Regional Effects of Cocaine on Phasic Dopamine Release in the Nucleus Accumbens:

A Role for the Hindbrain

By

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THESIS

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I. Introduction

A. Drug Addiction Remains a Pervasive Problem in America

Drug addiction has become an increasingly costly societal problem. In 2010, it was estimated that the overall cost of illicit drug use – accounting for healthcare, crime, and lost-work productivity – amounted to over $193 billion dollars, in the U.S. alone (NDIC, 2010). The specific cost of health care made up for $11 billion dollars of this surmounting cost (NDIC, 2010). While great efforts have been dedicated to finding a resolution to this problem, there still remains a large discrepancy between Americans in need of treatment and those who actually receive help. In 2013 it was approximated that 22.7 million Americans reported a need for drug or alcohol treatment, and only 2.5 million of those in need received care – at a specialty addiction clinic (SAMHA, 2014). It is clear that accessibility to affordable treatment of drug addiction is lacking for a majority of American in need. In order to reach a larger mass, we must find options that can be incorporated into general practice clinics. One method of doing so is to explore pharmacological options that aid in blocking the addicting effects of drugs. While therapeutics such as methodone exists for addictive opiates, they are still associated with dependence and can elicit similar addictive behavior (Kosten & George, 2002). There has yet to be similarly approved pharmaceutical treatments for other classes of illicit drugs of abuse such as psychostimulants, and more specifically cocaine (Rocha, 2003). *Understanding the specific mechanisms of addictive drugs, and how they act on reward circuits in the brain, would allow for more targeted treatments to aid in blocking the reinforcing and rewarding effects of drugs that come to be abused*. Strides have been made on this front for nicotine and tobacco addiction with pharmaceuticals such as bupropion. This particular therapy is not a substitute for nicotine; rather the drug binds to nicotinic

receptors and aids in preventing the urge to use tobacco (Jorenby et al., 2006). While clinical behavioral therapy will always be a critical component of remedying addiction, pharmacological treatments offer a more broad appeal for individuals that do not have access to specialty addiction treatment centers. Therefore, it is imperative that we continue to explore all of the mechanisms of drug action, in order to unmask possible targets for pharmaceutical therapies.

B. Psychostimulants are Powerfully Addictive Substances

Drug addiction begins with the initial reinforcing effects of drugs. Reinforcement learning is critical for survival such that it facilitates goal-directed behaviors toward stimuli of value such as a food, water and sex. However, it is also one of the strongest mechanisms that can lead to drug abuse. The mesolimbic dopamine system, and specifically the nucleus accumbens (NAc) plays a significant role in reinforcement learning such that fluctuations of incoming dopamine to the NAc provides a learning signal that encodes relevant information about rewards and associated cues (Schultz, 2002; 2007). Drugs of abuse also target the mesolimbic dopamine system. While different classes of addictive drugs vary in their primary pharmacological mechanisms, they all share an acute effect of increased dopaminergic signaling – most significantly seen in the NAc (Di Chiara & Imperato, 1988). The effect of increased dopaminergic activity has long been thought to be mechanistically responsible for the immediate reinforcing effects of drugs of abuse.

All drugs of abuse have a significant effect on dopaminergic signaling, however psychostimulants have an especially interesting relationship with the mesolimbic system. Psychostimulants include illicit drugs – cocaine and methamphetamine – as well as therapeutic drugs containing amphetamine salts, methylphenidate, and modafinil (Wood et al., 2014). The general effects of all psychostimulants include increased locomotion, arousal, wakefulness,

attention, and anorexia (Westfall & Westfall, 2006). When taken at low doses psychostimulants can be used as cognitive enhancers, as stimulant drugs enhance the perceptual salience of environmental stimuli (Everitt & Robbins, 2005). For example, rats that are given a noncontingent administration of amphetamine to the ventral striatum will increase their lever responding to unconditioned and unrewarded visual stimuli (Shin et al., 2010). Others have shown that rats trained to lever press for sucrose reward will increase lever pressing on an extinction trial when given an intra-accumbens injection of amphetamine (Wyvell & Berridge, 2000). While this mechanism is ultimately beneficial for learning and survival it is also one of the driving factors that make psychostimulants so addictive. Enhanced perception of salient cues paired with the rewarding effects of the drug itself allow for an exponential rate of reinforcement that can eventually lead to addiction (Covey et al., 2014; Parkinson et al., 1999; Di Chiara, 1999). As dopamine signaling is critical for the initial positive reinforcing effects of drugs – both behaviorally and pharmacologically – some introduction to the dopamine system is warranted.

C. Historical Perspective on Dopamine & Reward

Dopamine did not earn its title as an independent catecholeminergic neurotransmitter until the early 1960's when fluorescent histological studies provided neuroanatomical evidence for dopamine-based circuits (Carlsson et al., 1962). Shortly after its discovery, dopamine was proposed to be associated with aspects of reward (Phillips & Fibiger, 1973). One of the most prominent and first largely accepted hypotheses to come out of the field was the general anhedonia model, or the dopamine hypothesis of reward, which postulates that dopamine systems mediate the reinforcing properties of stimuli (Wise, 1982). Various studies have linked increased activity

of dopamine signaling with appetitive behavior towards a positively reinforced stimulus, such as natural rewards, intracranial self-stimulation (ICSS), and drugs of abuse (Wise, 1978; 1985; 1982).

While the general anhedonia model continues to be a widely recognized hypothesis today, there remain some faults. One of the most notable problems to arise for this model is the fact that hedonic elements of stimuli are maintained following lesions to dopamine areas. For example, rats receiving intra-oral infusions of palatable taste stimuli respond with stereotypical orofacial responses – termed taste reactivity (Grill & Norgren, 1978). Selective destruction of dopamine neurons, using 6-OHDA lesions, fail to alter appetitive taste reactivity to concentrated sucrose solution (Berridge & Robinson, 1998). Additionally, evidence has revealed that increases in dopamine are in fact anticipatory to the reward itself, as shown by studies focused on food (Roitman et al., 2004; Day et al., 2007), drug (Aragona et al., 2009; Fontana et al., 1993; Phillips et al., 2003), and ICSS reward (Owesson-White et al., 2008). This has lead to the belief that dopamine plays a role in associations of reward and may not be as critical for the hedonic properties of reward. Combined, these studies point to the fact that "reward" is not a unitary construct, and must derive from a set of complex interactions.

Following these discoveries, several widely recognized and competing hypotheses arose to explain the role of dopamine in motivation and reward. Following their lesioning studies, Robinson and Berridge proposed the incentive salience hypothesis, stating that reward processes can be divided into "wanting" and "liking", whereby dopamine modulates only the "wanting" of a stimulus, and "liking" is derived from a more complex interaction of learning and hedonic systems (Robinson & Berridge, 1998). Wolfram Shultz and colleagues recorded the electrophysiological responses of individual dopamine neurons and arrived at a different hypothesis. That is, dopamine plays a critical role in the regulation of reinforcement learning (Shultz, 1997). More specifically,

he and colleagues have observed that brief bursts of dopaminergic neural activity shift from primary rewards to the earliest reliable predictor of reward. The results led to the formulation of the reward prediction error hypothesis – that dopamine responses track the predictability of reward, which matches well with computational models of associative learning (Shultz, 1998). Lastly, an effort hypothesis has been proposed, where dopamine is responsible for initiating effort-related behavior needed to obtain a reward (Salamone & Correa, 2002). While the exact function of dopamine relating to reward processes is still hotly debated, it is clear that rewarding stimuli, including the initial exposure to drugs of abuse, is indeed associated with increased dopamine signaling.

D. Understanding the Essentials of Dopamine Signaling

Dopamine neurons lie almost exclusively in the midbrain. Within the midbrain there are multiple subgroups of dopamine neurons that project to different targets and play roles in different aspects of behavior. These subgroups include the retrorubral area (A8), substantia nigra pars compacta (SNc; A9), ventral tegmental area (VTA; A10), posterior hypothalamus (A11), arcuate nucleus (A12), and zona incerta (A13) (Dahlstrom & Fuxe, 1964). A9 is additionally categorized into ventral (A9v) and dorsal (A9d) tiers. A8, A9, and A10 are the most recognized of these groups as they pertain most closely to the classic functions of dopamine such that they service motor function and motivated behavior (Van den Heuvel et al., 2008). The remainder of the groups are primarily involved in hormone regulation (Björklund et al., 1975; Torre & Falorni 2007). A12 is particularly notable for it's role in inhibiting prolactin – a hormone secreted from the pituitary gland that enables lactation (Torre & Falorni 2007).

Midbrain dopaminergic cells project largely to structures found in the forebrain including the striatum and NAc (Anden et al., 1964). These projections can be broken down even further based on the subpopulation of dopamine cells, and specifically within the VTA. Cells found in the medial posterior portion of the VTA project to the medial prefrontal cortex (mPFC), the NAc core, and the medial NAc shell (Ikemoto, 2007; Lammel et al., 2008). Alternatively, cells found in the lateral portion of the VTA and SNc have projections to the NAc lateral shell, and dorsal striatum (Ikemoto 2007; Lammel et al., 2008). Beyond anatomical relation, these circuits have also been shown to have differential responses to drugs of abuse. NAc medial and lateral shell-projecting cells are selectively modified by cocaine, whereas mPFC-projecting cells fail to show changes in synaptic plasticity when exposed to cocaine (Lammel et al., 2011). Thus indicating a role for the NAc in the initial reinforcing effects of drugs of abuse.

The NAc is primarily comprised of inhibitory GABAergic medium spiny neurons (MSNs) that contain dopamine-binding receptors (Shuen et al., 2008). There are two classes of receptors to which dopamine can bind, and are differentiated with respect to their impact on adenylyl cyclase and cyclic AMP. D1-like receptors (D1, and D5) increase, and D2-like receptors (D2, D3, and D4) decrease cAMP production (Rankin et al., 2010). D1- and D2-like receptors can also be distinguished by their affinity, such that D1-like receptors have a low affinity, and D2-like receptors a high affinity to dopamine binding. While D2-like receptors are associated with responding to basal levels of dopamine, D1-like receptors are preferentially activated by brief bursts of highly concentrated dopamine release seen in phasic signaling (Goto & Grace 2007; Grace et al., 2007). The effects of drugs of abuse are largely thought to occur on D1-like receptors, however recent work suggests a role for D2-like receptors on behavioral responses to drugs of

abuse (Lobo & Nestler, 2011; Lobo et al., 2010). Nevertheless, postsynaptic dopamine receptors are only one example of targets accessed by drugs of abuse.

Synthesized from tyrosine, dopamine is packaged into synaptic vesicles for storage and transmission by vesicular monoamine transporters (VMATs) (Schuldiner et al, 1995). While VMATs provide tight regulation of available concentrations of dopamine, there is an additional mechanism for regulation of extracellular dopamine that is controlled by plasma membrane monoamine transporters. Specific to dopamine is the dopamine active transporter (DAT). DAT, found on the presynaptic cell membrane, is responsible for the rapid reuptake of dopamine back into the cell, following its release (Torres et al., 2003; Cragg & Rice, 2004). DAT is critical for dopamine homeostasis, and has long been thought to be the primary target for psychostimulant drugs such as cocaine.

