Published in final edited form as:

Biol Psychiatry. 2011 February 1; 69(3): 201–207. doi:10.1016/j.biopsych.2010.06.026.

Cocaine-insensitive dopamine transporters with intact substrate transport produced by self-administration

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Abstract

Background—Psychomotor stimulant drugs such as cocaine and amphetamine activate brain dopamine (DA) neurotransmission and support self-administration in humans and laboratory animals. Cocaine amplifies DA signaling by blocking the DA transporter (DAT) and this has been described as the most important mechanism underlying cocaine's reinforcing effects. Amphetamine has the added mechanism of reverse transport of intracellular DA through the DAT.

Methods—We used cocaine and amphetamine self-administration under a fixed-ratio 1 schedule followed by microdialysis in freely-moving rats to measure extracellular DA levels and fast scan cyclic voltammetry in brain slices to measure sub-second DA release and uptake parameters.

Results—Following a high dose (1.5 mg/kg, i.v.) cocaine self-administration paradigm (40 injections/day $\times$ 5 days), the DAT was markedly less sensitive to cocaine, as measured by microdialysis and voltammetry in the nucleus accumbens core. In contrast, the DAT substrate amphetamine retained the same efficacy at the DAT in cocaine self-administering animals, and amphetamine did not mimic cocaine's effect on the DAT when self-administered. A single session of cocaine self-administration caused a significant decrease in the ability of cocaine to inhibit the DAT, a finding which may provide a neurochemical basis for rapid tolerance. The effects of cocaine returned to normal within a few weeks following cessation of self-administration.

Conclusions—Here we, for the first time, demonstrate an *in vivo*, pharmacologically-induced alteration in the sensitivity of the DAT to cocaine that is specific to cocaine, spares DAT and substrate/releaser interactions, and is independent of maximal rate of DA uptake (V_{max}).

Keywords

Dopamine; Cocaine; Amphetamine; Self-Administration; Voltammetry; Microdialysis

Introduction

The dopamine transporter (DAT) removes dopamine (DA) from the extracellular space, thus terminating signaling, and it is also critically involved in the rewarding and stimulating

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effects of psychostimulants. Traditional DAT blockers like cocaine inhibit the uptake of DA without themselves being transported, while substrates like amphetamine have the added mechanism of being transported into DA terminals and ultimately causing the reverse transport of intracellular DA into the extracellular space through the DAT. Thus, both DAT blockers and substrates acutely increase DA levels and extend the magnitude and duration of DA signaling. Psychostimulant-induced DA elevations are thought to regulate motor, mood, motivation, attention, learning, and addiction processes (1,2). In the search for pharmacotherapies to treat cocaine addiction, it has been postulated that a drug which is effective in blocking the activity of cocaine at the DAT without altering DA uptake itself would be a good candidate. Thus, normal DA-dependent processes would be unaffected while cocaine addiction would be prevented or treated. However, no such pharmacotherapy has been developed to date.

Although the DAT was historically considered a static protein with little capacity for regulation, it was understood that modulation of DA levels in mesolimbic areas of the brain could produce corresponding changes in the reinforcing efficacy of drugs of abuse. Over the last few decades, however, it has become clear that DA transport is controlled through trafficking of DATs into and out of cell surface membranes (3,4,5), and this can happen on a time-scale of tens of seconds (6,7). Trafficking occurs through intracellular cascades controlling vesicular insertion and removal of DAT proteins from the plasma membrane. These events are regulated by phosphorylation, glycosylation, and other intracellular signaling systems (8,9,10). Thus, DAT function, and thereby extracellular DA levels, can be modulated through either pharmacological inhibition or alterations in cell-surface expression.

It is less clear, however, whether the intrinsic function of the DAT can be altered in the way that receptors can be desensitized or moved between high and low affinity states, independent of the number of plasma membrane DAT molecules. Nevertheless, research on DAT protein structure has suggested that different domains of the protein govern different aspects of its function. For example, there is debate regarding the degree of overlap of binding sites for various drugs, with a notable distinction between uptake inhibitors and substrates that can act as releasers (11,12,13). Evidence for such a distinction stems from molecular work which demonstrates that mutagenesis or substitution of various transmembrane domains or residues can alter DAT interactions with cocaine, but not amphetamine, or vice versa (14,15). For example, researchers have recently demonstrated that mutating three residues surrounding F105 on transmembrane 2 of the DAT results in insensitivity to cocaine, while DA uptake and substrate/releaser interactions are spared (16). Consequently, mice with a knock-in of these mutations do not self-administer cocaine, but self-administration (SA) of food, amphetamine and DA agonists remains intact (17).

