Sensitization of rapid dopamine signaling in the nucleus accumbens core and shell after repeated cocaine in rats

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Running Head: Rapid dopamine sensitization after cocaine
ABSTRACT

Repeated cocaine exposure and withdrawal leads to long term changes, including behavioral and dopamine sensitization to an acute cocaine challenge, that are most pronounced following long withdrawal periods. However, the changes in dopamine neurotransmission following short withdrawal periods are less well defined. To investigate dopamine neurotransmission after one day withdrawal, we used fast-scan cyclic voltammetry (FSCV) to determine whether repeated cocaine alters rapid dopamine release and uptake in the nucleus accumbens (NAc) core and shell. FSCV was performed in urethane anesthetized male Sprague-Dawley rats that had previously received 1 or 7 daily injections of saline or cocaine (15 mg/kg, i.p.). In response to acute cocaine, subjects showed increased dopamine overflow that resulted from both increased dopamine release and slowed dopamine uptake. One day cocaine pre-exposure, however, did not alter dopaminergic responses to a subsequent cocaine challenge. In contrast, 7d cocaine-treated subjects showed a potentiated rapid dopamine response in both the core and shell following an acute cocaine challenge. In addition, kinetic analysis during the cocaine challenge revealed a greater increase in apparent $K_m$ of 7d cocaine exposed subjects. Together, the data provide the first in vivo demonstration of rapid dopamine sensitization in the NAc core and shell after a short withdrawal period. In addition, the data clearly delineate cocaine’s release and uptake effects and suggest that the observed sensitization results from greater uptake inhibition in cocaine pre-exposed subjects.

Keywords: in vivo voltammetry; sensitization; cocaine; nucleus accumbens; carbon-fiber microelectrode
INTRODUCTION

Cocaine acts in the brain to slow dopamine uptake through inhibition of the dopamine transporter (DAT) (Ritz et al. 1987), and thus increases the amount of time that dopamine is present in the extra-neuronal space. Cocaine’s ability to increase dopamine overflow, particularly in the NAc, has been shown to play an important role in several models of addiction (Goeders and Smith 1983; Kalivas and Duffy 1993a; Ritz et al. 1988; Rodd-Henricks et al. 2002). Specifically, dopaminergic mechanisms in the NAc shell have been shown to alter motivation for rewards such as cocaine while dopamine in the NA core is important for goal directed behavior (Ikemoto 2007; Ito et al. 2000; Ito et al. 2004). In addition, repeated cocaine administration also leads to a progressive, or sensitized, increase of dopamine overflow (Heidbreder et al. 1996; Kalivas and Duffy 1993a; b), due to changes in the brain (Vanderschuren and Kalivas 2000; Wolf 1998). Specifically, cocaine has been shown to alter synaptic plasticity (Argilli et al. 2008; Borgland et al. 2004; Saal et al. 2003) and to modulate second messenger signaling proteins implicated in neuronal plasticity (Bibb et al. 2001; Nishi et al. 2000; Scheggi et al. 2004; Valjent et al. 2005).

An extensive microdialysis literature has revealed modulation of extracellular dopamine levels in the nucleus accumbens following acute and repeated cocaine exposure (reviewed in Pierce and Kalivas 1997). Normally, dopaminergic neurons fire at low frequency of~5 Hz (Hyland et al. 2002; Wightman and Zimmerman 1990), but these neurons also exhibit burst firing patterns that are characterized by multiple action potentials at frequencies of 20 Hz or more (Chiodo 1988; Grace and Bunney 1984; 1983). This burst firing leads to rapid and transient changes in dopamine concentration at terminals and this change in dopamine overflow can be readily observed using fast-scan cyclic voltammetry (FSCV) (Hyland et al. 2002;
Burst firing can be mimicked by high frequency stimulation of dopaminergic neurons (Sombers et al. 2009), and the time course of this evoked overflow has been shown to arise from the balance of uptake and release (Wightman and Zimmerman 1990). Here we examine the effect of repeated cocaine on these rapid dopamine dynamics.

Recent FSCV studies have demonstrated an important role for rapid dopamine signaling in reward-related behavior where dopamine concentration transients have been observed in response to cocaine and to cues that predict cocaine availability (Cheer et al. 2007a; Heien et al. 2005; Owesson-White et al. 2009; Phillips et al. 2003; Stuber et al. 2005). Given these data, the question addressed here is how repeated cocaine exposure may alter in vivo rapid dopamine dynamics. While previous voltammetry studies of sensitization have been performed in brain slices, in vivo voltammetry provides the advantage of addressing sensitization in the brain with much of the neuronal circuitry intact. In addition, the design allows for comparison of the NAc core and shell that receive topographically distinct inputs from different regions of the ventral tegmental area (reviewed in (Ikemoto 2007) and may exhibit differential responses to repeated cocaine exposure. Specifically, we used FSCV to monitor electrically stimulated dopamine overflow in the nucleus accumbens (NAc) core and shell of anesthetized rats. This experimental design has been used extensively to characterize dopamine uptake and release under different conditions (Jones et al. 1995; Mateo et al. 2004; Ng et al. 1991; Oleson et al. 2008; Walker et al. 2006; Wightman and Zimmerman 1990; Wu et al. 2001a). Further, this in vivo design is critical for kinetic analysis as the modeling of release and uptake employed here cannot be performed without electrically stimulated dopamine overflow. Our current results provide the first in vivo comparison and characterization of repeated cocaine effects on rapid dopamine overflow in the
core and shell. Further, the results show that the neurobiological changes associated with repeated cocaine exposure are sufficient to drive a sensitized rapid dopamine response to a subsequent cocaine challenge that originates from a greater inhibition of dopamine uptake in cocaine pre-exposed subjects.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats were supplied by Charles River Laboratories (Raleigh, NC) and weighed between 200-250 grams (7 to 8 weeks in age) upon arrival. Animals were fed ad libitum throughout the duration of the study and were housed 2 to 3 per cage on a light/dark cycle with lights on at 7 am and off at 7pm. Animal handling and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the University of North Carolina Animal Care and Use Committee.