E. Cocaine: a Drug Classically Associated with Direct Action on Dopamine Terminals

Cocaine is a psychostimulant that is derived from the coca plant typically found in South America. As with all psychostimulants cocaine causes an overall state of increased arousal, attentiveness and locomotion. Cocaine also increases heart rate, blood pressure and temperature. In the case of overconsumption, cocaine can cause cardiac arrest and death (Gold, 2005). In human users, cocaine can either be snorted in powder form, dissolved in water and injected, or used to create crystal like “crack” rocks that can be smoked. While it seems cocaine use has been on the decline in the U.S., in 2013 it was estimated that there are still approximately 1.5 million American users (SAMHSA, 2014). Like humans, rodents will readily self-administer cocaine and show similar addictive behaviors – a fact that has proven to be imperative for the study of cocaine and addiction alike. A landmark study demonstrated rats were just as capable as humans to reach

criteria for drug addiction based on their inability to refrain from cocaine self-administration, their willingness to work for cocaine reward, and their persistence in attaining drug when faced with an aversive consequence of an electric shock (Deroche-Gamonet et al., 2004). Just one experience with cocaine can elicit these powerful behavioral effects, as it was found that rats show cue-induced drug seeking behavior for up to one year after a single exposure to the drug (Ciccocioppo et al., 2004). Other species such as nonhuman primates and even worms show similar affinities to cocaine (Martelle et al., 2008; Rawls et al., 2010). Taken together, it is clear that cocaine can seize powerful control over behavior – even after just one experience with the drug.

The addictive properties of cocaine arise, at least initially, from its effects on dopamine signaling. Classically, cocaine has been characterized as a psychostimulant that binds to the transporters of biogenic amines such as dopamine, serotonin, and norepinephrine (Lucher & Ungless, 2006). It is most widely known for its action on the DAT. Cocaine binds to the DAT and blocks the reuptake of dopamine thus creating increases in the magnitude of release, time spent in extracellular space, and the diffusion of dopamine (Ritz et al., 1990). Its action on other biogenic amine transporters – serotonin and norepinephrine – functions in a similar manner. The transporter mechanism has become the common denominator in explaining the vast effects of cocaine both pharmacologically and behaviorally. For example, mice with functional but cocaine insensitive DAT lack locomotor effects, increased extracellular dopamine, and conditioned place preference in response cocaine – suggesting a critical role for the DAT in mediating the rewarding effects of cocaine (Chen et al., 2006). While it is clear that the DAT underlies primary effects, more recent revelations challenge the idea that cocaine's mechanism of action is this straightforward.

The effects of cocaine on dopaminergic cell excitability are more controversial than it's effects on the presynaptic DAT mechanism found at the terminal. The first electrophysiological

studies found that VTA dopaminergic cells decreased in firing rate following cocaine administration in anesthetized preparations (Einhorn et al., 1988; Hinerth et al., 2000; Mercuri et al., 1992). This agreed with the DAT mechanism, such that inhibition of reuptake allowed increased extracellular dopamine to act on D2 autoreceptors of dopamine neurons – thereby creating a negative feedback loop and decreasing the firing rate of the VTA. However recent data challenges this seemingly succinct story. When anesthetized rats were compared to awake rats a differential effect was demonstrated where VTA firing rates increased in awake but not in anesthetized rats following systemic cocaine administration (Koulchitsky et al., 2012). Even more controversial is a recent study suggesting that even in anesthetized animals there is a subpopulation of VTA neurons that increases in firing rate following cocaine administration (Mejias-Aponte et al, 2015). Firing rate remains only one example of the mysterious neural effects of cocaine.

Midbrain dopamine neurons exhibit both tonic and phasic firing patterns. While both patterns contribute to the ambient level of extracellular dopamine in their respective terminals (Owesson-White et al., 2012), phasic dopamine firing has a unique relationship with reward and salient reward-related cues. When presented with a primary reward or conditioned cue, dopamine neurons will show a rapid and synchronous firing response (Shultz, 1998) leading to brief bursts of extracellular dopamine release as demonstrated by fast-scan cyclic voltammetry (FSCV; Sombers et al., 2009). FSCV is an in vivo technique that allows for real-time monitoring of phasic bursts of dopamine release resulting from its high temporal resolution. FSCV has proven instrumental in uncovering not only the physiological mechanisms of dopamine signaling, but their behavioral relevance as well. These brief bursts of concentrated dopamine release observed through FSCV are referred to as transients and occur not only during reward-related events but spontaneously as well (Robinson et al., 2002; Wightman et al., 2007; Owesson-White et al., 2012).

Cocaine increases the duration of dopamine transients as a result of inhibited reuptake. However it not only mediates the duration of transient events but increases the amplitude and frequency as well (Aragona et al, 2008). While the DAT mechanism can be used to explain prolonged duration of transients and even amplitude, it does not suffice in reconciling the increased frequency of these events – as frequency is directly tied to the excitability of the dopamine neuron itself. Interestingly, recent work has shown that one way cocaine may be increasing dopamine neuron excitability is via $\alpha_1$adrenoreceptors (Goertz et al., 2014). While we know cocaine increases dopamine signaling, we do not yet know all of the means by it does so, let alone the effects it has on other systems.

F. Beyond Direct Action on the Dopamine Neuron

Since the inception of the classic mechanistic views, addiction research has focused primarily on midbrain dopamine and corresponding forebrain regions – ignoring the implications of other neural substrates affected by drugs of abuse. Due to the manner in which drugs are consumed their effects are not limited to this system alone. In fact, unless centrally administered to a precise location, drugs should be assumed to have wide reaching and global effects across brain and body. The narrow focus on midbrain and forebrain mechanisms in regards to addictive drugs has left other areas of the brain unappreciated.

Midbrain dopamine neurons receive inputs from a variety of regions across the brain. Some of the more commonly studied afferent projections arise from the amygdala, hippocampus, pallidum, and hypothalamus (Watabe-Uchida et al., 2012). A less appreciated source of projections to the mesolimbic system, and specifically in terms of addiction mechanisms, are inputs from the hindbrain. Recent work has shown that dopamine cell bodies found in the midbrain receive direct projections from structures located in the hindbrain. The pedunculpontine tegmental nucleus

(PPTg) and lateral dorsal tegmental nucleus (LDTg) are two structures found in the rostral hindbrain that have known direct excitatory cholinergic and glutamatergic projections to the VTA (Semba & Fibinger, 1992; West et al., 2003). Additionally, both of these structures show c-Fos activity following cocaine self-administration and experimenter delivered intra-venous cocaine (Zahm et al, 2009). Recent work has also demonstrated that lesions of the LDTg resulted in increased latency to initiate cocaine self-administration (Steidl et al, 2015). Thus hindbrain regions with known projections to the VTA are involved in cocaine-induced processes and are likely directly affected pharmacologically by cocaine. Caudal regions of the hindbrain are less understood in regards to their contribution to dopaminergic signaling and the effects of cocaine. Similar to the PPTg and LDTg, two regions of the caudal brainstem – the parabrachial nucleus (PBN) and the nucleus of the solitary tract (NTS) – show increases in c-Fos following peripherally administered cocaine (Grabus et al., 2004; Buffalari & Rinaman, 2014). The NTS is of particular interest, as it was recently discovered to have direction projections to the VTA via GLP-1 neurons (Alhadeff et al., 2012) and noradrenergic neurons (Mejias-Aponte et al., 2009). While evidence points to the caudal brainstem as a potential site for cocaine action, and furthermore as a site that may have modulatory actions on midbrain dopamine signaling, the consequences of cocaine in this particular region remains unclear.

The hindbrain is home to various neural substrates that are critical in maintaining vital functions for survival such as blood pressure, respiration, and energy homeostasis. While the hindbrain has not traditionally been an area of interest for addiction research, it poses the potential for mediating the effects of addictive drugs. The dense noradrenergic projections arising from the hindbrain are of specific interest, as they may be a means of modulating upstream dopaminergic activity. Addiction research has maintained a magnified interest on midbrain and forebrain

substrates, yet the hindbrain has gone largely unexplored. It is likely that substrates in this region may be involved in alternative mechanisms elicited by drugs of abuse, and in particular – cocaine.

G. Current Study: Regional Effects of Cocaine on Phasic Dopamine Release

This study aimed to determine if the effects of cocaine on phasic dopamine release extend beyond direct action on the dopamine neuron. Psychostimulants increase dopamine concentration in the NAc through the blockade of the DAT. However, they also increase the frequency of dopamine release events, a finding that cannot be explained by reuptake blockade alone. Rather, this effect may be mediated by systemic cocaine-induced increases in neural activity in brain regions that project to dopamine cell bodies resulting in increases in dopamine cell excitability. The hindbrain remains an underappreciated but potential site of action for cocaine and the modulation of phasic dopamine signaling. To further explore regionally selective actions of cocaine on phasic dopamine signaling, we administered cocaine into the lateral or fourth ventricles and compared the dopamine response to that of systemically delivered cocaine. Using FSCV in urethane anesthetized animals we first examined the effects of systemic cocaine on stimulated dopamine release, where we expected to see both an increase in amplitude and duration (an indication uptake) of electrically-evoked dopamine transients as systemic administration would allow for cocaine to act via traditional mechanisms including the DAT. This served as our comparison to central administration, as systemic cocaine most closely emulates the global effects that occur during self-administration. We then compared the effects of intracerebroventricular (I.C.V.) cocaine administration to the lateral ventricle. The flow of cerebral spinal fluid originates in the lateral ventricle (Sakka et al., 2011), therefore we expected to see a potentiation of magnitude and duration of stimulated dopamine release events as cocaine would be able to reach dopamine

terminals directly as well regions that act to enhance dopaminergic systems. Lastly, we examined the effects of I.C.V. cocaine administered to the fourth ventricle, as fourth ventricular infusions are restricted to sites of action in the hindbrain (Fitts & Masson, 1989; Hayes et al., 2009). If cocaine has regional effects on structures with known projections to the VTA such as the NTS, we expected fourth ventricular cocaine to modulate phasic dopamine release. While we now know there are direct projections arising from the hindbrain and terminating on the VTA, it is less understood how these projections influence dopamine function. These studies provide strong evidence for hindbrain-originating modulatory processes of phasic dopamine signaling in the midbrain.

II. Methods

A. Subjects

Forty-two experimentally naïve, male Sprague-Dawley rats (Charles River Laboratories, Chicago, IL) weighing approximately 300-400 g at testing were individually housed in plastic cages on a 12:12 light:dark cycle (lights on at 7 am). Experiments were conducted during the hours of their light cycle. Rats were maintained on *ad libitum* food and water daily. All rats were treated according to the guidelines recommended by the Animal Care Committee of the University of Illinois at Chicago.

B. Surgery

Rats were anesthetized with intraperitoneal (IP) urethane (1.5 mg/kg). Following anesthesia, the surgical area of the head was shaved and the animal was placed in a stereotaxic frame (KOPF Instruments; Tujenga, CA) where the surgical area was sterilized with Betadine scrub and alcohol pads. An incision running anterior to posterior was made and the surface of the skull was cleared and leveled between bregma and lambda. Animals were implanted with a guide cannula (Bioanalysical Systems, West Lafayette, IN) dorsal to the NAc (AP: +1.3 mm, ML: +1.0 mm), a chlorinated silver (Ag/AgCl) reference electrode in the cortex contralateral to the guide cannula and rats receiving intracerebral ventricular (I.C.V.) drug were implanted with an infusion cannula into either the lateral ventricle (AP: -1.0 mm, ML: -3.0 mm, DL: 3.0 mm, angled at 10°), or fourth ventricle (AP: -11.5 mm, ML: 0.0 mm, DL: -6.5 mm). Stainless steel screws were implanted to secure all implants with dental cement (Safco Dental Supply Company). A custom-made micromanipulator (UIC Machine Shop, Chicago, IL) was then placed into the guide cannula

and a carbon-fiber electrode was lowered into the NAc core. Simultaneously, a bipolar stimulating electrode (Plastics One Inc., Roanoke, VA) was lowered dorsal to the VTA (AP: -5.2 mm, ML: +1 mm, DV: -7.0 mm). The stimulating electrode was lowered in increments of 2 mm until electrically evoked dopamine release was detected at the carbon-fiber electrode in the NAc. Once an optimal depth (DV: -7.0 to -8.5 mm) was located, the stimulating electrode was cemented in place. Rats remained in the stereotaxic frame and experiments were conducted immediately following surgery.

