stimuli. As the ability to seek out and obtain rewards is critical for survival, disruptions in dopamine signaling within the mesolimbic pathway to the NAc can be highly maladaptive.

This circuit can be modulated by many factors. In particular, the present work shows the impact of self-administration of natural versus drug rewards as well as drug abstinence.

Furthermore, these factors are closely linked such that a change in processing drug rewards necessarily influences processing of natural rewards. This interaction may help to explain why addiction is so difficult to treat. Successful treatment must reset the aberrant processing of drugs without disrupting normal processing of other rewards. Therefore, a better understanding of the interplay between mesolimbic dopamine signaling, NAc cell firing, and natural vs. drug rewards will greatly improve therapeutic interventions for drug addiction.

REFERENCES

Adams, C. D. & Dickinson, A. (1981) Instrumental responding following reinforcer devaluation. *The Quarterly Journal of Experimental Psychology Section B*, **33**, 109-121.

Addy, N. A., Daberkow, D. P., Ford, J. N., Garris, P. A. & Wightman, R. M. (2010) Sensitization of Rapid Dopamine Signaling in the Nucleus Accumbens Core and Shell After Repeated Cocaine in Rats. *Journal of Neurophysiology*, **104**, 922-931.

Alexander, G. E. & Crutcher, M. D. (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in Neurosciences*, **13**, 266-271.

Alexander, G. E., DeLong, M. R. & Strick, P. L. (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*, **9**, 357-381.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders: DSM-IV. Washington, DC: American Psychiatric Association.

Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A. M. & Kimura, M. (1994) Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *Journal of Neuroscience*, **14**, 3969-3984.

Ariansen, J. L., Heien, M. L., Hermans, A., Phillips, P. E., Hernadi, I., Bermudez, M. A., Schultz, W. & Wightman, R. M. (2012) Monitoring extracellular pH, oxygen, and dopamine during reward delivery in the striatum of primates. *Frontiers in behavioral neuroscience*, **6**, 36.

Bäckström, P. & Hyytiä, P. (2007) Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, **192**, 571-580.

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X.-C., Toda, S. & Kalivas, P. W. (2003) Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat Neurosci*, **6**, 743-749.

Balleine, B. (1994) Effects of ibotenic acid lesions of the nucleus accumbens on instrumental action. *Behavioural Brain Research*, **65**, 181-193.

Belin, D., Belin-Rauscent, A., Murray, J. E. & Everitt, B. J. (2013) Addiction: failure of control over maladaptive incentive habits. *Current Opinion in Neurobiology*, **23**, 564-572.

Belle, A. M., Owesson-White, C., Herr, N. R., Carelli, R. M. & Wightman, R. M. (2013) Controlled Iontophoresis Coupled with Fast-Scan Cyclic Voltammetry/Electrophysiology in Awake, Freely Moving Animals. *ACS Chemical Neuroscience*, **4**, 761-771.

Berridge, K. C., Ho, C.-Y., Richard, J. M. & DiFeliceantonio, A. G. (2010) The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. *Brain Research*, **1350**, 43-64.

Berridge, K. C. & Robinson, T. E. (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, **28**, 309-369.

Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Hervé, D., Valjent, E. & Girault, J.-A. (2008) Opposing Patterns of Signaling Activation in Dopamine D1 and D2 Receptor-Expressing Striatal Neurons in Response to Cocaine and Haloperidol. *The Journal of Neuroscience*, **28**, 5671-5685.

Bocklisch, C., Pascoli, V., Wong, J. C. Y., House, D. R. C., Yvon, C., De Roo, M., Tan, K. R. & Luscher, C. (2013) Cocaine Disinhibits Dopamine Neurons by Potentiation of GABA Transmission in the Ventral Tegmental Area. *Science*, **341**, 1521-1525.

Brady, A. M. & O'Donnell, P. (2004) Dopaminergic Modulation of Prefrontal Cortical Input to Nucleus Accumbens Neurons In Vivo. *The Journal of Neuroscience*, **24**, 1040-1049.

Brog, J. S., Salyapongse, A., Deutch, A. Y. & Zahm, D. S. (1993) The patterns of afferent innervation of the core and shell in the "Accumbens" part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. *The Journal of Comparative Neurology*, **338**, 255-278.

Cacciapaglia, F., Wightman, R. M. & Carelli, R. M. (2011) Rapid Dopamine Signaling Differentially Modulates Distinct Microcircuits within the Nucleus Accumbens during Sucrose-Directed Behavior. *The Journal of Neuroscience*, **31**, 13860-13869.

Calipari, E. S., Ferris, M. J. & Jones, S. R. (2014) Extended access of cocaine self-administration results in tolerance to the dopamine-elevating and locomotor-stimulating effects of cocaine. *Journal of Neurochemistry*, **128**, 224-232.