Thus, “bottom-up” molecular approaches have succeeded in reducing the cocaine sensitivity of the DAT, in a specific manner that predictably affects behavior. In previous work, we demonstrated a “top-down”, pharmacologically-induced decrease in the sensitivity of the DAT to cocaine following high-dose binge cocaine SA (18). Our prior work also documented increased maximal rates of DA uptake (V_{max}), which have been theorized to influence DA response to cocaine. The impetus for the current investigation was to understand 1) whether reduced DA sensitivity in the nucleus accumbens (NAc) core is specific to cocaine or 2) whether reduced sensitivity can be induced by exposure to amphetamine and 3) how V_{max} in the NAc core is related to cocaine sensitivity (18). Here we, for the first time, demonstrate a pharmacologically-induced alteration in the sensitivity of the DAT to cocaine that is specific to cocaine, spares DAT and substrate/releaser interactions, and is independent of cocaine SA-induced changes in baseline V_{max} .

Methods and Materials

Subjects

Male, Sprague-Dawley rats (375 - 400 g; Harlan Laboratories) were used as subjects. All animals were maintained according to the National Institutes of Health (NIH) guidelines in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest University School of Medicine.

Self-administration procedures

The procedures for implantation of cannulae into the jugular vein and cocaine SA have been described previously (18). Following surgery, animals were singly-housed within chambers outfitted for both housing and SA procedures so that animals remained in chambers for the duration of SA procedures. Each animal was maintained on a reverse light cycle (3:00am-lights off; 3:00pm lights on), and all SA procedures occurred during the active/dark cycle (10:00am – 3:00pm). Each lever press resulted in the delivery of 1.5 mg/kg cocaine over 4 seconds. This dose was chosen because it is the most reinforcing dose, at the top of the dose-response curve in measures of reinforcing efficacy, and preferred over lower doses in choice studies (19). Concurrent with the start of each injection, the lever retracted and a stimulus light was activated for 20 seconds to signal a time-out period during which time responses produced no programmed consequence. The session was terminated after 40 injections. Typically under these conditions, animals acquired a stable pattern of cocaine SA within 2-5 days. Following establishment of stable responding, the animals were allowed to self-administer 40 injections/day for a period of 1 to 5 consecutive days for the time-course experiment, and 5 consecutive days for all remaining cocaine SA experiments.

All procedural aspects of amphetamine SA were identical to that of cocaine SA. To roughly match cocaine-induced dopamine overflow, preliminary studies demonstrated that an amphetamine dose of 0.56 mg/kg/infusion produced equal or greater levels of dopamine compared to cocaine-induced dopamine levels. In addition, animals were allowed to self-administer this dose of amphetamine for 5 hours per session, which corresponded to the average amount of time animals spent administering cocaine. Finally, 1 session was run per day for 5 consecutive days.

Microdialysis

Microdialysis surgery was performed concomitant with surgery for insertion of jugular vein catheter (for SA procedures). Microdialysis guide cannulae (CMA/Microdialysis AB, Stockholm, Sweden) were stereotactically implanted above the NAc core (anteroposterior, + 1.2 mm; lateral, 2.0 mm; ventral, 6.0 mm). Concentric microdialysis probes (2 mm membrane length, CMA/Microdialysis) were inserted the day after the last SA session, and approximately 16 h prior to the beginning of sample collection. Thus, rats were tested $\approx$ 48 h following their final SA session. The probes were continuously perfused at 0.8 μ l/min with artificial cerebrospinal fluid (aCSF; pH 7.4): NaCl 148 mM, KCl 2.7 mM, CaCl₂ 1.2 mM, MgCl₂ 0.85 mM. Once stable baselines were acquired, animals received either cocaine (1.5 mg/kg, i.v.) or amphetamine (0.56 mg/kg, i.v.) challenge and DA was monitored at 15 minute intervals until DA response returned to baseline.