Drug administration

After arrival, rats were allowed to acclimate to the facility for 5 to 7 days before drugs were administered. An intraperitoneal dose of 15 mg/kg cocaine was selected based on previous studies demonstrating behavioral and neurochemical changes with this dose in rats (Kalivas and Duffy 1993a). An intravenous dose of 0.3 mg/kg cocaine is sufficient to produce the feeling of a “high” in humans (Volkow et al. 1997) and is readily self-administered in rats (Stuber et al. 2005). While a 10 mg/kg i.p. dose results in cocaine concentrations in the brain similar to that observed with a 1.0 mg/kg intravenous dose (Orona et al. 1994), self-administration in humans...
and rodents is characterized by repeated exposure is short time intervals that would lead to a
greater cumulative dose. In our first experiment, rats received a single intraperitoneal (i.p.)
injection of saline vehicle (0.9% Sodium Chloride, Hospira, Lake Forest, IL) or 15 mg/kg
cocaine (NIDA, Bethesda, MD) in a non-home cage environment followed by a 1d withdrawal
period prior to electrochemical analysis with FSCV. In the second experiment, saline vehicle or
15 mg/kg cocaine was administered (i.p.) for 7 consecutive days in a non-home cage
environment followed by a 1d withdrawal and subsequent electrochemical analysis.

Surgery

Following the 1d withdrawal period, all rats were anesthetized with 1.5 g/kg urethane
(i.p.) (Sigma-Aldrich, St. Louis, MO) in preparation for in vivo voltammetric recordings.
Surgery was performed using a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) to
locate brain regions of interest for electrode placement. For electrical stimulation, a stainless-
steel bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) was placed in the ventral
tegmental area (VTA) using the following coordinates (A/P -5.2 mm, M/L + 0.5 to 1.5mm, D/V
8.0 mm below dura). To prepare carbon-fiber microelectrodes, glass capillaries (Stoelting Co,
Wood Dale, Illinois) were aspirated with a carbon-fiber (T650, Amoco, Greenville, SC, USA)
and then pulled with an electrode puller (Narishege International, Japan) to produce a glass
microelectrode with a 5 μm diameter at the tip and a protruding carbon-fiber. The carbon-fiber
was then cut at a length of 75 to 100 μm beyond the tip of the glass under a light microscope
(Olympus, Center Valley, PA) with a scalpel blade (Cahill et al. 1996). During the experiment,
carbon-fiber microelectrodes were placed either into the NAc core (A/P +1.2mm, M/L + 1.4mm,
D/V 6.5 to 7.5 mm below dura) or shell (A/P +1.7mm, M/L +0.8mm, D/V 6.5 to 7.5 mm below
dura). In addition, an Ag/AgCl reference electrode was placed in the contra lateral hemisphere of the brain 5 to 8 mm from the carbon-fiber electrode.

**Electrochemistry**

Background subtracted FSCV was used to monitor dopamine *in vivo*. To elicit dopamine overflow in the NAc, 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. During voltammetric recordings, the carbon-fiber microelectrode was held at -0.4 V versus the Ag/AgCl reference electrode. A triangular waveform was applied every 100 ms in which the potential was ramped from -0.4 V to 1.3 V and back to -0.4 V at a rate of 400 V/sec. The triangular waveform was generated with a multifunction data acquisition board (PCI 6052E, National Instruments, Austin, TX). Waveform acquisition, stimulation delivery, and data collection were synchronized using PCI-6711E (National Instruments, Austin, TX). Data processing and signal transduction were performed using custom built instrumentation (University of North Carolina, Department of Chemistry Electronics Facility) while cyclic voltammograms were visualized and analyzed with Tarheel CV software (ESA, Chelmsford, MA).

For each individual animal, a fresh carbon-fiber microelectrode was initially inserted 4 mm into the brain above the region of interest and the triangular waveform was applied at a frequency of 60 Hz for 5 to 10 minutes. 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 V to 1.3 V waveform (Takmakov et al. 2010). However, this process is not likely to alter dopamine release as cell firing is not affected by waveform application (Cheer
et al. 2005). Following the conditioning phase, waveform application was changed to 10 Hz and the electrode was lowered into the region of interest (NAc core or shell). Stimulating electrode placement was then optimized along the dorsal-ventral axis by the presence of a slight whisker twitch in response to a 60 Hz stimulation of the VTA. Microelectrode depth was subsequently optimized based on depth and the presence of a characteristic cyclic voltammogram for dopamine, with an oxidation peak at 0.6 V and a reduction peak at -0.2 V. In each experimental subject, pre-drug stimulations were performed using 20, 40 and 60 Hz stimulations. Each subject then received an acute challenge of 15 mg/kg cocaine (i.p) followed by 60 Hz stimulations of the VTA performed every 5 minutes until the cocaine effect on rapid dopamine overflow reached an asymptote. Once this point was reached, VTA stimulation (post-drug) was applied at 10, 20, 40 and 60 Hz to evaluate the cocaine effect at multiple stimulation frequencies.