Calipari, E. S., Ferris, M. J., Zimmer, B. A., Roberts, D. C. S. & Jones, S. R. (2013) Temporal Pattern of Cocaine Intake Determines Tolerance vs Sensitization of Cocaine Effects at the Dopamine Transporter. *Neuropsychopharmacology*, **38**, 2385-2392.

Cameron, C. M. & Carelli, R. M. (2012) Cocaine abstinence alters nucleus accumbens firing dynamics during goal-directed behaviors for cocaine and sucrose. *European Journal of Neuroscience*, **35**, 940-951.

Cardinal, R. N. (2002) Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. *Behavioral Neuroscience*, **116**, 553-567.

Carelli, R. & Deadwyler, S. (1994) A comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats. *The Journal of Neuroscience*, **14**, 7735-7746.

Carelli, R. M. (2000) Activation of accumbens cell firing by stimuli associated with cocaine delivery during self-administration. *Synapse*, **35**, 238-242.

Carelli, R. M. (2002) Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. 'natural' reinforcement. *Physiology & Behavior*, **76**, 379-387.

Carelli, R. M. & Ijames, S. G. (2001) Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule. *Brain Research*, **907**, 156-161.

Carelli, R. M., Ijames, S. G. & Crumling, A. J. (2000) Evidence That Separate Neural Circuits in the Nucleus Accumbens Encode Cocaine Versus "Natural" (Water and Food) Reward. *The Journal of Neuroscience*, **20**, 4255-4266.

Carelli, R. M. & Wightman, R. M. (2004) Functional microcircuitry in the accumbens underlying drug addiction: insights from real-time signaling during behavior. *Current Opinion in Neurobiology*, **14**, 763-768.

Carelli, R. M. & Wondolowski, J. (2003) Selective Encoding of Cocaine versus Natural Rewards by Nucleus Accumbens Neurons Is Not Related to Chronic Drug Exposure. *The Journal of Neuroscience*, **23**, 11214-11223.

Carelli, R. M. & Wondolowski, J. (2006) Anatomic distribution of reinforcer selective cell firing in the core and shell of the nucleus accumbens. *Synapse*, **59**, 69-73.

Carlezon, W. A. & Thomas, M. J. (2009) Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology*, **56**, Supplement 1, 122-132.

Chang, J. Y., Janak, P. H. & Woodward, D. J. (1998) Comparison of Mesocorticolimbic Neuronal Responses During Cocaine and Heroin Self-Administration in Freely Moving Rats. *The Journal of Neuroscience*, **18**, 3098-3115.

Cheer, J. F., Heien, M. L. A. V., Garris, P. A., Carelli, R. M. & Wightman, R. M. (2005) Simultaneous dopamine and single-unit recordings reveal accumbens GABAergic responses: Implications for intracranial self-stimulation. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 19150-19155.

Cheer, J. F., Wassum, K. M. & Wightman, R. M. (2006) Cannabinoid modulation of electrically evoked pH and oxygen transients in the nucleus accumbens of awake rats. *Journal of Neurochemistry*, **97**, 1145-1154.

Chen, B. T., Bowers, M. S., Martin, M., Hopf, F. W., Guillory, A. M., Carelli, R. M., Chou, J. K. & Bonci, A. (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. *Neuron*, **59**, 288-297.

Chesler, M. (2003) Regulation and Modulation of pH in the Brain. *Physiological Reviews*, **83**, 1183-1221.

Chesler, M. & Kaila, K. (1992) Modulation of pH by neuronal activity. *Trends in Neurosciences*, **15**, 396-402.

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L.-J., Shaham, Y., Marinelli, M. & Wolf, M. E. (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, **454**, 118-121.

Dalley, J. W. (2002) Nucleus accumbens dopamine and discriminated approach learning: interactive effects of 6-hydroxydopamine lesions and systemic apomorphine administration. *Psychopharmacology (Berlin, Germany)*, **161**, 425-433.

Dalley, J. W., Lääne, K., Theobald, D. E. H., Armstrong, H. C., Corlett, P. R., Chudasama, Y. & Robbins, T. W. (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. *Proceedings of the National Academy of Sciences*, **102**, 6189-6194.

Day, J. J., Jones, J. L., Wightman, R. M. & Carelli, R. M. (2010) Phasic Nucleus Accumbens Dopamine Release Encodes Effort- and Delay-Related Costs. *Biological Psychiatry*, **68**, 306-309.

Day, J. J., Roitman, M. F., Wightman, R. M. & Carelli, R. M. (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat Neurosci*, **10**, 1020-1028.

Day, J. J., Wheeler, R. A., Roitman, M. F. & Carelli, R. M. (2006) Nucleus accumbens neurons encode Pavlovian approach behaviors: evidence from an autoshaping paradigm. *European Journal of Neuroscience*, **23**, 1341-1351.