Fast-scan cyclic voltammetry

All voltammetry experiments were conducted the morning (active/dark cycle) following commencement of final SA session (18 hrs), with the exception of the time-course/recovery experiment which included 1, 3, and 14 days off. Coronal slices containing the NAc were prepared with a vibrating tissue slicer. A cylindrical carbon fiber microelectrode and a

bipolar stimulating electrode were placed into the core of the NAc. DA was evoked by a single, rectangular, electrical pulse (300 μ A, 2 ms), applied every 5 min. Extracellular DA was monitored at the carbon fiber electrode every 100 ms using fast-scan cyclic voltammetry (20) by applying a triangular waveform (-0.4 to $+1.2$ to -0.4 V vs Ag/AgCl, 300 V/s). Once the extracellular DA response was stable for three successive stimulations, cocaine (0.03 – 30 μ M) or amphetamine (0.1 – 10 μ M) was applied cumulatively to the brain slice. The doses were chosen in order to equate inhibition constants (K_i) in naïve animals (21). To evaluate the effects of cocaine, evoked levels of DA were modeled using Michaelis-Menten Kinetics, as a balance between release and uptake (22). Baseline voltammetry data were compared across groups using one-way ANOVAs. Data obtained after administration of cocaine were subjected to a two-way ANOVA with experimental group and concentration of cocaine as the factors. When significant interactions or main effects were obtained ($p < 0.05$), differences between groups were tested using bonferroni post-hoc tests.

Results

Reduced cocaine effect in rats following cocaine self-administration

Animals were allowed to self-administer for 5 days on a fixed-ratio 1 (FR1) schedule of reinforcement for 40 infusions per day. The inter-infusion interval decreased over the 5 days and the total session length was reduced ($n = 6$; $P < 0.01$). Upon completion of the prescribed 5 days of SA, baseline extracellular DA concentrations in the NAc core of cocaine self-administering animals (2.7 ± 0.73 nM; $n = 5$) were significantly lower than those of naïve animals (5.6 ± 0.87 nM; $n = 6$; $P < 0.05$), less than 24 hrs following the last self-administered cocaine infusion, measured by microdialysis. Systemic administration of a challenge dose of cocaine (1.5 mg/kg, i.v.) markedly increased DA levels in naïve rats but failed to produce significant changes in cocaine self-administering rats ($P < 0.01$; Fig. 1).

Significant reduction in dopamine uptake inhibition by cocaine but not amphetamine in animals following cocaine self-administration: time-course

To more fully characterize a mechanism for the blunted effects of cocaine in microdialysis, we investigated the kinetics of DA release and uptake from terminals in NAc brain slices using voltammetry. Fig. 2 depicts neurochemical responses before and after application of 10 μ M cocaine in slices from a naïve rat (Fig. 2A) and a rat with a 5-day history of cocaine SA (Fig. 2B). DA was evoked by a single electrical pulse (arrows). The descending portion of the DA signal represents the rate of DA uptake and its rising phase represents a combination of release and uptake (22). When cocaine was applied to the NAc of a naïve rat (top right panel), the descending portion of the curve is significantly less steep when compared to pre-drug, indicating inhibited uptake (Fig. 2A); and when compared to cocaine SA animals (Fig. 2B), indicating a greater effect of cocaine in naïve animals. Group data for naïve animals and animals that were tested within 24 hr following 5 days of SA is shown in Fig. 2C. Cocaine (0.03 – 30 μ M) significantly inhibited the DAT in naïve animals, producing a maximal apparent K_m value for DA uptake of 28.75 ± 3.14 μ M ($P < 0.0001$) (Fig. 2C). Apparent K_m refers to the observed affinity (in μ M) of DA for the transporter (22). Higher apparent K_m values correspond to lower DA uptake rates or increased DA uptake inhibition. Cocaine was much less effective at inhibiting DA uptake in animals following 5 days of SA with a maximal apparent K_m of 7.37 ± 1.38 μ M ($P < 0.01$).