 Histology

To verify microelectrode placement at the conclusion of the experiment, a 20 μA current was applied to the carbon-fiber microelectrode (supplementary figure 1). The lesion disrupts the tip of the carbon-fiber, therefore it was necessary to preserve some of the microelectrodes for post-calibration with known dopamine concentrations. In such instances, a tungsten wire was placed at the site of the carbon-fiber microelectrode and an electrolytic lesion was created as indicated above (supplementary figure 1). Each subject was then euthanized with a lethal dose of urethane (3 mg/kg, i.p.) and the brain was removed by dissection and stored in 10% formalin (Sigma-Aldrich, St. Louis, MO). Brain tissue was then sliced into 50 μm sections on a cryostat (Leica Microsystems Inc., Bannockburn, IL) and lesions were assessed under a light microscope (Olympus, Center Valley, PA), as indicated by representative placements in pictorial coronal
sections (Supp Fig 1). Subjects with lesions outside of the target region were excluded from analysis.

Data analysis

The electrochemical data presented in the study were background subtracted and filtered with Tarheel CV software (ESA, Chelmsford, MA). Data for each trial were represented in a two-dimensional graph with the y-axis corresponding to the applied potential of the waveform, the x-axis representing time and the z-axis (color scale) representing changes in current at the electrode (Fig 1B). Changes in current were thus visualized in the color plot and those attributable to dopamine were identified by its characteristic cyclic voltammogram. Since dopamine is oxidized at 0.6 V on the positive scan of the voltage, examination of current at this potential revealed changes in dopamine current versus time during the trial (I vs. t, Fig 1C).

Following the experiment, changes in current were converted to changes in dopamine concentration by post-calibration. The carbon-fiber microelectrode was removed from the brain and placed into a flow-injection analysis system in which known dopamine concentrations, ranging from 100 nM to 3 μM, were presented at the electrode surface. The calibration curve was generated using 20 different electrodes and gave a linear response across dopamine concentrations with an r-squared value of 0.9961 (data not shown). The equation generated from the linear response was used to determine electrode response of 12.4 nA/μM. The area of the carbon fiber cylinder was calculated to be 1221 μm² (based on 2.55 μm radius and 75 μm carbon fiber length) and calculated sensitivity of the electrode was 10.2 pA/μM/μm². This calibration was then used to determine the peak concentration of dopamine observed in the brain, [DA]_{max}, after stimulation of the VTA.
In accord with prior work, dopamine release was considered to be instantaneous with each stimulation pulse that was followed by uptake that is governed by Michaelis-Menton kinetics (Garris and Wightman 1995; Wu et al. 2001b). Specifically, the change in dopamine overflow during the stimulation was defined as follows: $d[DA]/dt_{\text{overflow}} = [DA]_p * f - d[DA]/dt_{\text{uptake}},$ where $[DA]_p$ was the amount of dopamine released per stimulation pulse, $f$ was the stimulation frequency and $d[DA]/dt_{\text{uptake}}$ was the rate of uptake. Thus, observed changes in rapid dopamine overflow during stimulation resulted from both dopamine release and uptake mechanisms. After stimulation, extracellular dopamine concentration was governed solely by uptake and was defined as $d[DA]/dt_{\text{overflow}} = -V_{\text{max}}/ ((K_m/[DA]) + 1),$ where $V_{\text{max}}$ was the maximal rate of uptake and $K_m$ was the dopamine concentration at which the rate of uptake is $\frac{1}{2}$ of $V_{\text{max}}.$

In our experimental design, the dopamine cell body region (VTA) was stimulated at various frequencies ranging from 10 to 60 Hz. During stimulation, a fraction of released dopamine is removed by uptake in the interval between stimulation pulses. For example, a stimulation consisting of 24 pulses applied at a 10 Hz frequency results in a 100 ms delay between each stimulus pulse. In contrast, 24 pulses at a 60 Hz frequency allows for a 16.67 ms pause between each pulse. By comparison, the lower stimulation frequencies provide more time for uptake between each stimulation pulse whereas higher stimulation frequencies provide less time for uptake between pulses. Thus, $[DA]_{\text{max}}$ obtained with low frequency stimulation results from a combination of uptake and release components whereas $[DA]_{\text{max}}$ values with high frequency stimulation are governed primarily by dopamine release. As a result, high stimulation frequencies lead to greater $[DA]_{\text{max}}$ values compared to lower stimulation frequencies. These
characteristics also allow for determination of release and uptake components contributing to the rapid dopamine signals.

As depicted in Figure 1A, high stimulation frequencies often lead to increases in dopamine concentration that continue after the completion of the stimulation (second white box on trace). This time delay is due to the kinetics associated with the electrode response time (Venton et al. 2002) which results in convoluted, or slowed, response (Garris and Wightman 1995; Venton et al. 2002). Since this time delay can be measured in vitro after removal from brain tissue, the convolution associated with in vivo measurements can be accounted for using a mathematical algorithm (Wu et al. 2001b). Accounting for this convolution also allows for subsequent determination of rate constants from in vivo FSCV recordings.