De Wit, H. & Stewart, J. (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, **75**, 134-143.

De Wit, H. & Wise, R. A. (1977) Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Canadian Journal of Psychology/Revue canadienne de psychologie*, **31**, 195-203.

Deneau, G., Yanagita, T. & Seevers, M. H. (1969) Self-administration of psychoactive substances by the monkey. *Psychopharmacologia*, **16**, 30-48.

Di Chiara, G. (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behavioural Brain Research*, **137**, 75-114.

Di Chiara, G. & Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences*, **85**, 5274-5278.

Di Ciano, P., Cardinal, R. N., Cowell, R. A., Little, S. J. & Everitt, B. J. (2001) Differential Involvement of NMDA, AMPA/Kainate, and Dopamine Receptors in the Nucleus Accumbens Core in the Acquisition and Performance of Pavlovian Approach Behavior. *The Journal of Neuroscience*, **21**, 9471-9477.

Di Ciano, P. & Everitt, B. J. (2001) Dissociable Effects of Antagonism of NMDA and AMPA/KA Receptors in the Nucleus Accumbens Core and Shell on Cocaine-seeking Behavior. *Neuropsychopharmacology*, **25**, 341-360.

Di Ciano, P. & Everitt, B. J. (2004a) Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology*, **47**, Supplement 1, 202-213.

Di Ciano, P. & Everitt, B. J. (2004b) Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. *European Journal of Neuroscience*, **19**, 1661-1667.

Everitt, B. J., Dickinson, A. & Robbins, T. W. (2001) The neuropsychological basis of addictive behaviour. *Brain Research Reviews*, **36**, 129-138.

Everitt, B. J., Parkinson, J. A., Olmstead, M. C., Arroyo, M., Robledo, P. & Robbins, T. W. (1999) Associative Processes in Addiction and Reward The Role of Amygdala-Ventral Striatal Subsystems. *Annals of the New York Academy of Sciences*, **877**, 412-438.

Everitt, B. J. & Robbins, T. W. (2000) Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology*, **153**, 17-30

Ferrario, C. R., Gorny, G., Crombag, H. S., Li, Y., Kolb, B. & Robinson, T. E. (2005) Neural and Behavioral Plasticity Associated with the Transition from Controlled to Escalated Cocaine Use. *Biological Psychiatry*, **58**, 751-759.

Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., Akers, C. A., Clinton, S. M., Phillips, P. E. M. & Akil, H. (2011) A selective role for dopamine in stimulus-reward learning. *Nature*, **469**, 53-57.

Fox, P. T. & Raichle, M. E. (1986) Focal Physiological Uncoupling of Cerebral Blood Flow and Oxidative Metabolism during Somatosensory Stimulation in Human Subjects. *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 1140-1144.

Fuchs, R., Evans, K. A., Parker, M. & See, R. (2004) Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, **176**, 459-465.

Fuchs, R., Ramirez, D. & Bell, G. (2008) Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, **200**, 545-556.

Gawin, F. H. (1991) Cocaine Addiction: Psychology and Neurophysiology. *Science*, **251**, 1580-1586.

Gawin, F. H. & Kleber, H. D. (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers: Clinical observations. *Archives of General Psychiatry*, **43**, 107-113.

Gerfen, C. R. (1988) Synaptic organization of the striatum. *Journal of electron microscopy technique*, **10**, 265-281.

Gerhardt, G. A. & Hoffman, A. F. (2001) Effects of recording media composition on the responses of Nafion-coated carbon fiber microelectrodes measured using high-speed chronoamperometry. *Journal of Neuroscience Methods*, **109**, 13-21.

Girault, J. & Greengard, P. (2004) THe neurobiology of dopamine signaling. *Archives of Neurology*, **61**, 641-644.

Goto, Y. & Grace, A. A. (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat Neurosci*, **8**, 805-812.

Graybiel, A. M., Aosaki, T., Flaherty, A. W. & Kimura, M. (1994) The basal ganglia and adaptive motor control. *Science*, **265**, 1826-1831.

Grigson, P. S. & Hajnal, A. (2007) Once is too much: Conditioned changes in accumbens dopamine following a single saccharin-morphine pairing. *Behavioral Neuroscience*, **121**, 1234-1242.

Grigson, P. S. & Twining, R. C. (2002) Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards. *Behavioral Neuroscience*, **116**, 321-333.

Grimm, J. W., Fyall, A. M. & Osincup, D. P. (2005) Incubation of sucrose craving: effects of reduced training and sucrose pre-loading. *Physiology & Behavior*, **84**, 73-79.