We also examined the time course for the reduction in cocaine's effect using dose-response curves (Fig. 3; 0.03 – 30 μ M). A single daily session of cocaine SA significantly decreased cocaine's efficacy in inhibiting DA uptake (Fig. 3A), with a maximal apparent K_m of 12.43 ± 3.6 μ M ($P < 0.01$; $n = 5$). Similarly, 3 days ($n = 4$) of cocaine SA reduced cocaine's effects

on DA uptake compared to naïve animals (Fig. 3A; maximal values of $10.43 \pm 3.62 \mu\text{M}$, $P < 0.01$). Pre-drug, baseline stimulated DA release remained constant across the three groups, and there was a general trend of decreased maximal rate of uptake across days. Specifically, V_{max} was significantly decreased following 1 day of SA ($P < 0.05$; Fig. 3A inset) with a significant linear trend towards recovering from 1 to 5 days ($P < 0.05$; Fig. 3A inset). This demonstrates that the absolute uptake rate and the ability of cocaine to inhibit the DAT are completely dissociated (see discussion).

We next examined the time course following cessation from 5 days of cocaine SA at 1, 3, or 14 days of abstinence (Fig. 3B). 1 and 3 days of deprivation were the only time-points when cocaine efficacy was significantly reduced ($P < 0.01$). After 14 days of withdrawal, the maximal value of inhibition of uptake (or apparent K_m) returned to control values, at 25.75 ± 4.15 (Fig. 3B).

Amphetamine retains the same ability to inhibit DA uptake following cocaine self-administration

Given the dissociation between baseline DA uptake and cocaine-induced inhibition, we investigated the kinetics of DA release and uptake following cocaine SA when the synthetic substrate, amphetamine, was applied to the slices. Similar to DA, we found that amphetamine ($0.2 - 10 \mu\text{M}$) retained the same efficacy at the DAT relative to controls (Fig. 4A), indicating a dissociation between substrate uptake and inhibition of uptake by cocaine.

Given no effect of cocaine SA on substrate uptake, we would expect no difference in DA overflow after amphetamine challenge between naïve and cocaine SA animals. Indeed, administration of a challenge dose of amphetamine (0.56 mg/kg , i.v.) increased DA levels equally in naïve and cocaine self-administering rats (Fig. 4B), suggesting that despite reduced cocaine effects, substrate-induced DA overflow remains intact.

Amphetamine self-administration does not change cocaine or amphetamine effects on DA uptake

The final set of experiments were conducted in animals that self-administered amphetamine to examine whether reduced effects of cocaine at the DAT are attributable to long term overflow of DA in the extracellular space and not to specific pharmacology of uptake blockade. Thus, the amphetamine dose was controlled to match cocaine-induced DA overflow and time spent administering amphetamine was matched to the average amount of time animals spent administering cocaine. DA concentrations in the NAc core of amphetamine SA animals ($2.3 \pm 0.27 \text{ fmol/sample}$; $n = 5$) was no different than cocaine SA animals ($2.7 \pm 0.73 \text{ fmol/sample}$; $n = 5$) and significantly lower than those of naïve animals ($5.6 \pm 0.87 \text{ fmol/sample}$; $n = 6$; $P < 0.05$). Unlike cocaine SA animals, cocaine challenge in the amphetamine SA animals increased DA overflow similar to naïve animals (Fig. 5A). Additionally, voltammetry demonstrated no effect of amphetamine SA on baseline DA uptake or on cocaine's ability to inhibit DA transport (Fig. 5B with inset). The representative voltammogram and color plot (Fig. 5C) demonstrates normal uptake inhibition with $10 \mu\text{M}$ cocaine (compare to Fig 2A). Finally, amphetamine challenge in these animals increased DA overflow equally to that in naïve and cocaine SA animals (Fig. 6A), with the corresponding ability of amphetamine to inhibit DA transport (Fig. 6B) in a manner consistent with naïve animals.

Discussion

The current study documents a reduced sensitivity of DA transporters to cocaine and reduced DA responses to a cocaine challenge in animals that self-administered high-dose

cocaine. We demonstrate that reduced cocaine effects are apparent after a single day of SA and last for several days following cessation of cocaine intake. Additionally, several findings demonstrate that reduced cocaine efficacy is independent of the ability of the DAT to transport DA and other substrates. The first is the fact that there is no relationship between changes in $V_{\max}$ and changes in the ability of cocaine to inhibit uptake across the administration time-course (i.e., across 1-5 days of administration). The second is that the ability of amphetamine to inhibit DA uptake remains intact following cocaine SA. Finally, unlike cocaine SA, amphetamine SA has no effect on the subsequent ability of cocaine or amphetamine to interact with the DAT and increase extracellular DA levels. These findings suggest for the first time that cocaine's effects at the DAT can be pharmacologically reduced *in vivo* while DAT substrate transport remains intact.