C. Fast-Scan Cyclic Voltammetry

FSCV allows for real-time monitoring of extracellular dopamine, along with other electroactive species. FSCV is performed at carbon-fiber electrodes (see Fortin *et al*., 2015 for further reference on electrode assembly). The voltage of the electrode is held at -0.4V relative to an Ag/AgCl reference electrode. A triangular waveform of voltage is then applied, and rapidly (400 V/s) scanned from a negative (-0.4V) to a positive (+1.3V) potential and back again. This voltage scan is applied every 100ms. As dopamine oxidizes (~+0.6 V on the positive-going scan) and reduces (~-0.2 V negative-going scan) at known points within this voltage range, it can be identified and dissociated from other electroactive species (**Figure 1A**). Moreover, since the oxidative current elicited by dopamine release is directly proportional to its concentration, dopamine concentration can be quantified (Heien *et al.*, 2004).

Carbon-fiber electrodes are connected to a blue wire that runs out of the back of the micromanipulator, allowing for connection to a removable "headstage". This "headstage" provides connection for all voltammetry-related implantations in order to supply voltage scans at the recording electrode, and a stimulating current to the stimulating electrode. Once the carbon-fiber

electrode is lowered into the NAc it is allowed equilibrate by repeatedly applying the triangular waveform at a rate of 60 Hz for 20 minutes, followed by 10 minutes at a rate of 10 Hz. Scanning the electrode in this manner allows for reduction of current drift and background noise. Once equilibrated, the background current picked up at the carbon-fiber electrode is stable between scans such that it can be subtracted out to reveal changes in current over time – such as that produced by dopamine release.

Using a microfluidic flow cell recording electrodes are calibrated after each experiment in order to test the sensitivity of the electrode with a known concentration of dopamine (**Figure 1B**) (Sinkala et al., 2012). Calibration is done by applying a flow of artificial cerebral spinal fluid (aCSF) buffer over the electrode, followed by a 5 second switch of flow to 1 uM dopamine in the same buffer. The resultant increase in current due to the oxidation of dopamine is measured and used to generate a calibration factor that can be applied to the data acquired in vivo (**Figure 1C**).

D. Drugs

Cocaine. Cocaine hydrochloride was dissolved into its vehicle (saline, 0.9%) to a concentration of 50.0 μg/1.0 μl saline for I.C.V. manipulations. This dose was chosen based on its ability to induce a conditioned place preference when given to the lateral ventricle (Morency & Beninger, 1986). Rats received microinjections of 1 μl of this concentration to either the lateral or fourth ventricle. Rats that received systemic (I.P.) cocaine received a concentration of 2.5 mg/kg body weight dissolved into saline (0.9%). This dose was selected to match the magnitude of effect elicited by ICV cocaine on the peak of evoked dopamine release based on early pilot data of the ventricular effects on dopamine release.

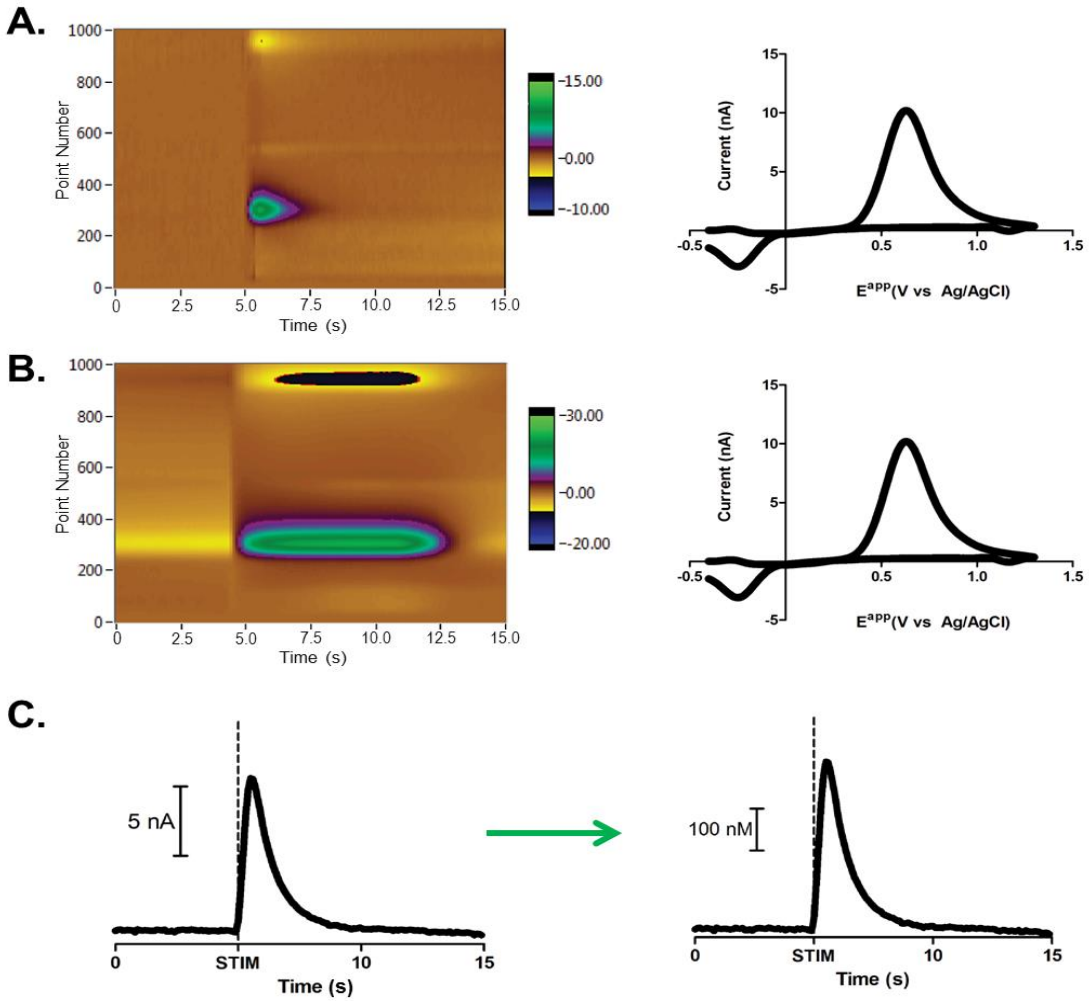

Figure 1. *Visualization, confirmation, and quantification of dopamine release using FSCV.* **(A).** *Left*: Background-subtracted color plot of electrically-evoked dopamine release at 5s, where changes of current are represented by color and the range of applied potentials (y-axis) are plotted as a function of time (x-axis). *Right:* Cyclic voltammogram (CV) representing characteristic changes in background-subtracted current (y-axis) produced by the oxidation of dopamine as a function of applied potential (x-axis) following electrical stimulation. **(B).** *Left*: Background-subtracted color plot of a known concentration of dopamine applied to a carbon-fiber electrode beginning at 5s within a microfluidic flow cell during calibration. *Right*: Cyclic voltammogram of

oxidative current produced by a known concentration of dopamine during calibration. Conventions are identical to those of **Figure 1A** for both *Left* and *Right***. (C).** *Left:* Trace of current (y-axis) elicited by electrically-evoked dopamine release as a function of time (x-axis) where "STIM" represents the beginning of electrical stimulation. *Right:* Trace of calculated concentration (y-axis) of dopamine release across time (x-axis). As oxidative current and concentration are directly proportional, quantification of concentration is determined by using a calibration factor calculated from a known concentration of dopamine during calibration flow cell recordings.

E. Experimental Paradigm

Experiments were performed the same day as the surgery described above. As FSCV parameters needed for monitoring evoked dopamine release were set during surgery, experiments began immediately after implantations were secured. Rats remained in the stereotaxic frame following surgery and the carbon-fiber electrode that was lowered into the NAc during surgery was used for the remainder of the experiment. During the experiment a train of current pulses (24 pulses, 60 Hz, 120 μA, 4ms/pulse) was delivered to the VTA every 3 minutes. Dopamine concentration was measured during the 5 seconds prior to and the 15 seconds after the onset of VTA stimulation. Each stimulation evoked a sharp rise in dopamine concentration followed by an exponential decay (**Figure 1C**). Peak dopamine concentration ([DA] max) as well as the latency for dopamine concentration to decay from peak concentration by 50% were determined for each stimulation – the latter being a measure of the rate of reuptake by the DAT (España et al., 2008). Once a stable baseline of peak release was achieved – as determined by 3 consecutive recordings within 10% of each other – animals received drug or vehicle either systemically (I.P.) or centrally to either the lateral or fourth ventricle (I.C.V.). Stimulations continued for 1 hour following drug or vehicle administration.

F. Histology

After experiments were completed a stainless steel electrode was lowered through the guide cannula to the depth of the recording site. Current (1.0 mA, 0.4 s) was applied to create a lesion in order to localize the recording site. Additionally, 1 μl of india ink (Fischer Scientific) was infused into the infusion cannula to verify placement of either the lateral or fourth ventricle for rats that underwent I.C.V. manipulations. In order to preserve the efficacy of the ink in the

ventricles, rats underwent live-decapitation. No further anesthesia was required due to the deep plane of anesthesia achieved with urethane. Rats that did not have infusion cannulas implanted also underwent live-decapitation. Brains were immediately extracted and stored in 4% formalin for at least 24 hours before being mounted and sliced in a 20°C cryostat (LEICA CM1850). Brain slices were mounted on Poly-L-Lysine subbed slides (American Master*Tech Scientific, Inc.) and placements were verified and photographed using bright field microscopy (Olympus BX43 Fluorescence Research Microscope). All recordings were determined to be located in the core of the NAc, as summarized by **Figure 2**.

G. Data Analysis

Data ([DA] max; 50% decay rate) from each rate was expressed as a percentage of the average baseline. Separate statistical comparisons for each of these measures were conducted for each injection/infusion site (systemic, lateral ventricle, fourth ventricle). For each measure within a given injection/infusion site, a 2-way mixed analysis of variance (ANOVA) – where drug treatment (cocaine vs. saline) was a between-subjects variable and time (23 samples) was a within-subjects variable – was calculated. Significant interaction terms were further explored using Bonferroni-corrected t-tests at each time point.