**Analysis of release and uptake kinetics**

To determine the values of $[DA]_p$, $V_{max}$, and $K_m$, a simplex minimization algorithm (Press et al. 1989) was used as previously described (Wu et al. 2001b). Briefly, pre-drug dopamine responses at one location (NAc core or shell) in the anesthetized rat brain were obtained using stimulation frequencies of 20, 40 and 60 Hz (Fig 1B). The current versus time response was then converted to dopamine concentration versus time using values obtained during post-calibration (Fig 1C). Simulated curves for 20, 40 and 60 Hz stimulations were then constructed based on initial estimates of values for $[DA]_p$, $V_{max}$, and $K_m$. The simulated curves were convoluted with a measured delay to generate a modeled curve of dopamine concentration versus time. Next, the modeled curves were compared to curves measured in vivo (Fig 1D) and simplex minimization was repeated until the best fit for all three kinetic parameters ($[DA]_p$, $V_{max}$, and $K_m$) was obtained. After an acute challenge of the competitive inhibitor, cocaine, $V_{max}$ was held constant
at the pre-drug value for each subject and $[DA]_p$ and $K_m$ were determined with simplex
minimization using 60 Hz stimulation frequencies.

Statistics

An independent samples t-test was performed to compare pre-drug $[DA]_{\text{max}}$ responses in
subjects with previous saline or cocaine exposure. To measure the effect of acute cocaine
exposure on rapid dopamine responses, pre-drug $[DA]_{\text{max}}$ values were normalized to 100% and
compared to post-drug responses after an acute cocaine challenge. A 1-sample t-test was
performed in a within subjects design to determine significant differences between pre-drug and
post-drug $[DA]_{\text{max}}$ values. In addition, an independent samples t-test was used to compare acute
cocaine responses in subjects that had received either saline or cocaine pre-exposure prior to the
FSCV experiment. Changes in $[DA]_p$ and $K_m$ after an acute cocaine challenge were represented
as a percent change from pre-drug values and statistical significance between treatment groups
was determined using a 2 X 2 univariate ANOVA with brain region (NAc core or shell) and pre-
exposure (saline or cocaine) variables. For all experiments, $p < 0.05$ was considered significant.
All statistical tests were performed using SPSS 14.0 software (SPSS, Chicago, IL).

RESULTS

One day cocaine pre-exposure does not alter dopamine responses in the nucleus accumbens core
during a drug-free state

To evaluate the effects of 1d cocaine exposure on rapid dopamine dynamics, we first
examined $[DA]_{\text{max}}$ responses in subjects that previously received 1d saline/cocaine followed by a
24h withdrawal period. The experimental timeline consisted of a 1d pre-exposure with a 24h
withdrawal prior to FSCV recordings (Fig 2A). Baseline FSCV recordings were first performed before drug administration (Fig 2A). Dopamine responses in the NAc core revealed no significant differences between [DA]_{max} in subjects pre-treated with saline compared to cocaine for all of the frequencies tested (p > 0.05, independent samples t-test, Fig 2B). Similarly, [DA]_{max} in the NAc shell showed no significant difference between saline and cocaine treated subjects at all tested frequencies (p > 0.05, independent samples t-test, Fig 2C). Thus, 1d pre-exposure to cocaine did not alter [DA]_{max} responses in either the NAc core or shell following a subsequent cocaine challenge.

Acute cocaine administration increase [DA]_{max} in the nucleus accumbens before and after 1d cocaine pre-exposure

A single cocaine exposure has been shown to increase both basal dopamine levels (Kalivas and Duffy 1990) and rapid dopamine dynamics in the NAc (Heien et al. 2005). Here we sought to determine whether a single cocaine exposure could alter rapid dopamine responses to a subsequent cocaine challenge. In the saline control group (1d saline), [DA]_{max} showed a significant increase after acute cocaine (black traces) in the NAc core compared to the pre-drug baseline (gray traces, Fig 3A). This increase was evident at all tested stimulation frequencies (p < 0.05, 1 sample t-test) with the exception of the 20 Hz stimulation frequency (p = 0.07, 1 sample t-test). Likewise, 1d previous cocaine exposure 24h prior did not alter the [DA]_{max} response to an acute cocaine challenge in the NAc core as 1d cocaine treated subjects showed increased [DA]_{max} after acute cocaine (p < 0.05 at 10Hz, 20 Hz, and 60 Hz; 1-sample t-test, Fig 3A) with no significant difference compared to 1d saline treated subjects (p > 0.05 at all frequencies, independent samples t-test, Fig 3A). Similar effects were also observed in the NAc
shell where an acute cocaine challenge led to increased $[DA]_{\text{max}}$ compared to pre-drug values in both 1d saline and 1d cocaine treated subjects ($p < 0.05$, 1-sample t-test at a frequencies tested, Fig 3B). Consistent with the results in the NAc core, 1d pre-exposure to cocaine did not alter responses in the NAc shell to an acute cocaine challenge when compared to 1d saline pre-exposure ($p > 0.05$, independent samples t-test, Fig 3B). Together, the data demonstrate that a 1d pre-exposure to cocaine is not sufficient to alter rapid dopamine responses to cocaine as assessed by FSCV. In terms of the rapid dopamine response to cocaine, the increase in $[DA]_{\text{max}}$ after acute cocaine occurred at almost all tested frequencies in the core and shell. These results suggest that cocaine was able to increase the $[DA]_{\text{max}}$ responses that resulted from a combination of uptake and release components (low frequency stimulations) and as well as those that were governed primarily by release (high frequency stimulations).