Early microdialysis studies demonstrated that the ability of cocaine challenge to increase DA levels in the NAc was blunted following several days of high-dose cocaine SA (23). Subsequent work suggested that one possible mediator of decreased DA levels following repeated administration may be increased maximal rates of DA uptake ($V_{\max}$) (24). We have replicated the blunted effect of cocaine challenge on animals that self-administer cocaine using microdialysis, and extended this work to demonstrate for the first time a reduced efficacy of cocaine at the DAT with no increase in the baseline rate of DA uptake. The dissociation of DAT inhibition, manifested as decreased affinity of DA for the DAT (increased app. K_m) and maximal uptake rate ($V_{\max}$) is important in this case given a recent demonstration that increasing $V_{\max}$ in cultured cells, mediated by increased expression of DAT on the cell surface, is associated with decreased potency of cocaine at inhibiting DA uptake (25). Based on this evidence, it could be argued that our previously published demonstration of decreased potency following 24 hr access to cocaine for 10 days could be attributed to the accompanying increased $V_{\max}$ in that study (18). Here, we have dissociated $V_{\max}$ for DA uptake and efficacy of cocaine using a model of cocaine SA that we suspected would not increase $V_{\max}$ based on preliminary data. In fact, a persistent decrease in cocaine efficacy is present across different SA paradigms which either increase (18) or decrease $V_{\max}$, so the decreased cocaine sensitivity is not attributable to fluctuations in baseline rate of DA uptake.

Additionally, we have discovered that the reduction in cocaine's ability to inhibit DAT function occurs and plateaus after only 1 day of binge-like cocaine SA, such that there are only minor differences between 1, 3, and 5 days of access to cocaine. This time-course is dissimilar to that of $V_{\max}$, wherein the maximal rate of DA uptake slows after 1 day of SA and gradually recovers after extended experience and cessation of SA. This is congruent with our previous report where 10 day access to cocaine SA increased $V_{\max}$ relative to baseline (18). The rapid slowing and subsequent upward linear trajectory of DA uptake suggests a biphasic response of $V_{\max}$. This suggests that time spent administering drug may be one reconciling factor for equivocal data on the regulation of DAT following cocaine administration (24,26,27,28). The upward linear trajectory of $V_{\max}$, when plotted across time spent administering cocaine, exceeds the $V_{\max}$ in the naïve group when an animal is withdrawn from cocaine SA for 3 days. The decreased apparent K_m resolves later, between 3 and 14 days. As noted above, the dissimilar time-course between $V_{\max}$ and K_m within this study is an additional factor suggesting a dissociation between baseline uptake rates and cocaine efficacy.

The possibility of dissociations of cocaine efficacy and $V_{\max}$ have been shown in cell culture systems that express DAT. For example, specific mutations in DAT amino acid sequence markedly reduce cocaine sensitivity but do not alter DA transport activity (16), and have been shown to decrease cocaine-related behavior of knock-in mice when these mutations are expressed *in vivo* (17,29). This indicates that cocaine sensitivity is separable

from DA/substrate transport; however, such changes have not been reported in wild type (i.e., genetically unaltered) animals until now. Thus, this is the first study, to our knowledge, to demonstrate functional regulation of DAT that is independent of maximal rate of DA uptake at baseline.

Given the dissociation between uptake rate of the substrate DA and reduced cocaine efficacy, we then predicted that the efficacy of the synthetic substrate amphetamine would not be diminished in our cocaine SA animals. Indeed, amphetamine's efficacy in cocaine SA animals was found to be identical to that in naïve animals, which is consistent with the theory that reduced efficacy of cocaine is independent of DAT's ability to transport substrates, whether endogenous or synthetic. For additional confirmation, we demonstrated that amphetamine SA does not affect cocaine or substrate interactions with the DAT. Since we attempted to match the amount and time-course of elevated DA in amphetamine SA animals to that of cocaine SA animals, we confirmed that neither high extracellular DA nor amphetamine reduces the ability of cocaine to inhibit DAT.