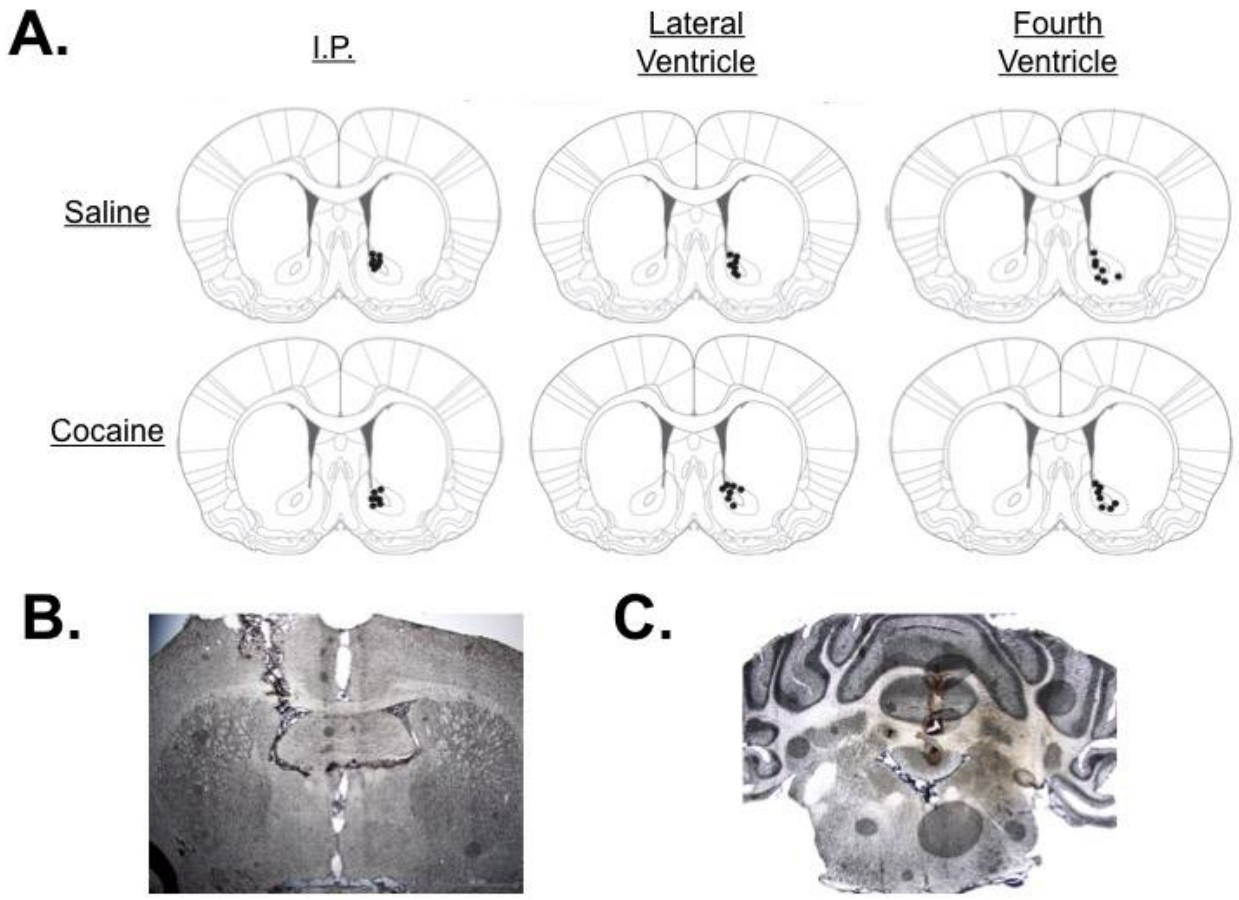

Figure 2. *Histological verification of recording and infusion sites.* **A.** Electrolytic lesions of FSCV recording sites in the NAc of each subject are represented as black dots and are summarized by treatment (saline, cocaine) and location of treatment (I.P., lateral ventricle, fourth ventricle). Each experimental group contains an n = 7. **B.** Representative example of lateral ventricular cannula placement and visualization of India ink infused into the lateral ventricle. **C.** Representative example of fourth ventricular cannula placement and visualization of India ink to infused into the fourth ventricle.

III. Results

A. Systemic cocaine potentiates the magnitude and duration of electrically-evoked dopamine release events in the NAc.

We aimed to investigate regional effects of cocaine on electrically-evoked dopamine release in anesthetized rats. Electrical stimulation evokes a sharp rise in dopamine due to release but the magnitude is also limited due to reuptake throughout the stimulation. In addition, dopamine 'spikes' decrease in an exponential manner due to reuptake. Thus, it was hypothesized that if systemic cocaine (2.5 mg/kg I.P.) increased dopamine concentration solely via reuptake blockade then it would increase the magnitude of dopamine release as well as the duration following electrical stimulation of the dopamine cell bodies in the VTA relative to saline. Data were normalized to average baseline measurements and expressed as percent change for all subjects as to account for differences across subjects due to a variety of factors that could include carbon-electrode sensitivity, number of release sites in the NAc captured, and stimulation site of the VTA. Systemic cocaine reliably increased the magnitude of electrically-evoked dopamine release events in the NAc. The effects of drug treatment on magnitude are represented by **Figure 3A** where the average [DA]max at each of the 23 time points is expressed as percent change from baseline. Analysis revealed that the magnitude of electrically-evoked dopamine release varied as a function of time, [$F(1, 23) = 3.60$, $p < 0.001$], as well as a function of drug treatment, [$F(1, 23) = 19.45$, $p < 0.001$]. More importantly, a significant interaction was found such that cocaine-treated rats showed an increase in magnitude of dopamine at specific time points related to saline-treated rats, [$F(1, 23) = 9.26$, $p < 0.001$]. Post-hoc Bonferroni t-tests revealed differences between cocaine and

saline treatment beginning at 12 minutes post-drug administration ($p < 0.05$), and continued to be significant until the end of the session at 60 minutes ($p < 0.001$).

Systemic cocaine also reliably increased the duration of electrically-evoked dopamine release events across time. Duration was calculated as the average latency for the signal to decay to half maximum (50% decay) and is expressed as a percent change from baseline in **Figure 3B**. The duration of dopamine release events varied both as a function of time, [$F(1, 23) = 7.11$, $p < 0.001$], as well as by drug treatment, [$F(1, 23) = 32.02$, $p < 0.001$]. As with magnitude, a significant interaction was found such that cocaine-treated rats showed an increase in duration of release events at specific time points relative to saline-treated rats, [$F(1, 23) = 6.98$, $p < 0.001$]. Post-hoc analysis revealed that differences between cocaine- and saline-injected rats emerged at 12 minutes ($p < 0.05$) and remained significant through the remainder of the session ($p < 0.05$). In **Figure 3C**, since significant differences emerged at 12 minutes (denoted by rectangle in Figure 2A and Figure 2B) following injection, the average dopamine spike evoked during the final baseline stimulation is plotted with the average dopamine spike evoked 12 minutes after injection for saline and cocaine injected rats. A summary of both magnitude and duration effects at the 12 minute time point for both systemic cocaine and control groups is depicted in **Figure 3D**.

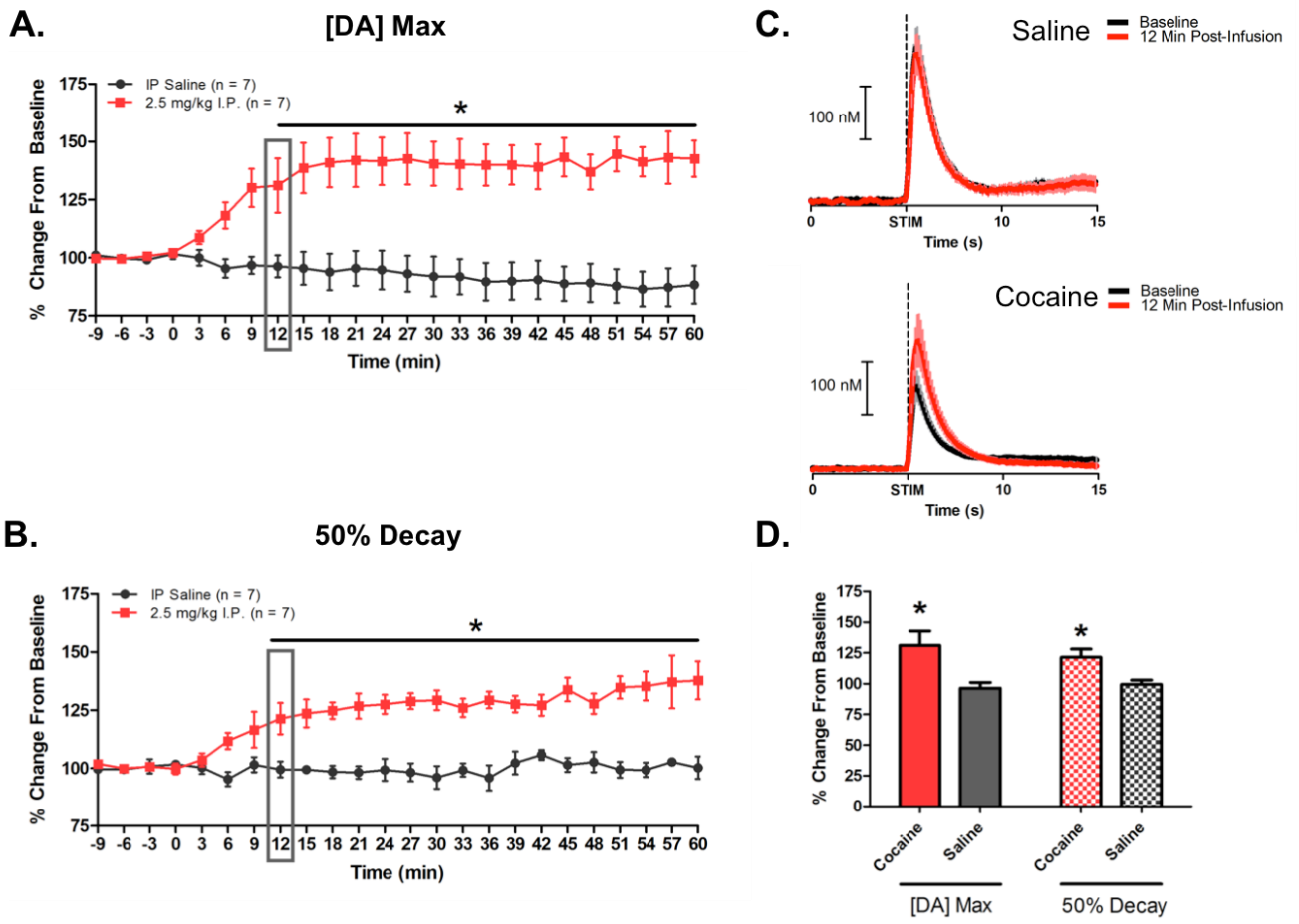

Figure 3. *Systemic cocaine potentiates both magnitude and duration of electrically-evoked dopamine release events in the NAc.* **(A).** Average [DA]max at each of the 23 time points and expressed as percent change from baseline. Symbols are the means and error bars represent ±1 standard error of the mean (SEM) for saline (black filled circle and line) and cocaine (red filled squares and line) injected rats. Significance is represented as * = $p < 0.05$. **(B).** Average latency for the signal to decay to half maximum (50% decay) expressed as a percentage of baseline. Conventions are identical to those of **Figure 3A**. **(C).** Average dopamine spike evoked during the final baseline stimulation (black trace) is plotted with the average dopamine spike evoked 12 minutes after injection (red trace) for saline (top) and cocaine (bottom) injected rats. SEM is represented as a lighter shade surrounding corresponding bolded lines. **(D).** Summary of average

[DA] max and 50% decay at 12 minutes post-injection expressed as percent change. Bars represent means and error bars represent ±1 SEM for saline (dark gray) and cocaine (red) treatment. Significance is represented as * = $p < 0.05$.