Altered rapid dopamine release and uptake in 7d saline versus 7d cocaine treated subjects

To address whether a longer cocaine exposure could alter rapid dopamine responses in the NAc core and shell, we first examined $[DA]_{\text{max}}$ responses in 7d treated subjects during a drug-free state (Fig 4A). In the NAc core, analysis of $[DA]_{\text{max}}$ revealed no significant differences in $[DA]_{\text{max}}$ between 7d saline and 7d cocaine treated subjects ($p > 0.05$ at all tested frequencies, independent samples t-test, Fig 4B). In the NAc shell, 7d cocaine exposure did not alter $[DA]_{\text{max}}$ responses during the drug-free state ($p > 0.05$ at all tested frequencies, independent samples t-test, Fig 4B). Thus, similar to 1d cocaine pre-exposure (Fig 2), 7d pre-exposure to cocaine did not alter $[DA]_{\text{max}}$ responses in the NAc core or shell following a cocaine challenge.

Seven day cocaine pre-exposure alters rapid dopamine responses to an acute cocaine challenge
Repeated exposure to cocaine is sufficient to alter basal dopamine levels in response to an acute cocaine challenge as assessed by microdialysis (Kalivas and Duffy 1993a). In the current study, we hypothesized that 7d repeated cocaine pre-exposure would also alter rapid dopamine responses to a subsequent cocaine challenge. In the NAc core of 7d pretreated subjects, comparison of [DA]_{max} in pre-drug (grey traces, Fig 5A) versus acute cocaine (black traces, Fig 5A) responses revealed a significant difference at several of the stimulation frequencies tested (p < 0.05 for 10 Hz, 40 Hz, and 60 Hz; 1-sample t-test, Fig 5A). Similarly, 7d cocaine treated subjects showed increased [DA]_{max} in the NAc core in response to an acute cocaine challenge (p < 0.05 for all frequencies tested, 1-sample t-test, Fig 5A). In contrast to the 1d experiment, however, 7d cocaine treated subjects showed a potentiated cocaine response in the NAc core with 10 Hz and 20 Hz VTA stimulation frequencies (p < 0.05, independent samples t-test, Fig 5A). Thus, while rapid dopamine increases after an acute cocaine challenge was observed at both low (10 Hz) and high (60 Hz) stimulation frequencies, the sensitized responses after 7d cocaine exposure was restricted to low stimulation frequencies.

In separate animals, we also examined rapid dopamine responses in the NAc shell of 7d saline or cocaine treated subjects. Both 7d saline and 7d cocaine treated subjects showed increased [DA]_{max} after acute cocaine (Fig 5B, black trace) compared to their pre-drug responses (Fig 5B, gray trace) at all frequencies tested (p < 0.05, 1-sampled t-test, Fig 5B). In addition, 7d cocaine treated subjects showed a potentiated response to cocaine at the 10 Hz and 20 Hz stimulation frequencies (p < 0.05, independent samples t-test, Fig 5B). This sensitized response in the shell mimicked those observed in the NAc core of 7d cocaine treated subjects. In light of the components that contribute to [DA]_{max} in our experimental design, the sensitization that was
observed with low stimulation frequencies suggests that this effect of repeated cocaine likely resulted from uptake mechanisms as opposed to release mechanisms.

To further examine the possible role of release and uptake components in the sensitized rapid dopamine response, we determined $K_m$ and $[DA]_p$ values after the acute cocaine challenge. Here, we used kinetic analysis to determine whether the potentiated increase in $[DA]_{\text{max}}$ after 7d cocaine pre-exposure was due to changes in release, uptake or both. The analysis revealed that an acute cocaine challenge increased $[DA]_p$ in the NAc core and shell of 7d saline pre-exposed subjects ($p < 0.05$, 1-sample t-test, Fig 6A) and in the NAc shell of 7d cocaine pre-exposed subjects ($p < 0.05$, 1-sample t-test, Fig 6A). The acute cocaine challenge also increased the apparent $K_m$ value in the shell of 7d saline treated subjects ($p < 0.05$, 1-sample t-test, Fig 6B) and in the core and shell of 7d cocaine treated subjects ($p < 0.05$, 1-sample t-test, Fig 6B).

Furthermore, subjects that had previously received cocaine for 7 days showed a potentiated increase in apparent $K_m (F_{3,25} = 5.942, p < 0.05 \text{ vs. cocaine subjects, Fig 6B})$. Together, the data suggest that the changes associated with cocaine’s ability to alter the apparent $K_m$ value played a greater role in the sensitized response to an acute cocaine challenge. Given that such effects were not observed with a 1d pre-exposure, the ability of a cocaine challenge to augment the $[DA]_{\text{max}}$ appears to be dependent on the duration of the intermittent cocaine pre-exposure.
The work presented here provides the first _in vivo_ characterization and comparison of acute and repeated cocaine effects on rapid dopamine dynamics in the NAc core and shell. Specifically we demonstrate that 7d cocaine exposure and 1d withdrawal is sufficient to potentiate rapid dopamine signals in both sub-regions of the NAc after an acute cocaine challenge. Given that experiments were performed in anesthetized animals, the data illustrate that this potentiation can occur of contextual cues at the time of the cocaine challenge. Thus, we propose that repeated cocaine exposure leads to underlying changes in the mesolimbic dopamine system that modulate rapid dopamine responses to subsequent cocaine exposure. The fact this potentiation was not observed with 1d cocaine pre-exposure suggests that these underlying neurobiological changes emerge with longer cocaine exposure. Specifically, the 7d sensitization was restricted to low stimulation frequencies (10 and 20 Hz), where rapid dopamine overflow is governed more strongly by uptake, and was not observed at higher stimulation frequencies where overflow is primarily governed by release. Consistent with this observation, kinetic analysis revealed a potentiated change in apparent $K_m$ for uptake measured _in vivo_ during an acute cocaine challenge demonstrating an increased potency of cocaine’s uptake inhibiting actions in pretreated animals.