Grimm, J. W., Hope, B. T., Wise, R. A. & Shaham, Y. (2001) Neuroadaptation: Incubation of cocaine craving after withdrawal. *Nature*, **412**, 141-142.

Groenewegen, H. J., Wright, C. I. & Beijer, A. V. (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system? *Progress in brain research*, **107**, 485-511.

Groves, P. M. (1983) A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. *Brain Research*, **286**, 109-132.

Groves, P. M., Linder, J. C. & Young, S. J. (1994) 5-hydroxydopamine-labeled dopaminergic axns: Three-dimensional reconstructions of axons, synapses and postsynaptic targets in rat neostriatum. *Neuroscience*, **58**, 593-604.

Heien, M. L. A. V., Johnson, M. A. & Wightman, R. M. (2004) Resolving Neurotransmitters Detected by Fast-Scan Cyclic Voltammetry. *Analytical Chemistry*, **76**, 5697-5704.

Heikkila, R. E., Orlansky, H. & Cohen, G. (1975) Studies on the distinction between uptake inhibition and release of [3H]dopamine in rat brain tissue slices. *Biochemical Pharmacology*, **24**, 847-852.

Hollander, J. A. & Carelli, R. M. (2005) Abstinence from Cocaine Self-Administration Heightens Neural Encoding of Goal-Directed Behaviors in the Accumbens. *Neuropsychopharmacology*, **30**, 1464-1474.

Hollander, J. A. & Carelli, R. M. (2007) Cocaine-Associated Stimuli Increase Cocaine Seeking and Activate Accumbens Core Neurons after Abstinence. *The Journal of Neuroscience*, **27**, 3535-3539.

Hollerman, J. R. & Schultz, W. (1998) Dopamine neurons report an error in the temporal prediction of reward during learning. *Nat Neurosci*, **1**, 304-309.

Hooks, M. S. (1994) Behavioral and neurochemical sensitization following cocaine self-administration. *Psychopharmacology (Berlin, Germany)*, **115**, 265-272.

Huang, A. C. W. & Hsiao, S. (2002) Haloperidol attenuates rewarding and aversively conditioned suppression of saccharin solution intake: Reevaluation of the anhedonia hypothesis of dopamine blocking. *Behavioral Neuroscience*, **116**, 646-650.

Janak, P. H., Chang, J.-Y. & Woodward, D. J. (1999) Neuronal spike activity in the nucleus accumbens of behaving rats during ethanol self-administration. *Brain Research*, **817**, 172-184.

Jones, J. L., Wheeler, R. A. & Carelli, R. M. (2008) Behavioral responding and nucleus accumbens cell firing are unaltered following periods of abstinence from sucrose. *Synapse*, **62**, 219-228.

Jones, S. R., Garris, P. A. & Wightman, R. M. (1995) Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics*, **274**, 396-403.

Kalivas, P. W. (2009) The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*, **10**, 561-572.

Kalivas, P. W. & McFarland, K. (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology*, **168**, 44.

Kalivas, P. W., McFarland, K., Bowers, S., Szumlinski, K., Xi, Z.-X. & Baker, D. (2003) Glutamate Transmission and Addiction to Cocaine. *Annals of the New York Academy of Sciences*, **1003**, 169-175.

Kalivas, P. W. & O'Brien, C. (2007) Drug Addiction as a Pathology of Staged Neuroplasticity. *Neuropsychopharmacology*, **33**, 166-180.

Kalivas, P. W., Volkow, N. & Seamans, J. (2005) Unmanageable Motivation in Addiction: A Pathology in Prefrontal-Accumbens Glutamate Transmission. *Neuron*, **45**, 647-650.

Kalivas, P. W. & Volkow, N. D. (2005) The Neural Basis of Addiction: A Pathology of Motivation and Choice. *Am J Psychiatry*, **162**, 1403-1413.

Kawagoe, K. T. & Wightman, R. M. (1994) Characterization of amperometry for in vivo measurement of dopamine dynamics in the rat brain. *Talanta*, **41**, 865-874.

Kebabian, J. W. & Calne, D. B. (1979) Multiple receptors for dopamine. *Nature*, **277**, 93-96.

Keithley, R. B., Carelli, R. M. & Wightman, R. M. (2010) Rank Estimation and the Multivariate Analysis of in Vivo Fast-Scan Cyclic Voltammetric Data. *Analytical Chemistry*, **82**, 5541-5551.

Kelley, A. (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience & Biobehavioral Reviews*, **27**, 765-776.

Kilpatrick, M. R., Rooney, M. B., Michael, D. J. & Wightman, R. M. (2000) Extracellular dopamine dynamics in rat caudate–putamen during experimenter-delivered and intracranial self-stimulation. *Neuroscience*, **96**, 697-706.