B. Lateral ventricular cocaine potentiates the magnitude but not duration of electrically-evoked dopamine release events in the NAc.

Lateral ventricular cocaine was examined for its regional effects on electrically-evoked dopamine release in the NAc. It was expected that, like systemic cocaine, it too would increase both the magnitude and duration of dopamine release via dopamine transporter blockade. Indeed, lateral ventricular cocaine reliably increased the magnitude of electrically-evoked dopamine release. The average [DA]max at each time point is represented as percent change from baseline for both saline- and cocaine-treated rats in **Figure 4A**. Analysis revealed a main effect of both time, [$F(1, 23) = 5.80$, $p < 0.001$], and drug treatment [$F(1, 23) = 12.50$, $p < 0.01$]. Additionally, a significant interaction was found where animals receiving lateral ventricular cocaine showed an increase of magnitude across time compared to saline-treated animals, [$F(1, 23) = 4.86$, $p < 0.001$]. Post-hoc analysis revealed this interaction was significant between the 6 minute ($p < 0.05$) and 30 minute ($p < 0.05$) time points. Therefore lateral ventricular cocaine increases the magnitude of electrically-evoked dopamine release in NAc in a rapid, yet brief manner as compared to systemic cocaine.

Unlike the effects on magnitude, lateral ventricular cocaine failed to significantly effect the duration of electrically-evoked dopamine release events. The average latency to reach 50% decay is expressed as percent change from baseline for each time point in **Figure 4B**. While statistical analyses revealed a main effect of time, [$F(1, 23) = 4.15$, $p < 0.001$], there was no significant effect of drug treatment, [$F(1, 23) = 1.34$, p = n.s.], nor was there an interaction of cocaine exposure across time, [$F(1, 23) = 1.33$, p = n.s.]. Therefore lateral ventricular cocaine did not significantly effect the duration of electrically-evoked dopamine release in the NAc. As systemic cocaine became significant only after 12 minutes post drug administration, this time point was used for all

experimental groups to compare differences in both magnitude and duration of electrically-evoked dopamine release events. Average dopamine spikes comparing stimulation during the final baseline and 12 minutes post-treatment from both cocaine- and saline-treated rats are illustrated in **Figure 4C**. A summary for both [DA]max, and 50% decay is represented in **Figure 4D** as the average percent change from baseline at 12 minutes post-treatment for both saline- and cocaine-injected rats.

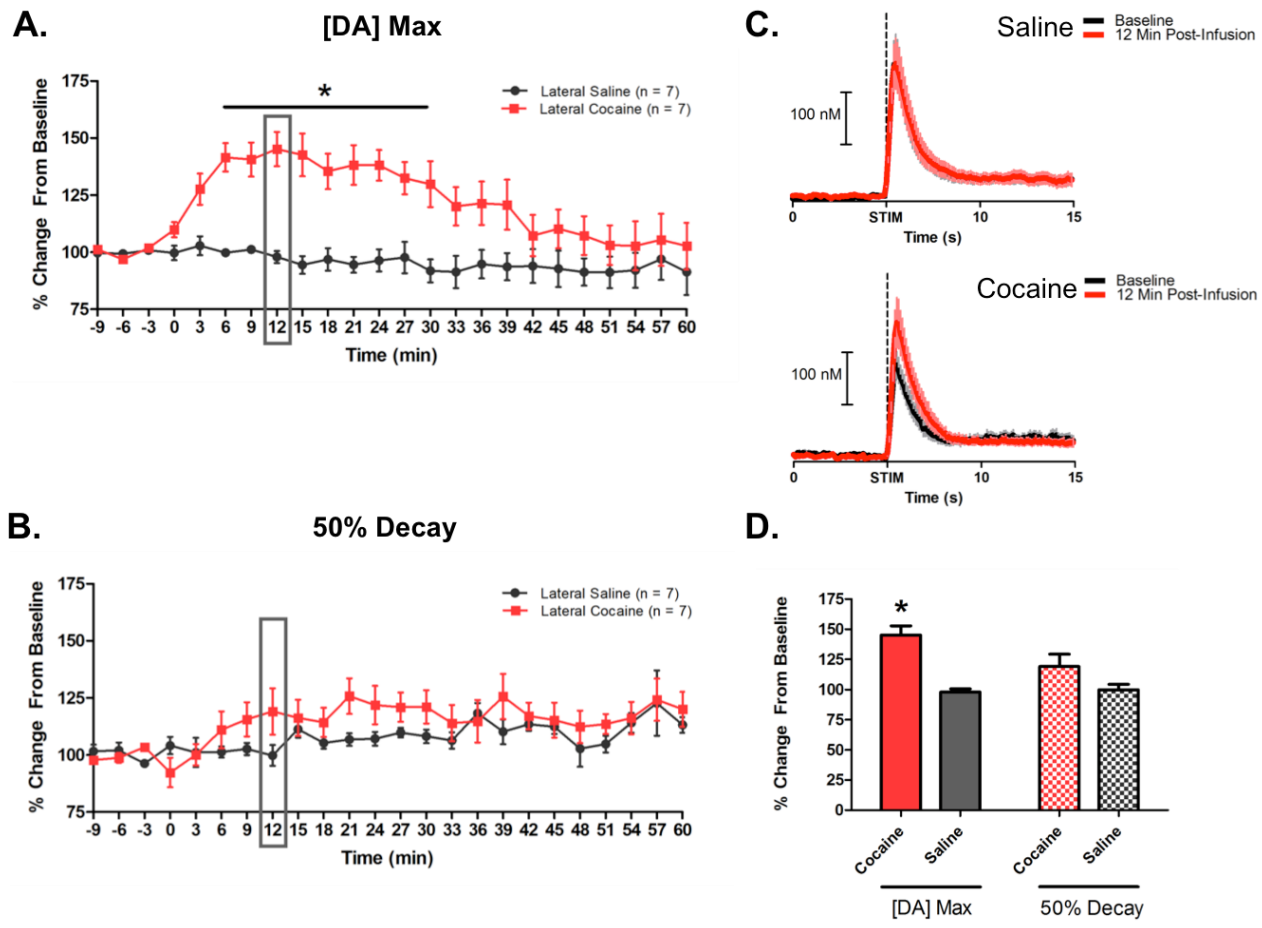

Figure 4. *Lateral ventricular cocaine potentiates the magnitude but not duration of electrically-evoked dopamine release events in the NAc.* **(A).** Average [DA]max at each of the 23 time points and expressed as percent change from baseline. Symbols are the means and error bars represent ±1 standard error of the mean (SEM) for saline (black filled circle and line) and cocaine (red filled squares and line) injected rats. Significance is represented as * = $p < 0.05$. **(B).** Average latency for the signal to decay to half maximum (50% decay) expressed as a percentage of baseline. Conventions are identical to those of **Figure 4A**. **(C).** Average dopamine spike evoked during the final baseline stimulation (black trace) is plotted with the average dopamine spike evoked 12 minutes after injection (red trace) for saline (top) and cocaine (bottom) injected rats. SEM is

represented as a lighter shade surrounding corresponding bolded lines. **(D).** Summary of average [DA] max and 50% decay at 12 minutes post-injection expressed as percent change. Bars represent means and error bars represent ±1 SEM for saline (dark gray) and cocaine (red) treatment. Significance is represented as * = $p < 0.05$.

C. Fourth ventricular cocaine potentiates the magnitude but not duration of electrically-evoked dopamine release events in the NAc

Cocaine administered to the fourth ventricle was used to explore hindbrain activation and it's regional effects on electrically-evoked dopamine release. If cocaine has regional effects to increase excitatory drive to dopamine cell bodies and has action beyond reuptake blockade, then the effects of cocaine delivered to a region remote to dopamine terminals could reveal such increased drive. Fourth ventricular cocaine reliably increased the magnitude of electrically-evoked dopamine release events in the NAc. The effect of cocaine – as compared to saline – on magnitude can be seen in **Figure 5A**, where [DA]max is represented as a percent change from baseline across each time point recorded. Statistical analyses revealed a main effect of time, [$F(1, 23) = 6.39$, $p < 0.001$], as well as a main effect of drug treatment, [$F(1, 23) = 6.15$, $p < 0.05$]. These effects were modulated by a significant interaction, [$F(1, 23) = 5.11$, $p < 0.001$], such that fourth ventricular cocaine increased the magnitude of dopamine release events over time compared to saline-treated animals. Post-hoc Bonferroni t-tests proved this interaction to be significant from 6 minutes following drug administration ($p < 0.05$) to 21 minutes ($p < 0.05$). Therefore, similar to lateral ventricular cocaine, cocaine administered to the fourth has a rapid, yet brief effect on the magnitude of electrically-evoked dopamine in the NAc compared to systemic cocaine.

Fourth ventricular cocaine failed to show a significant effect on the duration of electrically-evoked dopamine release. Latency to reach 50% decay is represented as a percent change from baseline across time in **Figure 5B**. Analyses revealed a main effect of time, [$F(1, 23) = 2.52$, $p < 0.001$] but no significant main effect of drug treatment, [$F(1, 23) = 1.65$, p = n.s.] or interaction of drug treatment over time on duration, [$F(1, 23) = 0.907$, p = n.s.]. Thus, while fourth ventricular cocaine significantly modulates the magnitude of electrically-evoked dopamine release in the

NAc, it has no significant effect on the duration of such events. Average dopamine spikes for both cocaine- and saline-treated animals at the final baseline recording and 12 minutes post drug administration are illustrated in **Figure 5C**. Additionally, a summary of both fourth ventricular saline and cocaine effects on [DA]max and 50% decay at the 12-minute time point are represented in **Figure 5D**.