This study builds on the microdialysis literature concerning extracellular dopamine responses to cocaine and extends the evidence for neurochemical sensitization to rapid dopamine signaling. Indeed, previous studies have demonstrated increased extracellular dopamine in the NAc following acute cocaine exposure in cocaine naïve rats, an effect which is accompanied by increased locomotor activity (Heidbreder et al. 1996; Kalivas and Duffy 1990; Parsons and Justice 1993). In our work with rapid dopamine, acute cocaine in naïve animals also led to an
increase in [DA]_{max}. Previous microdialysis studies have shown that repeated cocaine exposure for 4 to 10 days can elicit a sensitized extracellular dopamine response to an acute cocaine challenged presented after 24 hour withdrawal (Kalivas and Duffy 1993a; b; Parsons and Justice 1993; Pettit et al. 1990). Sensitized extracellular dopamine levels after 24 hour cocaine withdrawal are often correlated with locomotor behavior and while we did not examine behavior in the current study, we used a dose (15 mg/kg, i.p.) that has been previously demonstrated to initiate behavioral sensitization (Kalivas and Duffy 1993a). It should be noted, however, that the evidence for extracellular dopamine sensitization is mixed for withdrawal periods of less than one week while withdrawal periods of two weeks or greater lead to a more robust extracellular dopamine sensitization (Heidbreder et al. 1996; Hooks et al. 1994; Hurd et al. 1989; Kalivas and Duffy 1993a; Pierce and Kalivas 1997; Segal and Kuczenski 1992). Nevertheless, the observed rapid dopamine sensitization in the absence of contextual cues and behavior during the challenge suggests that repeated cocaine exposure is sufficient to induce changes in the neural mechanisms governing rapid dopamine signaling responses to a cocaine challenge after a 24 hour withdrawal.

The primary distinctive aspect of our sensitization study is the use of FSCV and kinetic analysis, which allows for the examination of both release and uptake components of the dopamine signal. To date, there are a limited number of voltammetric analyses that have addressed dopaminergic effects of chronic cocaine exposure. In one previous study, [DA]_{max} was assessed in rats following a 10d cocaine exposure and 24h withdrawal. In that work, Ng and colleagues (1991) used a prolonged length of stimulation (10 sec) that led to a steady-state [DA]_{max} responses during stimulation with the goal of depleting the dopamine releasable pool. Importantly, they compared the maximal amount of dopamine available for release and observed a 44% [DA]_{max} increase in cocaine treated versus saline treated subjects. In fact, the difference in
cocaine treated subjects emerged after 7 to 8 seconds of stimulation, and the authors suggested that the potentiated increase in [DA]_{max} arose from a greater pool of available dopamine in cocaine treated animals (Ng et al. 1991). In contrast, such changes were not detected in our design where shorter stimulation periods (0.4 to 2.4 sec) were employed. Thus our work illustrates that the [DA]_{max} potentiation can emerge in the presence of cocaine, even if it is not apparent during the drug-free state. While previous voltammetric analysis was limited to the NAc core, we extended the work to reveal sensitization in both the core and the shell of the NAc. Given that DATs are more densely expressed in the NAc core (Nirenberg et al. 1997), one might suggest that cocaine would have a greater effect on dopamine transmission in the NAc shell due to its ability to inhibit a greater number of transporters in this lower density region. In addition, studies have shown that rats will self-administer cocaine directly to the NAc shell, but not to the NA core. Despite these anatomical and functional differences, we observed similar cocaine-mediated sensitization in both sub regions of the NAc suggesting that repeated cocaine exposure is sufficient to potentiate cocaine effects in both the core and shell. However, differences in repeated cocaine effects may emerge with different withdrawal periods or with the addition of behavioral analysis. Indeed, rapid dopamine sensitization can persist after long withdrawal periods as a sensitized [DA]_{max} response to a cocaine challenge has been observed in NAc slices following a 14d withdrawal (Williams et al. 1995). Thus, our voltammetry data provides the foundation for future combined voltammetry and behavioral examinations of rapid dopamine effects after short and long withdrawal periods.