Decreased cocaine efficacy at the DAT may be the functional consequence of binge cocaine intake, and may be a key mechanism underlying self-reports of decreased euphoria or tolerance produced by repeated psychostimulant use in human addicts (30). In fact, the DA system has been described as hypoactive or "burned out" in this population (31,32,33). While these factors may in fact be mechanistically linked to tolerance, it has yet to be fully understood why both humans and animals will increase rates of cocaine intake despite a blunted euphoric experience or blunted cocaine efficacy, respectively. While our goal was not specifically to model escalation of cocaine intake as it is understood in the existing literature (34), our animals did increase their rate of intake over each day. Given this, and the fact that the blunted effect of cocaine was fully present and plateaued after a single day of cocaine SA, it is unlikely that the reduction in cocaine efficacy at the DAT alone can account for escalation of cocaine intake. Nevertheless, to borrow from receptor literature, this rapid "desensitization" of the DAT may be a necessary prerequisite for mechanisms that are more directly related to escalation of cocaine intake. For example, it has been documented that animals titrate their cocaine intake to "preferred" levels, adjusting infusion rates accordingly (35,36,37). Our model demonstrates that DAT tolerance to cocaine effects, not sensitization, is present as animals escalate their rate of intake. Therefore, it is possible that escalation of FR infusion rates within subjects over time may be accounted for in part by tolerance to drug that an animal attempts to "overcome" in order to achieve previously experienced reward. The idea that animals are motivated to compensate for neurobiological hypofunction is a historical tenet in the drug use literature, and includes theories of opponent processes and allostasis (38). Alternatively, tolerance to aversive effects at high doses might also be postulated to promote greater intake.

In the search for pharmacotherapies to treat cocaine addiction, it has been postulated that a drug which is effective in blocking the binding of cocaine to the DAT but having no DA uptake-inhibiting effects itself would be a good candidate. Our studies show for the first time that dissociation of the effects of cocaine and uptake of DA is possible in vivo, albeit so far only through binge cocaine SA. The molecular mechanism of the effect is not yet known, however there are several possibilities that would support such an effect. The first is the discovery of site-specific DAT mutations that greatly reduce cocaine binding but leave DA uptake largely intact (16). These mutations highlight residues that may be necessary for cocaine-like drug binding, but not substrate binding. Thus, reduced effects of cocaine at the DAT in cocaine experienced animals may be associated with cocaine-specific binding site alterations that are independent of substrate binding. Nevertheless, more recent models demonstrate considerable overlap between cocaine-like and substrate binding deep within

the pore of the DAT protein (39), making the situation appear to be more complex than two mutually exclusive binding sites for substrates and cocaine.

Additional mechanisms could include shifts in inward vs. outward facing DAT (40), DAT glycosylated vs. non-glycosylated (41), or oligomer/monomer ratios (25). Indeed, increasing numbers of reports over the last decade have shown that DATs can form oligomers, which in turn have been hypothesized to affect DA uptake and inhibitor potency (25).

In summary, these studies demonstrate that decreased sensitivity to cocaine across variable maximal DA uptake rates characterizes the DA system of rats that have been exposed to high dose self-administration of cocaine. Decreased sensitivity of the DAT as the mechanism of presynaptic tolerance to cocaine is a previously understudied neural adaptation. While reports of altered DAT numbers and altered rate of DA uptake abound (42), none have described a decreased efficacy at the DAT that is specific to cocaine. This may be due to allosteric alterations, conformational or covalent modifications, or oligomerization of DATs which allow DA to be taken up normally, but render cocaine ineffective at inhibiting uptake. Defining the molecular mechanism(s) responsible for SA-induced “desensitization” of the DAT to cocaine will be critical in advancing our understanding of DAT regulation *in vivo* and potentially for developing pharmacological agents for the treatment of cocaine addiction.

Acknowledgments

The present research was supported by grants RO1DA021325 (SR Jones), P50DA006634 (SR Jones and DCS Roberts), R01 DA14030 (DCS Roberts), and T32DA007246 (MJ Ferris) from the National Institutes of Health. The authors would like to thank Leanne Thomas and Jason Locke for their technical assistance.