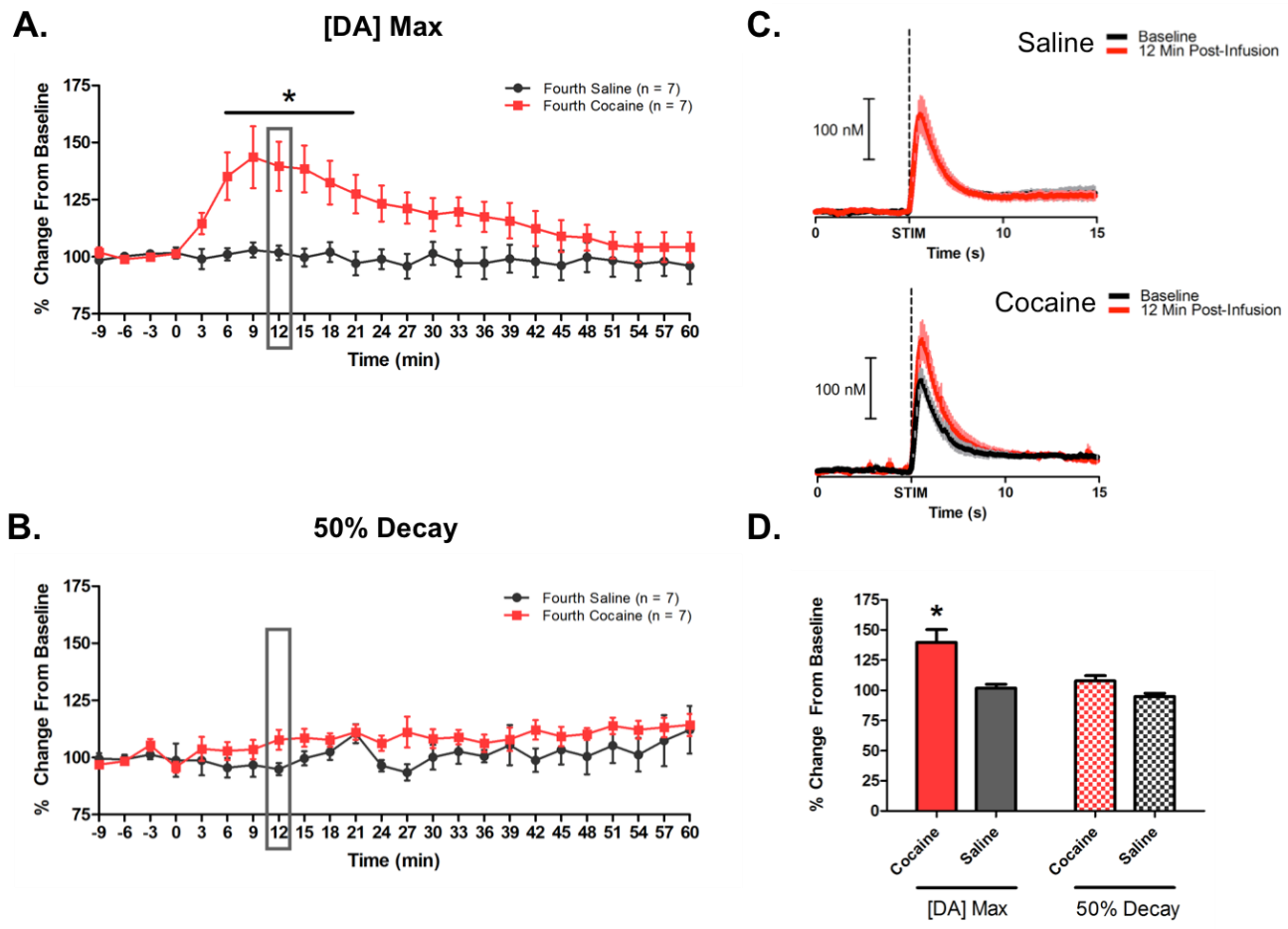

Figure 5. *Fourth ventricular cocaine potentiates the magnitude but not duration of electrically-evoked dopamine release events in the NAc.* **(A).** Average [DA]max at each of the 23 time points and expressed as percent change from baseline. Symbols are the means and error bars represent ±1 standard error of the mean (SEM) for saline (black filled circle and line) and cocaine (red filled squares and line) injected rats. Significance is represented as * = $p < 0.05$. **(B).** Average latency for the signal to decay to half maximum (50% decay) expressed as a percentage of baseline. Conventions are identical to those of **Figure 5A**. **(C).** Average dopamine spike evoked during the final baseline stimulation (black trace) is plotted with the average dopamine spike evoked 12 minutes after injection (red trace) for saline (top) and cocaine (bottom) injected rats. SEM is

represented as a lighter shade surrounding corresponding bolded lines. **(D).** Summary of average [DA] max and 50% decay at 12 minutes post-injection expressed as percent change. Bars represent means and error bars represent ±1 SEM for saline (dark gray) and cocaine (red) treatment. Significance is represented as * = $p < 0.05$.

IV. Discussion

Mesolimbic dopamine signaling is essential to the initial reinforcing properties of drugs of abuse and the formation of drug addiction. The primary mechanisms by which different classes of addictive drugs potentiate dopaminergic signaling have largely been elucidated, however there remain additional effects on dopamine action that cannot be explained by their traditional actions alone. *This study aimed to probe cocaine action in different parts of the neuraxis for effects on NAc dopamine signaling to uncover possible alternative targets of cocaine action that may be involved in the formation of drug addiction.* Indeed we found that cocaine administered to the fourth ventricle – which restricted cocaine action to the hindbrain – potentiated the magnitude ([DA] max) of electrically evoked dopamine release in the NAc. This effect occurred in the absence of an effect on the duration (50% decay) of evoked release and hence cannot be explained by dopamine reuptake blockade – the traditional explanation for cocaine action on dopamine signaling. The rapid and uniform time course of this effect was almost identical to that of lateral ventricular cocaine, where it too augmented magnitude but had no effect on duration of evoked dopamine release events in the NAc. Both centrally administered effects of cocaine were compared to a systemic delivery (2.5 mg/kg) where, not surprisingly, we observed an effect on cocaine on both magnitude and duration of evoked dopamine release events. Systemic cocaine delivery followed a slightly longer onset yet prolonged time course overall. These data provide evidence for additional mechanisms of cocaine action that include action in the hindbrain and suggest that this underappreciated region may be involved in processes underlying addiction via modulation of upstream dopaminergic signaling.

Previous work has shown that cocaine increases both the magnitude and duration of electrically-evoked dopamine release in the NAc in both slice preparations (Jones et al., 1995; Jones et al., 1995) and in vivo when given systemically (Suaud-Chagny et al., 1995; Jones et al., 1995; Heien et al., 2005). Therefore our results from systemic cocaine exposure were to be expected, however served as a critical baseline comparison for centrally administered groups as well as confirmation of the effect under our FSCV protocol. The effect of increased duration is of particular interest in the systemic group, as it was not shown in the central groups. As previously mentioned, cocaine is traditionally thought to increase extracellular dopamine by acting on the DAT and blocking the reuptake of dopamine into the cell (Torres et al., 2003; Cragg & Rice 2004). As a result of inhibiting the DAT, the latency for dopamine to be cleared from the extracellular space is increased, therefore when cocaine is delivered systemically it should have access to dopamine terminals to inhibit the DAT and increase the duration of electrically-evoked dopamine release events in the NAc. In awake animals, dopamine release events occur spontaneously (Robinson et al., 2002; Wightman et al., 2007; Owesson-White et al., 2012). While the current study did not examine these 'naturally occurring' release events, cocaine has been shown to increase their frequency – a phenomenon that cannot be explained by DAT blockade alone. As frequency relates to cell excitability (Sombers et al., 2009; Owesson-White et al., 2012), it has been suggested that cocaine activates excitatory inputs to the VTA thereby enhancing the ability of dopaminergic cells to release neurotransmitter more readily and frequently (Covey et al., 2014). The results of both lateral and fourth ventricular cocaine speak to this hypothesis.

The idea that cocaine activates central mechanisms is hardly debated, however it is important to note that cocaine must pass the blood brain barrier in order to elicit an unconditioned response of increased dopaminergic signaling (Porter-Stransky et al., 2011). In other words, the

effects of cocaine on dopamine release cannot be attributed to an indirect activation initiated by the peripheral action of cocaine. Furthermore, centrally administered cocaine is sufficient in eliciting similar behavioral effects seen with systemic doses. For example, cocaine administered to the lateral ventricle has been shown to generalize to discriminative properties of a stimulus that was previously trained by systemic administration (Wood et al., 1987). Additionally, the dose of I.C.V. cocaine used in the current study was also shown to induce a conditioned place preference in rats (Morency & Beninger, 1986). This work along with the global spread of I.C.V. infusions to the lateral ventricle make it no surprise that lateral ventricular cocaine potentiates the magnitude of electrically-evoked dopamine release. However, the lack of uptake blockade was surprising as it was assumed cocaine would reach dopamine terminals in the NAc. It is plausible that the I.C.V. infusion of cocaine diffused rapidly within the ventricular system before reaching dopaminergic terminals to access the DAT thereby explaining the lack of effect on duration. Nevertheless, the lack of effect on uptake provides additional support for the idea that cocaine activates excitatory input to dopaminergic cells thus allowing for an increase in magnitude of electrically-evoked dopamine release following lateral ventricular administration of cocaine.

The idea of cocaine indirectly activating the dopaminergic system via excitatory inputs was probed even further by examining the role of the hindbrain. Cocaine has the ability to activate substrates lying within the hindbrain as it has been shown to induce cFOS expression in the PPTg and LDTg (Zahm et al., 2009), as well as the PBN (Grabus et al., 2004). Furthermore, various regions within the hindbrain provide dense excitatory noradrenergic input to dopaminergic cells (Mejias-Aponte et al., 2009) – a plausible target for cocaine action and upstream excitatory drive. The significant effect fourth ventricular cocaine had on magnitude but not duration of electrically-evoked dopamine release in the NAc further supports the idea that cocaine can activate VTA-

projecting substrates, leading to increased excitability of dopaminergic cells via said projections, thus allowing for neurotransmitter to be more readily released – in contrast to the idea that cocaine increases extracellular dopamine only via DAT blockade. However, these results do not speak to the exact mechanism driving increased excitability of VTA dopaminergic cells.

Cocaine not only activates dopaminergic systems, but noradrenergic and serotonergeric systems as well. As previously mentioned, the hindbrain maintains a large noradrenergic presence and provides dense excitatory input to midbrain dopamine neurons. This system serves as a potential explanation for the fourth ventricular effects of cocaine on midbrain dopamine release – whereby cocaine activates noradrenergic substrates within the hindbrain that then directly excite upstream dopaminergic cells thus increasing the magnitude of dopamine release when the VTA is electrically stimulated. While there are several classes of adrenergic receptors ($\alpha1$, $\alpha2$, and β) $\alpha1$ adrenoreceptors are believed to be most closely involved with psychostimulant action (Weinshenker & Schroeder, 2007). Activation of $\alpha1$-receptors is believed to be responsible for psychostimulant-induced increases in VTA firing patterns (Zhou et al., 2006). Recent work supports this further by demonstrating that cocaine activates $\alpha1$-receptors in the ventral midbrain resulting in increased dopaminergic activity (Goertz et al., 2014). While the ventricular system provided a gross approach to exploring regional differences of cocaine action, it fails to provide specificity of the mechanisms at work. To explore the noradrenergic hypothesis further a systemic pretreatment of Prazosin – an $\alpha1$-adrenoceptor antagonist – was used in an attempt to block the fourth ventricular effects of cocaine on electrically-evoked dopamine release in the NAc. However, rather than blunting the potentiating effects of hindbrain-administered cocaine on dopamine release, Prazosin augmented the effect on magnitude even further. It was later revealed that Prazosin alone had a potentiating effect on electrically-evoked dopamine release in

anesthetized animals. As Prazosin was given systemically it likely was activating an array of processes that potentially have counteracting effects. While α1-receptor activation has been shown to potentiate dopaminergic activity, there has also been work arguing for an inhibitory role of α1-activation on dopamine signaling (Paladini & Williams, 2004). It is clear that more work must be done to elucidate the means by which norepinephrine influences dopaminergic signaling, and though general α1 antagonism proved unsuccessful in explaining fourth ventricular cocaine effects on midbrain dopamine release, the noradrenergic hypothesis should not be ruled out, as the mechanisms at work could be localized and specific to certain regions and conditions.

In addition to noradrenergic mechanisms there remains another likely candidate for modulating hindbrain-induced increases in phasic dopamine release. The NTS not only shows increased cFOS following systemic cocaine (Grabus et al., 2004) but it also has direct GLP-1 projections to the VTA (Alhadeff et al., 2012). Recently it has been proposed that GLP-1 receptors play a role in mediating the rewarding effects of cocaine, such that animals given the GLP-1 agonist exendin-4 showed significant decreases in self-administration behavior for cocaine reward (Sørenson et al., 2015). Fourth ventricular cocaine may be acting on the NTS and initiating slightly more complex mechanisms to influence VTA excitability, as compared to the proposed noradrenergic hypothesis. Regardless of exact mechanism, it is clear that substrates within the hindbrain are not only activated by cocaine, but also capable of modulating upstream dopaminergic signaling.