By using FSCV and kinetic analysis to distinguish release and uptake components of the dopamine signal, our work provides unique insight into cocaine’s mechanism of action at a level that cannot be described using standard microdialysis techniques. While cocaine effects on
dopamine dynamics are often described in terms of DAT inhibition, our observed increase in rapid dopamine release, $[DA]_p$, after cocaine is consistent with other voltammetry studies using electrical stimulation (Lee et al. 1998; Venton et al. 2006; Walker et al. 2006). There are several potential mechanisms by which cocaine could increase dopamine release. Indeed, in freely moving animals, intravenous cocaine administration increases the frequency and amplitude of transient dopamine events in the NAc shell (Aragona et al. 2008; Cheer et al. 2007b; Sombers et al. 2009). These effects can be blocked by lidocaine-mediated inhibition of the VTA and likely result from cocaine-mediated increases in burst firing of dopamine neurons (Sombers et al. 2009). In addition to these VTA mechanisms, another factor that could contribute to dopamine release is the synapsin dependent mobilization of vesicles from the reserve pool to the readily releasable pool. Indeed, recent work from our laboratory with synapsin knock out mice revealed a reduced dopaminergic response to cocaine that is due to an attenuation of cocaine’s ability to increase $[DA]_p$ (Venton et al. 2006). Thus, synapsin mechanisms likely contribute to the cocaine-mediated increase in $[DA]_p$ that was observed in the current study as well.

Importantly, our kinetic data during the acute cocaine challenge provide a unique in vivo demonstration of the enhanced cocaine-mediated uptake inhibition in cocaine pre-exposed animals. Our in vivo results are consistent with previous in vitro voltammetry data from 7d cocaine treated subjects that showed greater uptake inhibition after bath application of cocaine in NAc slices, an effect which persisted after a 2 week withdrawal period. To date, however, the majority of the data has suggested that DAT cocaine effects are due to changes in trafficking and expression rather than changes in activity or function (Kahlig and Galli 2003; Zahniser and Sorkin 2004; 2009). Indeed, changes in DAT cell surface expression and dopamine clearance have been demonstrated following cocaine exposure both in vivo and in vitro (Cass et al. 1993;
However, our findings of a potentiated uptake inhibition during a cocaine challenge serve to complement previous *in vitro* literature (Izenwasser and Cox 1990; Lee et al. 1998) and suggests that changes in DAT function and activity may also play an important role in dopaminergic responses to repeated cocaine. In addition, such DAT effects are also likely to affect extracellular dopamine responses to cocaine. For instance, stronger DAT inhibition in the NAc would allow for potentiated increases in extracellular dopamine levels. In addition to these effects in the NAc, the potentiated $K_m$ shift could also modulate rapid and extracellular dopamine overflow in the VTA to influence downstream dopaminergic responses in the NAc.

Overall, the data presented in the current work provides evidence for *in vivo* rapid dopamine sensitization in both the NAc core and shell after repeated cocaine exposure and clearly distinguishes the contribution of release and uptake components to this sensitization. Given that repeated exposure to cocaine is a characteristic of self-administration, the effects of repeated cocaine on rapid dopamine overflow may in fact influence drug-seeking behavior in the self-administration task. Indeed, both burst firing of dopamine neurons in the VTA and rapid dopamine overflow in the NAc have been shown to play an important role in responses to reward-predictive cues (Cheer et al. 2007a; Hyland et al. 2002; Phillips et al. 2003; Schultz 1998). Furthermore, changes in the potency of cocaine’s effects on DAT during self-administration could modulate the hedonic value of the drug, especially given the fact that cocaine-induced DAT inhibition contributes to euphoria in humans (Volkow et al. 1999). With such implications, the current demonstration of rapid dopamine sensitization highlights the need for additional studies to further examine the mechanisms of such effects as well as their behavioral consequences.
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FIGURE LEGENDS

Figure 1: *In vivo* rapid dopamine overflow at the carbon-fiber microelectrode. (A) Dopamine overflow in the NAc shell after electrical stimulation of the VTA. At high stimulation frequencies, a continual rise in dopamine concentration even after the stimulation has ceased (2nd white box on trace). This slowed, or convoluted, response is due to the kinetics of the electrode response time and can be accounted for using a mathematical algorithm. (B) Pre-drug rapid dopamine responses at multiple stimulation frequencies represented in a 3-dimensional image in which current is indicated in false color. (C) Dopamine concentration versus time plots obtained using the 0.6 V current (dashed line in B) associated with the oxidation of dopamine and calculated using post-calibration values. A simplex minimization algorithm was employed in order to determine values for [DA]_p, V_max, and K_m. (D) Comparison of modeled data (obtained with simplex minimization) to data measured *in vivo*.

Figure 2: Drug-free [DA]_max responses after 1d saline or cocaine followed by 24h withdrawal. (A) Experimental timeline for 1d drug-exposure and FSCV recordings. (B, C) Pre-exposure to 1d cocaine did not alter peak dopamine responses in the NAc core or shell compared to 1d saline treated subjects. [DA]_max in the NAc core (n=9) (B) and NAc shell (n=16) (C) after 10, 20, 40 and 60 Hz stimulations of the VTA. [DA]_max data presented as ± SEM.

Figure 3: Cocaine pre-exposure for 1d does not alter rapid dopamine responses in the NAc core or shell after an acute cocaine challenge. (A) Rapid dopamine responses in the NAc core after application of various stimulation frequencies to the VTA (n=9). Dopamine responses are
represented as concentration vs. time for both the pre-drug state (grey trace) and after an acute cocaine challenge (black trace). Both pre-exposure groups showed an increase in $[\text{DA}]_{\text{max}}$ after acute cocaine at all frequencies tested ($^* p < 0.05$ vs. baseline, 1 sample t-test) with the exception of the 20 Hz response in the saline treated subjects ($^+ p < 0.07$ vs. baseline, 1-sample t-test). No significant differences were observed when comparing acute cocaine responses in 1d saline versus 1d cocaine treated subjects ($p > 0.05$, independent samples t-test). (B) Rapid dopamine responses in the NAc shell after application of various stimulation frequencies to the VTA (n=16). Both 1d saline and 1d cocaine treated subjects showed increased $[\text{DA}]_{\text{max}}$ following an acute cocaine challenge at all frequencies tested ($^* p < 0.05$, 1-sample t-test) with no difference between the two treatment groups ($p > 0.05$, 1-sample t-test). $[\text{DA}]_{\text{max}}$ data presented as $\pm$ SEM.