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Figure 1.

Lack of cocaine effect in the NAc core of rats following cocaine SA. The effect of cocaine (1.5 mg/kg, i.v.) on extracellular DA was measured in the NAc core of naïve animals (■) and rats following 5 days of cocaine SA (□). ***, $P < 0.001$; *, $P < 0.05$ compared with cocaine self-administering rats (Bonferroni post-hoc test).

Figure 2.

Significant reduction in DA uptake inhibition by cocaine in animals following cocaine SA. This figure shows the effect of cocaine on representative electrically evoked (1 pulse) DA signals in NAc core slices from naïve (**A**) and cocaine self-administering rats (**B**). The color plots represent the voltammetric currents (encoded in color in the z-axis) plotted against the applied potential (y-axis) and time (x-axis). Directly above the color plots are the faradaic currents at the oxidation potential for DA (i.e., concentration-time plots). Electrical stimulation (arrows) rapidly induced DA release in the NAc. There is a decrease in uptake following cocaine as depicted by increases in intensity and time course of the oxidative current (plotted as green on color plots). This inhibition is noticeable in naïve animals but significantly reduced in cocaine self-administering rats. The background-subtracted cyclic voltammograms indicative of DA are shown as insets to the upper right of the concentration-time plots. **C.** Group data demonstrating that cocaine, applied to brain slices containing the NAc, is less able to inhibit DA uptake following 5 days of cocaine SA ($\square$) compared to naïve rats ($\blacksquare$), measured as apparent K_m .

Figure 3.

SA history determines cocaine effects on baseline maximal rate of DA uptake (V_{max} ; insets) and cocaine-induced uptake inhibition (apparent K_m). **A.** One day of cocaine SA is enough to cause a significant decrease in the ability of cocaine to inhibit DA uptake in the NAc (*, $P < 0.05$). Cocaine effects remained significantly altered following 3 or 5 days of cocaine SA. **B.** Time-course of recovery with 1, 3, or 14 days of abstinence following 5 days of SA. Following 3 days of withdrawal, tolerance to the effects of cocaine persists (*, $P < 0.05$). The effect of cocaine on DA uptake returned to control values following 14 days of withdrawal. Note: 5 days of SA and 1 day abstinence are the same animals, also shown to be significantly reduced in Fig. 2C. **Insets:** Maximal rate of DA uptake (V_{max}) values for SA history and following cessation values of apparent K_m . *, $P < 0.05$ compared with naïve rats (Dunnett's multiple comparison test).

Figure 4.

Amphetamine retains the same potency at inhibiting DA uptake following cocaine SA. **A.** Following 5 days of cocaine SA ($\square$), amphetamine, applied to brain slices containing the NAc, retained the same potency as in naïve rats ($\blacksquare$). The graph shows the effect of amphetamine on DA uptake, measured as apparent K_m . **B.** The effect of amphetamine on extracellular DA was measured in the NAc core of naïve animals ($\blacksquare$) and rats following 5 days of cocaine SA ($\square$).

Figure 5.

Cocaine retains the same potency at inhibiting DA uptake following amphetamine SA. **A.** The effect of cocaine on extracellular DA was measured in the NAc core of naïve animals (■) and rats following 5 days of amphetamine SA (□). **B.** Following 5 days of amphetamine SA (□), cocaine applied to brain slices containing the NAc retained the same potency as in naïve rats (■), measured as apparent K_m . **Inset:** Maximal rate of DA uptake (V_{max}) values for animals that self-administer amphetamine are no different than naïve animals. **C:** This figure shows the effect of cocaine on representative electrically evoked (1 pulse; arrows) DA signals in NAc core slices from amphetamine self-administering rats (compare to naïve rats shown in Fig 2A).

Figure 6.

Amphetamine retains the same potency at inhibiting DA uptake following amphetamine SA.

A. The effect of amphetamine on extracellular DA was measured in the NAc core of naïve animals (■) and rats following 5 days of amphetamine SA (□). **B.** Following 5 days of amphetamine SA (□), amphetamine applied to brain slices containing the NAc retained the same inhibition of DA uptake as in naïve rats (■), measured as apparent K_m .