Kim, D. S., Duong, T. Q. & Kim, S. G. (2000) High-resolution mapping of iso-orientation columns by fMRI. *Nature neuroscience*, **3**, 164-169.

Kiyatkin, E. A. & Gratton, A. (1994) Electrochemical monitoring of extracellular dopamine in nucleus accumbens of rats lever-pressing for food. *Brain Research*, **652**, 225-234.

Koob, G. & Nestler, E. (1997) The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci*, **9**, 482-497.

Kreitzer, A. C. & Malenka, R. C. (2008) Striatal Plasticity and Basal Ganglia Circuit Function. *Neuron*, **60**, 543-554.

Lu, L., Grimm, J. W., Hope, B. T. & Shaham, Y. (2004) Incubation of cocaine craving after withdrawal: a review of preclinical data. *Neuropharmacology*, **47**, Supplement 1, 214-226.

Lu, L., Grimm, J. W., Shaham, Y. & Hope, B. T. (2003) Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *Journal of Neurochemistry*, **85**, 1604-1613.

Mateo, Y., Lack, C. M., Morgan, D., Roberts, D. C. S. & Jones, S. R. (2005) Reduced Dopamine Terminal Function and Insensitivity to Cocaine Following Cocaine Binge Self-Administration and Deprivation. *Neuropsychopharmacology*, **30**, 1455-1463.

McCutcheon, J. E., Beeler, J. A. & Roitman, M. F. (2012) Sucrose-predictive cues evoke greater phasic dopamine release than saccharin-predictive cues. *Synapse*, **66**, 346-351.

McFarland, K., Davidge, S. B., Lapish, C. C. & Kalivas, P. W. (2004) Limbic and Motor Circuitry Underlying Footshock-Induced Reinstatement of Cocaine-Seeking Behavior. *The Journal of Neuroscience*, **24**, 1551-1560.

McFarland, K. & Kalivas, P. W. (2001) The Circuitry Mediating Cocaine-Induced Reinstatement of Drug-Seeking Behavior. *The Journal of Neuroscience*, **21**, 8655-8663.

McFarland, K., Lapish, C. C. & Kalivas, P. W. (2003) Prefrontal Glutamate Release into the Core of the Nucleus Accumbens Mediates Cocaine-Induced Reinstatement of Drug-Seeking Behavior. *The Journal of Neuroscience*, **23**, 3531-3537.

Michael, D. J. & Wightman, R. M. (1999) Electrochemical monitoring of biogenic amine neurotransmission in real time. *Journal of Pharmaceutical and Biomedical Analysis*, **19**, 33-46.

Mirenowicz, J. & Schultz, W. (1994) Importance of unpredictability for reward responses in primate dopamine neurons. *Journal of Neurophysiology*, **72**, 1024-1027.

Mogenson, G. J., Jones, D. L. & Yim, C. Y. (1980) From motivation to action: Functional interface between the limbic system and the motor system. *Progress in Neurobiology*, **14**, 69-97.

Morris, G., Arkadir, D., Nevet, A., Vaadia, E. & Bergman, H. (2004) Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron*, **43**, 133-143.

Nicola, S. M. & Deadwyler, S. A. (2000) Firing Rate of Nucleus Accumbens Neurons Is Dopamine-Dependent and Reflects the Timing of Cocaine-Seeking Behavior in Rats on a Progressive Ratio Schedule of Reinforcement. *The Journal of Neuroscience*, **20**, 5526-5537.

Nicola, S. M., Surmeier, D. J. & Malenka, R. C. (2000) Dopaminergic Modulation of Neuronal Excitability in the Striatum and Nucleus Accumbens. *Annual Review of Neuroscience*, **23**, 185-215.

Nicola, S. M., Taha, S. A., Kim, S. W. & Fields, H. L. (2005) Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. *Neuroscience*, **135**, 1025-1033.

Nicola, S. M., Yun, I. A., Wakabayashi, K. T. & Fields, H. L. (2004) Cue-Evoked Firing of Nucleus Accumbens Neurons Encodes Motivational Significance During a Discriminative Stimulus Task. *Journal of Neurophysiology*, **91**, 1840-1865.

Nicolaysen, L. C., Pan, H.-T. & Justice Jr, J. B. (1988) Extracellular cocaine and dopamine concentrations are linearly related in rat striatum. *Brain Research*, **456**, 317-323.

O'Donnell, P. (2003) Dopamine gating of forebrain neural ensembles. *European Journal of Neuroscience*, **17**, 429-435.

O'Donnell, P. & Grace, A. A. (1993) Dopaminergic modulation of dye coupling between neurons in the core and shell regions of the nucleus accumbens. *The Journal of Neuroscience*, **13**, 3456-3471.