The validity of ventricular flow remains a final, yet unlikely explanation for the fourth ventricular effects of cocaine on electrically-evoked dopamine release in the NAc. Skeptics may believe that cocaine administered to the fourth ventricle could be traveling up the ventricular system or seeping through the intracisternal space and acting via more traditional mechanisms.

This is unlikely for several reasons. First, cerebral spinal fluid is classically seen to flow caudally from the lateral ventricles to the third then fourth ventricle. Hayes et al. (2009) confirmed this when looking at the effects of hindbrain-delivered leptin on pAMPK levels where they found fourth ventricular infusions of leptin decreased pAMPK levels in the NTS (caudal hindbrain) but not in the hypothalamus. As the hypothalamus has an abundance of leptin receptors the lack of effect of fourth ventricular leptin suggests that it was restricted to the caudal hindbrain and did not flow up to more rostral areas surrounding the third ventricle. Additionally, a seminal study on thirst regulation showed lateral ventricular infusions of angiotensin promoted robust drinking behavior, however, similar infusions to the fourth ventricle failed to do so (Fitts & Masson, 1989) – again demonstrating a lack of upstream flow of CSF within the ventricular system. Beyond the published work that refutes the idea of back flow, the use of india ink verification in our histology proves that ink – and presumably I.C.V. cocaine – was restricted to the hindbrain as it was not visible more rostrally in the third or lateral ventricles. Lastly, the similarity in time course of both lateral and fourth ventricular cocaine on evoked dopamine release illustrates the quick action cocaine has on hindbrain processes. If it were the case that cocaine was influencing dopamine signaling via alternative routes other than the hindbrain, it could be assumed the effect would have a delayed onset.

This study serves as a critical first step in uncovering less appreciated effects of cocaine, and addictive drugs in general. This is the first evidence that the caudal hindbrain has a functional role in modulating upstream dopamine signaling when exposed to cocaine. While we cannot explicitly point to the mechanism at large, these results elucidate the full extent of cocaine action and provide insight on regions that have otherwise been overlooked. Having a deep understanding

of the exhaustive effects of cocaine will ultimately support the development of therapeutic aids in blocking the rewarding and reinforcing effects of cocaine that maintain drug addiction.

V. Animal Care Approval

All procedures proposed for this experimentation have been approved by the University of Illinois Animal Care Committee under protocol 15-004.

VI. Funding Mechanisms

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VI. References

Alhadeff AL, Rupprecht LE, Hayes MR (2012). GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology*. 153(2):647-58.

Anden NE, Carlsson A, Dahlstroem A, Fuxe K, Hillarp NA, Larsson K (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sci*. 3:523-530.

Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM (2008). Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is due to a direct increase in phasic dopamine release events. *J Neurosci. 28*(35), 8821–8831.

Aragona BJ, Day JJ, Roitman MF, Cleaveland NA, Wightman RM, Carelli RM (2009). Regional specificity in the real-time development of phasic dopamine transmission patterns during acquisition of a cue–cocaine association in rats. *Eur. J. Neurosci*. 30:1889–1899.

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

Björklund A, Lindvall O, Nobin A (1975). Evidence of an incerto-hypothalamic dopamine neuron system in the rat. *Brain Res*. 89(1):29-42.

Blessing WW, Willoughby JO (1985). Inhibiting the rabbit caudal ventrolateral medulla prevents baroreceptor-initiated secretion of vasopressin. *J Physiol.* 367: 253-265.

Buffalari DM, Rinaman L (2014) Cocaine self-administration and extinction alter medullary noradrenergic and limbic forebrain cFos responses to acute, noncontingent cocaine injections in adult rats. *Neuroscience*. 281C:241-250.

Carlsson A, Falck B, Hillarp NA (1962). Cellular localization of brain monoamines. *Acta Physiol Scand Suppl*. 56: 1-28.

Chen R, Tilley MR, Wei H, Zhou R, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, Han DD, Gu HH (2006). Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc Natl Acad Sci USA.* 103(24):9333-8.

Ciccocioppo R, Martin-Fardon R, Weiss F (2004). Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. *Nat Neurosci.* 7(5):495-6.

Covey DP, Roitman MF, Garris PA (2014). Illicit dopamine transients: reconciling actions of abused drugs. *Trends Neurosci.* 37(4): 200-210.

Cragg SJ, Rice ME (2004). Dancing past the DAT at the DA synapse. *Trends Neurosci*. 27(5):270-

277.

Dahlstroem A, Fuxe K (1964). Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand*. 232:1-55.

Day JJ, Roitman MF, Wightman RM, and Carelli RM. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci*. 10: 1020–1028.

Delfs JM, Zhu Y, Druhan JP, Aston-Jones GS (1998). Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat. *Brain Res*. 806: 127–140.

Deroche-Gamonet V, Belin D, Piazza PV (2004). Evidence for addiction-like behavior in the rat. *Science*. 305(5686):1014-7.

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

Di Chiara G, North RA (1992). Neurobiology of opiate abuse. *Trends Pharmacol Sci*. 13:185-193.

Di Chiara G, Tanda G, Bassareo V, Pontieri F, Acquas E, Fenu S, Cadoni C, Carboni E (1999). Drug addiction as a disorder of associative learning. Role of nucleus accumbens shell/extended amygdala dopamine. *Ann N Y Acad Sci*. 877:461-85.

Einhorn LC, Johansen PA, White FJ (1988). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci.* 8:100–112.

España RA, Roberts DC, Jones SR (2008). Short-acting cocaine and long-acting GBR-12909 both elicit rapid dopamine uptake inhibition following intravenous delivery. *Neuroscience*. 155(1):250-257.

Everitt BJ, Robbins TW (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci*. 8(11):1481-9.

Fibiger HC, Phillips AG (1974). Role of dopamine and norepinephrine in the chemistry of reward. *J Psychiatr Res*. 11: 135-143.

Fitts DA, Masson DB (1989). Forebrain sites of action for drinking and salt appetite to angiotensin or captopril. *Behav Neurosci*. 103(4):865-72.

Fontana DJ, Post RM, and Pert A (1993). Conditioned increases in mesolimbic dopamine overflow by stimuli associated with cocaine. *Brain Res*. 629: 31–9.

Fortin SM, Cone JJ, Ng-Evans A, McCutcheon JE, Roitman MF (2015). Sampling phasic dopamine signaling with fast-scan cyclic voltammetry in awake, behaving rats. *Curr Protoc Neurosci.* 70:7.25.1-7.25.20.

Geisler S, Marinelli M, DeGarmo B, Becker ML, Freiman AJ, Beales M, Meredith GE, Zahm DS (2008). Prominent activation of brainstem and pallidal afferents of the ventral tegmental area by cocaine. *Neuropsychopharm*. 33(11): 2688-2700.

Goertz BR, Wanat MK, Gomez JA, Brown ZJ, Phillips PEM, Paladini CA (2014). Cocaine increases dopaminergic neuron and motor activity via midbrain alpha-1 adrenergic signaling. *Neuropsychopharm.* 1-12.

Gold MS (2005). Cocaine (and crack): Clinical aspects. In: Lowinson, J.H., ed. *Substance Abuse: A Comprehensive Textbook, 4th edition*, Baltimore: Williams & Wilkins, pp. 218-251.

Goto Y, Grace AA (2005). Dopamine-dependent interactions between limbic and prefrontal cortical plasticity in the nucleus accumbens: disruption by cocaine sensitization. *Neuron*. 47(2): 255–266.

Grace, A. A., Floresco, S. B., Goto, Y., and Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci*. 30:220–227.

Grabus SD, Glowa JR, Riley AL (2004). Morphine- and cocaine-induced c-Fos levels in Lewis and Fischer rat strains. *Brain Res.* 998(1):20-8.

Grenhoff J, Nisell M, Ferre S, Aston-Jones G, Svensson TH (1993). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *J Neural Transm Gen Sect.* 93: 11-25.

Grenhoff J, North RA, Johnson SW (1995). Alpha 1-adrenergic effects on dopamine neurons recorded intracellularly in the rat midbrain slice. *Eur J Neurosci.* 7:1707-1713.

Grill HJ, Norgren R (1978). The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats. *Brain Res*. 143(2):281-97.

Hayes MR, Skibicka KP, Bence KK, Grill HJ (2009). Dorsal hindbrain 5'-adenosine monophosphate-activated protein kinase as an intracellular mediator of energy balance. *Endocrinology*. 150(5):2175-82.

Heien ML, Johnson MA, Wightman RM (2004). Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Anal Chem*. 76:5697-5704

Heien ML, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Wassume KM, Wightman RM (2005). Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc Natl Acad Sci USA.* 102(29):10023-8.

Hinerth MA, Collins HA, Baniecki M, Hanson RN, Waszczak BL (2000). Novel in vivo electrophysiological assay for the effects of cocaine and putative 'cocaine antagonists' on dopamine transporter activity of substantia nigra and ventral tegmental area dopamine neurons. *Synapse.* 38:305–312.

Hnasko TS, Sotak BN, Palmiter RD (2005). Morphine reward in dopamine-deficient mice. *Nature*. 438:854-857.

Ikemoto S (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev*. 56(1):27-78.

Jones SR, Garris PA, Kilts CD, Wightman RM (1995). Comparison of dopamine uptake in the basolateral amygdaloid nucleus, caudate-putamen, and nucleus accumbens of the rat. *J Neurochem*. 64(6):2581-9.

Jones SR, Garris PA, Wightman RM (1995). Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *J Pharmacol Exp Ther*. 274(1):396-403.

Jones BE, Halaris AE. McIlhany M, Moore RY (1977). Ascending projections of the locus coeruleus in the rat. I. Axonal transport in central noradrenaline neurons. *Brain Res*. 127: 1-21.

Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR (2006). Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA* 296 (1): 56–63.

Judd LL, Marston MG, Attkisson C, Berrettini W, Buc NL, Bunney BS, Murphy CBG, Dominguez RA, Friedel RO< Drummond HE, (1998). Effective medical treatment of opiate addiction. *Journal of the American Medical Association.* 280(22):1936-1943.

Kahveci N, Gulec G, Ozluk K (2006). Effects of intracerebroventricularly-injected morphine on anxiety, memory retrieval and locomotor activity in rats: Involvement of vasopressinergic system and nitric oxide pathway. *Pharm Biochem Behav*. 85: 859-867.

Koob GF (1992). Drugs of abuse: anatomy, pharmacology and fuction of reward pathways. *Trends Pharmacol Sci*. 13: 177–184.

Kosten TR, George TM (2002). The neurobiology of opioid dependence: implications for treatment. *Sci & Prac Perspec.* 1: 13-20.

Koulchintsky S, De Backer B, Quertemont E, Charlier C, Seutin V (2012). Differential effects of cocaine on dopamine neuron firing in awake and anesthetized rats. *Neuropsychopharmcol*. 37:1559-1571.

Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J (2008). Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron*. 57(5):760-73.

Lammel S, Ion DI, Roeper J, Malenka RC (2011). Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. *Neuron*. 70(5):855-62.

Liprando LA, Miner LH, Blakely RD, Lewis DA, Sesack SR (2004). Ultrastructural interactions between terminals expressing the norepinephrine transporter and dopamine neurons in the rat and monkey ventral tegmental area. *Synapse.* 52: 233-244.