Figure 4: Drug-free $[\text{DA}]_{\text{max}}$ responses after 7d saline or cocaine followed by 24h withdrawal. A) Experimental timeline for 1d drug-exposure and FSCV recordings. (B, C) Pre-exposure to 7d cocaine did not alter peak dopamine responses in the NAc core or shell when compared to 7d saline treated subjects. $[\text{DA}]_{\text{max}}$ in the NAc core (n= 9) (B) and NAc shell (n=17) (C) in response to electrical stimulation of the VTA. $[\text{DA}]_{\text{max}}$ data presented as $\pm$ SEM.

Figure 5: Cocaine pre-exposure for 7d potentiates the NAc core and shell rapid dopamine response to an acute cocaine challenge. (A) Concentration versus time traces of rapid dopamine responses in the NAc core obtained after application of various stimulation frequencies to the VTA (n=9). Both groups showed increases in $[\text{DA}]_{\text{max}}$ following an acute cocaine challenge at all frequencies tested ($^* p < 0.05$ vs. baseline, 1-sample t-test) with the exception of the 20 Hz stimulation frequency in 7d saline treated subjects ($p < 0.06$ vs. baseline,
1-sample t-test). Subjects previously exposed to 7d cocaine revealed a potentiated response to the cocaine challenge when compared to 7d saline treated subjects (*p < 0.05 for 10 and 20 Hz stimulations, independent samples t-test). (B) Concentration versus time traces in the NAc shell after application of various stimulation frequencies to the VTA (n=17). An acute cocaine challenge led to an increased [DA]max response at all frequencies tested in both 7d saline and 7d cocaine treated subjects (p < 0.05 vs. baseline, 1-sample t-test). Subjects that were pre-exposed to 7d cocaine also showed a potentiated response to the acute cocaine challenge at 10 and 20 Hz stimulation frequencies compared to 7d saline treated subjects (*p < 0.05, independent samples t-test). [DA]max data presented as ± SEM.

Figure 6: Cocaine pre-exposure for 7d leads to a greater uptake inhibition during an acute cocaine challenge. (A) One day saline and cocaine treated subjects showed increased [DA]p in response to an acute cocaine challenge (*p < 0.05 vs. baseline, **p < 0.005 vs. baseline, ***p < 0.001 vs. baseline, 1-sample t-test). (B) All groups also showed a significant increase in apparent K_m after the acute cocaine challenge (*p < 0.05 vs. baseline, **p < 0.005 vs. baseline, 1-sample t-test) with the exception of the NAc core response in 1d cocaine subjects. In addition, 7d cocaine treated subjects showed a potentiated change in apparent K_m while cocaine was on board as revealed by a main effect of cocaine (F_{3,25} = 5.942, *p < 0.05, univariate ANOVA). Data presented as ± SEM.
A) Carbon-fiber microelectrode response (in vivo)

Convoluted response

15 sec Measured data

B) Color plot

20 Hz stim 40 Hz stim 60 Hz stim 1 60 Hz stim 2

C) Concentration versus time

0.2 μM 2 sec

20 Hz stim 40 Hz stim 60 Hz stim 1 60 Hz stim 2

Curve fitting with simplex minimization

D) Comparison of raw versus modeled data

- Raw data
- Modeled data

Figure 1
Figure 2

A) Experimental timeline

1 day drug administration

24 hour withdrawal

Post-drug analysis

Cocaine challenge

FSCV

B) $[\text{DA}]_{\text{max}}$ in NA core (drug-free state)

C) $[\text{DA}]_{\text{max}}$ in NA shell (drug-free state)
A) Acute cocaine effect [DA]_{max} in NA core (1d)

B) Acute cocaine effect [DA]_{max} in NA shell (1d)
A) Experimental timeline

- 7 day drug administration
- 24 hour withdrawal
- Post-drug analysis
- Cocaine challenge
- Pre-drug analysis
- FSCV

B) $[\text{DA}]_{\text{max}}$ in NA core (drug-free state)

C) $[\text{DA}]_{\text{max}}$ in NA shell (drug-free state)

Figure 4
Figure 5

A) Acute cocaine effect on $[DA]_{\text{max}}$ in NA core (7d)

B) Acute cocaine effect on $[DA]_{\text{max}}$ in NA shell (7d)

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**B) Acute cocaine effect on $[DA]_{\text{max}}$ in NA shell (7d)**

- **Pre-drug**
- **Acute cocaine (15 mg/kg)**

Pre-exposure:
- 1d Sal
- 1d Coc
- 10 Hz Stim
- 20 Hz Stim
- 40 Hz Stim
- 60 Hz Stim

$[DA]_{\text{max}}$ (% of control):
- 10 Hz Stim
- 20 Hz Stim
- 40 Hz Stim
- 60 Hz Stim

* * *

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**Figure 5**
7d pre-exposure

A.) $[\text{DA}]_p$ after acute cocaine challenge

B.) $K_m$ apparent after acute cocaine challenge

Figure 6