O'Donnell, P., Greene, J., Pabello, N., Lewis, B. L. & Grace, A. A. (1999) Modulation of Cell Firing in the Nucleus Accumbens. *Annals of the New York Academy of Sciences*, **877**, 157-175.

Owesson-White, C. A., Ariansen, J., Stuber, G. D., Cleaveland, N. A., Cheer, J. F., Mark Wightman, R. & Carelli, R. M. (2009) Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. *European Journal of Neuroscience*, **30**, 1117-1127.

Pan, W.-X., Schmidt, R., Wickens, J. R. & Hyland, B. I. (2005) Dopamine Cells Respond to Predicted Events during Classical Conditioning: Evidence for Eligibility Traces in the Reward-Learning Network. *The Journal of Neuroscience*, **25**, 6235-6242.

Parkinson, J. A., Olmstead, M. C., Burns, L. H., Robbins, T. W. & Everitt, B. J. (1999) Dissociation in Effects of Lesions of the Nucleus Accumbens Core and Shell on Appetitive Pavlovian Approach Behavior and the Potentiation of Conditioned Reinforcement and Locomotor Activity byd-Amphetamine. *The Journal of Neuroscience*, **19**, 2401-2411.

Pavlov, I. P. (1927). Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Oxford: Oxford University Press.

Paxinos, G. & Watson, C. (2007) *The Rat Brain in Stereotaxic Coordinates*, 6th edn. New York, Elsevier.

Pennartz, C. M. A., Groenewegen, H. J. & Lopes da Silva, F. H. (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: An integration of behavioural, electrophysiological and anatomical data. *Progress in Neurobiology*, **42**, 719-761.

Peoples, L. L., Lynch, K. G., Lesnock, J. & Gangadhar, N. (2004) Accumbal Neural Responses During the Initiation and Maintenance of Intravenous Cocaine Self-Administration. *Journal of Neurophysiology*, **91**, 314-323.

Peters, J. & Kalivas, P. (2006) The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology*, **186**, 143-149.

Phillips, P. E. M., Robinson, D. L., Stuber, G. D., Carelli, R. M. & Wightman, R. M. (2003a) Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. *Methods in molecular medicine*, **79**, 443-464.

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

Pickens, C. L., Airavaara, M., Theberge, F., Fanous, S., Hope, B. T. & Shaham, Y. (2011) Neurobiology of the incubation of drug craving. *Trends in Neurosciences*, **34**, 411-420.

Pu, L., Liu, Q.-s. & Poo, M.-m. (2006) BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. *Nat Neurosci*, **9**, 605-607.

Raichle, M. E. (1998) Behind the scenes of functional brain imaging: A historical and physiological perspective. *Proceedings of the National Academy of Sciences*, **95**, 765-772.

Ramamoorthy, S., Samuvel, D. J., Balasubramaniam, A., See, R. E. & Jayanthi, L. D. (2010) Altered dopamine transporter function and phosphorylation following chronic cocaine self-administration and extinction in rats. *Biochemical and Biophysical Research Communications*, **391**, 1517-1521.

Rescorla, R. A. (1992) Depression of an instrumental response by a single devaluation of its outcome. *The Quarterly Journal of Experimental Psychology Section B*, **44**, 123-136.

Richardson, N. R. & Gratton, A. (1996) Behavior-Relevant Changes in Nucleus Accumbens Dopamine Transmission Elicited by Food Reinforcement: An Electrochemical Study in Rat. *The Journal of Neuroscience*, **16**, 8160-8169.

Robbins, T. W. & Everitt, B. J. (2002) Limbic-Striatal Memory Systems and Drug Addiction. *Neurobiology of Learning and Memory*, **78**, 625-636.

Roberts, D. C. S., Corcoran, M. E. & Fibiger, H. C. (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacology Biochemistry and Behavior*, **6**, 615-620.

Robinson, T. E. & Berridge, K. C. (1993) The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews*, **18**, 247-291.

Robinson, T. E. & Berridge, K. C. (2000) The psychology and neurobiology of addiction: an incentive–sensitization view. *Addiction*, **95**, S91-S117.

Robinson, T. E., Gorny, G., Mitton, E. & Kolb, B. (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse*, **39**, 257-266.

Roitman, M. F., Stuber, G. D., Phillips, P. E. M., Wightman, R. M. & Carelli, R. M. (2004) Dopamine Operates as a Subsecond Modulator of Food Seeking. *The Journal of Neuroscience*, **24**, 1265-1271.

Roitman, M. F., Wheeler, R. A. & Carelli, R. M. (2005) Nucleus Accumbens Neurons Are Innately Tuned for Rewarding and Aversive Taste Stimuli, Encode Their Predictors, and Are Linked to Motor Output. *Neuron*, **45**, 587-597.