Lobo MK, Covington HE III, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science*. 330:385–390.

Lobo MK, Nestler EJ (2011). The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front Neuroanat.* 5:41.

Luscher C, Ungless, MA (2006). The mechanistic classification of addictive drugs. *PLoS Med*. 3(11):e437.

Martelle JL, Czoty PW, Nader MA (2008). Effect of time-out duration on the reinforcing strength of cocaine assessed under a progressive-ratio schedule in rhesus monkeys. *Behav Pharmacol*. 19(7):743-746.

McNicol E. (2008). Opioid side effects and their treatment in patients with chronic cancer and noncancer pain. *J Pain Palliat. Care Pharmacother*., 22: 270–281.

Mejias-Aponte CA, Drouin C, Aston-Jones G (2009). Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. *J Neuro*. 29(11): 3613-3626.

Mejias-Aponte CA, Ye C, Bonci A, Kiyatkin EA, Morales M (2015). A subpopulation of neurochemically-identified ventral tegmental area dopamine neurons is excited by intravenous cocaine. *J Neurosci.* 35(5):1965-78.

Mercuri NB, Calabresi P, Bernardi G (1992). The electrophysiological actions of dopamine and dopaminergic drugs on neurons of the substantia nigra pars compacta and ventral tegmental area. *Life Sci.* 51:711–718.

Mogenson GJ, Jones DL, Yim CY. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol*. 14, 69–97.

Morency MA, Beninger RJ (1986). Dopaminergic substrates of cocaine-induced place conditioning. *Brain Res.* 399:33-41.

National Drug Intelligence Center (2010). National Threat Assessment: The Economic Impact of Illicit Drug Use on American Society. Washington, DC: United States Department of Justice.

National Institute on Drug Abuse. Trends & Statistics Retrieved from http://www.drugabuse.gov/related-topics/trends-statistics on January 11, 2015

Nestler EJ, Hope BT, Widnell KL (1993). Drug addiction: a model for the molecular basis of neural plasticity. *Neuron*. 11: 995– 1006.

Nestler EJ, Alreja M, Aghajanian GK (1994). Molecular and cellular mechanisms of opiate action: studies in the rat locus coeruleus. *Brain Res.* 35: 521–528.

Owesson-White CA, Cheer JF, Beyene M, Carelli RM, Wightman RM. (2008). Dynamic changes in accumbens dopamine correlate with learning during intracranial self-stimulation. *Proc. Natl. Acad. Sci. U. S. A.* 105:11957–62.

Owesson-White CA, Roitman MF, Sombers LA, Belle AM, Keithley RB, Peele JL, Carelli RM, Wightman RM (2012). Sources contributing to the average extracellular concetration of dopamine in the nucleus accumbens. *J Neurochem*. 121(2):252-62.

Paladini CA, Wiliams JT (2004). Noradrenergic inhibition of midbrain dopamine neurons. *J Neurosci.* 24:4568-4575.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (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 by D-amphetamine. *J Neurosci*. 19(6):2401-11.

Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984). Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology*. 84:167-173.

Phillips AG, Fibiger HC (1973). Deficits in stimulation-induced feeding after intraventricular administration of 6-hydroxydopamine in rats. *Behav Biol*. 9(6): 749-54.

Phillips PEM, Stuber GD, Heien MLAV, Wightman RL, and Carelli RM (2003). Subsecond dopamine release promotes cocaine seeking. *Nature*. 422: 614–8.

Porter-Stransky KA, Wescott SA, Hershman M, Badrinarayan A, Vander Weele CM, Lovic V, Aragona BJ (2011). Cocaine must enter the brain to evoke unconditioned dopamine release in the nucleus accumbens shell. *Neurosci Lett*. 504:13-17.

Rankin ML, Hazelwood LA, Free RB, Namkung Y, Rex EB, Roof RA, Sibley DR (2010). Molecular pharmacology of the dopamine receptors. In: Iversen LL, Iversen SD, Dunnett SB, Bjorklund A, eds. *Dopamine Handbook.* New York, NY: Oxford University Press: 63-87.

Rawls SM, Patil T, Yuvasheva E, Raffa RB (2010). First evidence that drugs of abuse produce behavioral sensitization and cross sensitization in planarians. *Behav Pharmacol.* 21(4):301-13.

Rinaman L (2003). Hindbrain noradrenergic lesions attenuate anorexia and alter central cFos expression in rats after gastric viscerosensory stimulation. *J Neurosci.* 23(31): 10084-10092.

Ritz MC, Cone EJ, Kuhar MJ (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure activity study. *Life Sci.* 46:635–645.

Robinson DL, Heien ML, Wightman RM (2002). Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. *J Neurosci*. 22(23):10477-86.

Robinson TE, Berridge KC (2008). The incentive sensitization theory of addiction: some current issues. *Phil. Trans. R. Soc*. B363:3137–3146.

Rocha B (2003). Stimulant and reinforcing effects of cocaine in monoamine transporter knockout mice. *Europ J Pharmacol*. 479:107-115.

Rocha B, Fumagalli F, Gainetdinov RR, Jones S, Ator R, Giros B, Miller GW, Caron MG (1998). Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci.* 1:132-137.

Roitman MF, Stuber GD, Phillips PEM, Wightman RM, Carelli RM (2004). Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci. 24*, 1265–71.

Sakka L, Coll G, Chazal J (2011). Anatomy and physiology of cerebrospinal fluid. *Eur Ann Otorhinolaryngol Head Neck Dis*. 128(6):309-16.

Salamone J, Correa M (2002). Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Brain Res.* 137:3-25.

Schuldiner S, Shirvan A, Linial M (1995). Vesicular neurotransmitter transporters: from bacteria to humans. *Physiol Rev.* 75(2):369-92.

Schultz W (1997). Dopamine neurons and their role in reward mechanisms. *Curr Opin Neurobiol*. 7:191–7.

Schultz W (1998). Predictive reward signal of dopamine neurons. *J Neurophysiol.* 80:1-27.

Schultz W (2002). Getting formal with dopamine and reward. *Neuron.* 36:241-263.

Schultz W (2007). Multiple dopamine functions at different time courses. *Annu. Rev. Neurosci.* 30:259-88.

Semba K, Fibiger HC (1992). Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. *J Comp Neurol.* 323:387–410

Shin R, Cao J, Webb SM, Ikemoto S (2010). Amphetamine administration into the ventral striatum facilitates behavioral interaction with unconditioned visual signals in rats. *PLoS One*. 5(1):e8741.

Simansky KJ, Bourbonais KA, Smith GP (1985). Food-related stimuli increase the ratio of 3,4-dihydroxyphenylacetic acid to dopamine in the hypothalamus. *Pharmacol Biochem. Behav*. 23: 253–258.

Sinkala E, McCutcheon JE, Schuck MJ, Schmidt E, Roitman MF, Eddington DT (2012). Electrode calibration with a microfluidic flow cell for fast-scan cyclic voltammetry. *Lab Chip*. 12(13):2403-8.

Simon H, Le Moal M, Stinus I, Calas A (1979). Anatomical relationships between the ventral mesencephalic tegmentum – a 10 region and the locus coeruleus as demonstrated by anterograde and retrograde tracing techniques. *J Neural Transm.* 44: 77-86.

Shuen JA, Chen M, Gloss B, Calakos N (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J Neurosci.* 28:2681–5.

Sombers LA, Beyene M, Carelli RM, Wightman RM (2009). Synaptic overflow of dopamine in the nucleus accumbens arise from neuronal activity in the ventral tegmental area. *J Neurosci.* 29(6):1735-42.

Sommermeyer H, Frielingsdorf J, Knorr A (1985). Effects of prazosin on the dopaminergic neurotransmission in rat brain. *Eur J Pharmacol.* 276: 267-270.

Sørensen G, Reddy IA, Weikop P, Graham DL, Stanwood GD, Wortwein G, Galli A, Fink-Jensen A (2015). The glucagon-like peptide (GLP-1) receptor agonist exendin-4 reduces cocaine self-administration in mice. *Physiol Behav*. 149:262-8.

Steidl S, Cardiff KM, Wise RA (2015). Increased latencies to initiate cocaine self-administration following laterodorsal tegmental nucleus lesions. *Behav Brain Res*. 287:82-8.

Suaud-Chagny MF, Dugast C, Chergui K, Msghina M, Gonon F (1995). Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems in vivo. *J Neurochem*. 65(6):2603-11.

Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality. (September 4, 2014). *The NSDUH Report: Substance Use and Mental Health Estimates from the 2013 National Survey on Drug Use and Health: Overview of Findings*. Rockville, MD

Torre DL, Falorni A (2007). Pharmacological causes of hyperprolactinemia. *Ther Clin Risk Manag*. 3(5):929-51.

Torres GE, Gainetdinov RR, Caron MG (2003). Plasma membrane monoamine transporters: structure regulation and function. *Nat Rev Neuro.* 4(1):13-25.

Van den Heuvel DM, Pasterkamp RJ (2008). Getting connected in the dopamine system. *Prog Neurobiol*. 85(1):75-93.

Watabe-Uchida MI, Zhu L, Ogawa SK, Vamanrao A, Uchida N (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron*. 74(5):858-73.

Weinshenker D, Schroeder JP (2007). There and back again: a tale of norepinephrine and drug addiction. *Neuropsychopharm.* 32: 1433-1451.

West AR, Floresco SB, Charara A, Rosenkranz JA, Grace AA (2003). Electrophysiological interactions between striatal glutamatergic and dopaminergic systems. *Ann N Y Acad Sci*. 1003:53-74.

Westfall TC, Westfall DP (2006). Adrenergic agonists and antagonists. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 11th Edition. Brunton LL, Lazo JS, and Parker KL eds. The McGraw-Hill Companies, New York.

Wightman RM, Heien ML, Wassum KM, Sombers LA, Aragona BJ, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Carelli RM (2007). Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur J Neurosci*. 26(7):2046-54.

Wise RA (1978). Catecholamine theories of reward: a critical review. *Brain Res*. 152:215-47

Wise RA (1985). The anhedonia hypothesis: Mark III. *Behav Brain Sci*. 8:178-86.

Wise RA (1982). Neuroleptics and operant behavior: the anhedonia hypothesis. *Behav Brain Sci.* 5:39-87.

Wood DM, Retz KC, Emmett-Oglesby MW (1987). Evidence of a central mechanism mediating tolerance to the discriminative stimulus properties of cocaine. *Pharmacol Biochem Behav*. 28(3):401-6.

Wood S, Sage JR, Shuman T, Anagnostaras SG (2014). Psychostimulants and cognition: a continuum of behavioral and cognitive activation. *Pharmacol Rev*. 66:193-221.

Wybell CL, Berridge KC (2000). Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. *J Neurosci.* 1;20(21):8122-30.

Zahm DS, Becker ML, Freiman AJ, Strauch S, Degarmo B, Geisler S, Meredith GE, Marinelli M (2010). Fos after single and repeated self-administration of cocaine and saline in the rat: emphasis on the Basal forebrain and recalibration of expression. *Neuropsychopharm*. 35(2):445-463.

Zhou Y, Bunney BS, Shi WX (2006). Differential effects of cocaine on firing rate and pattern of dopamine neurons: role of alpha1 receptors and comparison with L-dopa and apomorphine. *J Pharmacol Exp Ther*. 317(1):196-201.