Roop, R. G., Hollander, J. A. & Carelli, R. M. (2002) Accumbens activity during a multiple schedule for water and sucrose reinforcement in rats. *Synapse*, **43**, 223-226.

Salamone, J. D. (1994) The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. *Behavioural Brain Research*, **61**, 117-133.

Schoenbaum, G. & Setlow, B. (2003) Lesions of Nucleus Accumbens Disrupt Learning about Aversive Outcomes. *The Journal of Neuroscience*, **23**, 9833-9841.

Schultz, W., Dayan, P. & Montague, P. R. (1997) A Neural Substrate of Prediction and Reward. *Science*, **275**, 1593-1599.

Sinha, R. (2013) The clinical neurobiology of drug craving. *Current Opinion in Neurobiology*, **23**, 649-654.

Skinner, B. F. (1938). The behavior of organisms: An experimental analysis. New York: Appleton.

Snyder, S. H. (2011) What dopamine does in the brain. *Proceedings of the National Academy of Sciences*, **108**, 18869-18871.

Stratford, T. R. (1997) Injections of nociceptin into nucleus accumbens shell or ventromedial hypothalamic nucleus increase food intake. *Neuroreport*, **8**, 423-426.

Stratford, T. R. & Kelley, A. E. (1997) GABA in the Nucleus Accumbens Shell Participates in the Central Regulation of Feeding Behavior. *The Journal of Neuroscience*, **17**, 4434-4440.

Sugam, J. A., Day, J. J., Wightman, R. M. & Carelli, R. M. (2012) Phasic Nucleus Accumbens Dopamine Encodes Risk-Based Decision-Making Behavior. *Biological Psychiatry*, **71**, 199-205.

Takmakov, P., Zachek, M. K., Keithley, R. B., Bucher, E. S., McCarty, G. S. & Wightman, R. M. (2010) Characterization of Local pH Changes in Brain Using Fast-Scan Cyclic Voltammetry with Carbon Microelectrodes. *Analytical Chemistry*, **82**, 9892-9900.

Thompson, J. K., Peterson, M. R. & Freeman, R. D. (2003) Single-Neuron Activity and Tissue Oxygenation in the Cerebral Cortex. *Science*, **299**, 1070-1072.

Thorndike, E. L. (1933) A Proof of the Law of Effect. *Science*, **77**, 173-175.

Toda, S., McGinty, J. F. & Kalivas, P. W. (2002) Repeated cocaine administration alters the expression of genes in corticolimbic circuitry after a 3-week withdrawal: a DNA macroarray study. *Journal of Neurochemistry*, **82**, 1290-1299.

Tran-Nguyen, L. T. L., Fuchs, R. A., Coffey, G. P., Baker, D. A., Odell, L. E. & Neisewander, J. L. (1998) Time-Dependent Changes in Cocaine-Seeking Behavior and Extracellular Dopamine Levels in the Amygdala during Cocaine Withdrawal. *Neuropsychopharmacology*, **19**, 48-59.

Ungless, M. A., Whistler, J. L., Malenka, R. C. & Bonci, A. (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature*, **411**, 583-587.

UNODC (2011) World Drug Report. *United Nations Office on Drugs and Crime*, Vienna.

Urbanics, R., Leniger-Follert, E. & Lübbers, D. (1978) Time course of changes of extracellular H+ and K+ activities during and after direct electrical stimulation of the brain cortex. *Pflügers Archiv*, **378**, 47-53.

Valjent, E., Bertran-Gonzalez, J., Hervé, D., Fisone, G. & Girault, J.-A. (2009) Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends in Neurosciences*, **32**, 538-547.

Venton, B. J., Michael, D. J. & Wightman, R. M. (2003) Correlation of local changes in extracellular oxygen and pH that accompany dopaminergic terminal activity in the rat caudate–putamen. *Journal of Neurochemistry*, **84**, 373-381.

Volkow, N. D. (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse (New York, N.Y.)*, 1**4**, 169-177.

Volkow, N. D., Fowler, J. S. & Wang, G.-J. (1999) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Journal of Psychopharmacology*, **13**, 337-345.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Tomasi, D., Telang, F. & Baler, R. (2010) Addiction: Decreased reward sensitivity and increased expectation sensitivity conspire to overwhelm the brain's control circuit. *BioEssays*, **32**, 748-755.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Hitzemann, R., Chen, A. D., Dewey, S. L. & Pappas, N. (1997) Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature*, **386**, 830-833.

Voorn, P., Jorritsma-Byham, B., Van Dijk, C. & Buijs, R. M. (1986) The dopaminergic innervation of the ventral striatum in the rat: A light- and electron-microscopical study with antibodies against dopamine. *The Journal of Comparative Neurology*, **251**, 84-99.

Wagner, F. A. & Anthony, J. C. (2002) From First Drug Use to Drug Dependence: Developmental Periods of Risk for Dependence upon Marijuana, Cocaine, and Alcohol. *Neuropsychopharmacology*, **26**, 479-488.

Wang, B., Shaham, Y., Zitzman, D., Azari, S., Wise, R. A. & You, Z.-B. (2005) Cocaine Experience Establishes Control of Midbrain Glutamate and Dopamine by Corticotropin-Releasing Factor: A Role in Stress-Induced Relapse to Drug Seeking. *The Journal of Neuroscience*, **25**, 5389-5396.

Weeks, J. R. (1962) Experimental Morphine Addiction: Method for Automatic Intravenous Injections in Unrestrained Rats. *Science*, **138**, 143-144.

Wheeler, R. A., Aragona, B. J., Fuhrmann, K. A., Jones, J. L., Day, J. J., Cacciapaglia, F., Wightman, R. M. & Carelli, R. M. (2011) Cocaine Cues Drive Opposing Context-Dependent Shifts in Reward Processing and Emotional State. *Biological Psychiatry*, **69**, 1067-1074.

Wheeler, R. A., Twining, R. C., Jones, J. L., Slater, J. M., Grigson, P. S. & Carelli, Regina M. (2008) Behavioral and Electrophysiological Indices of Negative Affect Predict Cocaine Self-Administration. *Neuron*, **57**, 774-785.

White, F. J. (1998) Drug addiction: Cocaine and the serotonin saga. *Nature*, **393**, 118-119.

Wightman, R. M., Amatorh, C., Engstrom, R. C., Hale, P. D., Kristensen, E. W., Kuhr, W. G. & May, L. J. (1988) Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience*, **25**, 513-523.

Wightman, R. M. & Robinson, D. L. (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. *Journal of Neurochemistry*, **82**, 721-735.

Wightman, R. M. & Zimmerman, J. B. (1990) Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Research Reviews*, **15**, 135-144.

Wilson, C. J. & Kawaguchi, Y. (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *The Journal of Neuroscience*, **16**, 2397-2410.

Wise, R. A. (1997) Drug Self-Administration Viewed as Ingestive Behaviour. *Appetite*, **28**, 1-5.

Wise, R. A. (2004) Dopamine, learning and motivation. *Nat Rev Neurosci*, **5**, 483-494.

Wise, R. A. & Bozarth, M. A. (1982) Action of drugs of abuse on brain reward systems: An update with specific attention to opiates. *Pharmacology Biochemistry and Behavior*, **17**, 239-243.

Wise, R. A. & Rompre, P. P. (1989) Brain Dopamine and Reward. *Annual Review of Psychology*, **40**, 191-225.

Wyvell, C. L. & Berridge, K. C. (2000) Intra-Accumbens Amphetamine Increases the Conditioned Incentive Salience of Sucrose Reward: Enhancement of Reward "Wanting" without Enhanced "Liking" or Response Reinforcement. *The Journal of Neuroscience*, **20**, 8122-8130.

Wyvell, C. L. & Berridge, K. C. (2001) Incentive Sensitization by Previous Amphetamine Exposure: Increased Cue-Triggered "Wanting" for Sucrose Reward. *The Journal of Neuroscience*, **21**, 7831-7840.

Xi, Z.-X., Ramamoorthy, S., Baker, D. A., Shen, H., Samuvel, D. J. & Kalivas, P. W. (2002) Modulation of Group II Metabotropic Glutamate Receptor Signaling by Chronic Cocaine. *Journal of Pharmacology and Experimental Therapeutics*, **303**, 608-615.

Xi, Z.-X., Ramamoorthy, S., Shen, H., Lake, R., Samuvel, D. J. & Kalivas, P. W. (2003) GABA Transmission in the Nucleus Accumbens Is Altered after Withdrawal from Repeated Cocaine. *The Journal of Neuroscience*, **23**, 3498-3505.

Yonetani, M., Huang, C. C., Lajevardi, N., Pastuszko, A., Delivoriapapadopoulous, M. & Anday, E. (1994) Effect of Acute Cocaine Injection on the Extracellular Level of Dopamine, Blood Flow, and Oxygen Pressure in Brain of Newborn Piglets. *Biochemical Medicine and Metabolic Biology*, **51**, 91-97.

Yorgason, J. T., España, R. A. & Jones, S. R. (2011) Demon Voltammetry and Analysis software: Analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *Journal of Neuroscience Methods*, **202**, 158-164.

Zahm, D. S. (1999) Functional-anatomical Implications of the Nucleus Accumbens Core and Shell Subterritories. *Annals of the New York Academy of Sciences*, **877**, 113-128.

Zahm, D. S. & Brog, J. S. (1992) On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience*, **50**, 751-767.