Published in final edited form as:

Eur J Neurosci. 2013 August ; 38(4): 2628–2636. doi:10.1111/ejn.12266.

Paradoxical tolerance to cocaine after initial supersensitivity in drug-use prone animals

Mark J. Ferris, Ph.D.¹, Erin S. Calipari¹, James R. Melchior¹, David C.S. Roberts, Ph.D.¹, Rodrigo A. España, Ph.D.^{1,2}, and Sara R. Jones, Ph.D.¹

¹Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, North Carolina 27157

²Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129

Abstract

There is great interest in outlining biological factors and behavioral characteristics that either predispose or predict vulnerability to substance use disorders. Response to an inescapable novel environment has been shown to predict a “drug-use prone” phenotype that is defined by rapid acquisition of cocaine self-administration. Here, we show that response to novelty can also predict neurochemical and behavioral effects of acute and repeated cocaine. We used cocaine self-administration under a fixed-ratio one schedule followed by fast scan cyclic voltammetry in brain slices to measure sub-second dopamine release and uptake parameters in drug-use prone and resistant phenotypes. Despite no significant differences in stimulated release and uptake, animals with high responses to a novel environment had dopamine transporters that were more sensitive to cocaine-induced uptake inhibition, which corresponded to greater locomotor activating effects of cocaine. These animals also acquired cocaine self-administration more rapidly, and after five days of extended access cocaine self-administration, high responding animals showed robust tolerance to DA uptake inhibition by cocaine. The effects of cocaine remained unchanged in animals with low novelty responses. Similarly, the rate of acquisition was negatively correlated with DA uptake inhibition by cocaine after self-administration. Thus, we show that tolerance to cocaine-induced inhibition of DA uptake coexists with a behavioral phenotype that is defined by increased preoccupation with cocaine as measured by rapid acquisition and early high intake.

Keywords

Dopamine; Individual Differences; Voltammetry; Self-Administration; Dopamine Transporter; Rat

Introduction

Exposure to drugs of abuse does not universally engender addiction. For example, less than 20% of individuals who have taken drugs ultimately become addicted or develop a substance use disorder (SAMHSA, 2008) as defined by the Diagnostic and Statistical Manual of Mental Disorders (American Psychological Association, 2004). There has been great interest in outlining biological factors and behavioral characteristics that either

Corresponding author: Sara R. Jones, Ph.D., Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157, Phone: 336-716-5504, Fax: 336-716-8501, srjones@wakehealth.edu.

Conflict of Interest

All authors have no conflict of interest to declare.

predispose or predict vulnerability to substance use disorders. There has been significant progress in developing animal models to understand this vulnerability (Piazza et al., 1989; Deroche-Gamonet et al., 2004; Blanchard et al., 2009; Beckmann et al., 2011). For example, there is a strong relationship between a rodent's propensity to seek and engage novelty as measured by locomotor response in an inescapable novel environment (Blanchard et al., 2009; Dellu et al., 1996), and the rate at which they will acquire self-administration of psychostimulant drugs such as cocaine (Piazza et al., 1989; Davis et al., 2008; Mantsch et al., 2001).

This suggests a strong link between novelty seeking and vulnerability to substance abuse, which corroborates work showing the same relationship in humans (Wills et al., 1998; Dellu et al., 1996). Recently, the validity of response to novelty as a predictor of drug abuse vulnerability was refined to be predictive of a "drug-use prone"/rapid acquisition phenotype as opposed to an "addiction prone"/compulsive phenotype (Deroche-Gamonet et al., 2004; 2008; Belin et al., 2011).

Differences in mesolimbic dopamine (DA) function between animals with either high or low response to novelty (HR and LR, respectively) have been documented (Verheij and Cools, 2011). HR animals possess higher intracellular (Verheij et al., 2008) and extracellular (Antoniou et al., 2008; Hooks et al., 1992) DA levels in addition to higher cocaine-, amphetamine-, and stress-induced DA overflow in microdialysis studies (Hooks et al., 1991; 1992; Rouge-Pont et al., 1998). HR animals also show higher metabolite/DA ratios in the nucleus accumbens (NAc) and striatum (Piazza et al., 1991). Others have shown greater numbers of TH-positive cells in the substantia nigra and ventral tegmental area of HR animals (Jerzemowska et al., 2012).

Despite considerable advancement in outlining a hyperdopaminergic mesolimbic DA system in HR animals relative to LR animals, almost no work has investigated response to novelty as a predictor of individual sensitivity to changes in rapid DA release and uptake signals produced by cocaine self-administration. Our laboratory has recently found that a history of high-dose cocaine self-administration produces tolerance to cocaine effects at the DA transporter (DAT) (Mateo et al., 2005; Ferris et al., 2011; 2012). Therefore, the purpose of the current investigation was to investigate whether 1) HR and LR rats differ in subsecond DA release and uptake kinetics and responses to acute cocaine administration, and 2) whether HR and LR rats differ in plasticity of DA terminals following a history of cocaine self-administration.

Materials and Methods

Subjects

Male, Sprague-Dawley rats (375 – 400 g; Harlan Laboratories) were used as subjects. A total of 22 animals underwent cocaine self-administration and subsequent voltammetry experiments, while a subset (n=12) of these animals were prescreened for their locomotor response to a novel environment. Two additional but separate sets of naïve animals (n=11–12 each) were used to relate response to novelty to the locomotor activating effects of cocaine and response to novelty to the effects of cocaine on uptake inhibition using voltammetry. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine, and experiments were carried out in accordance with the National Institutes of Health guidelines regarding the care and use of animals for experimental procedures.

Locomotor assessment

Animals were allowed a minimum of seven days to acclimate to the housing environment and light cycle prior to the start of experiments. Each animal was maintained on a reversed light cycle (3:00am-lights off; 3:00pm lights on), and all locomotor testing occurred during the dark cycle (9:00AM). We chose this time point for two reasons; 1) so that the locomotor assessment coincided with the time when animals would self-administer cocaine, and 2) because animals are generally awake and more active during this time of the dark cycle. We avoided the light portion of the cycle because we wanted to prevent sleep from contributing to variability in locomotor activity. Animals were first transferred to the locomotor testing room (with lights off) and allowed to habituate within their home cages for one hour. Animals were then placed in activity monitors (Med Associates, St. Albans, Vermont) and their horizontal activity was monitored for 90 minutes. As stated previously, a subset of animals that were tested for locomotor activating effects of cocaine were given an intraperitoneal (10 mg/kg, i.p.) injection of cocaine after novelty assessment and were recorded an additional 60 minutes. The activity chambers were acrylic boxes measuring 43 × 43 × 30 cm and contained two infrared beam arrays. Horizontal activity was measured by beam breaks which were recorded by a computer. Animals were then returned to their home cage and colony until the start of self-administration procedures, which occurred within one week of locomotor activity assessment.

Self-administration procedures

The procedures for implantation of cannulae into the jugular vein and cocaine self-administration have been described previously (Mateo et al., 2005; Ferris et al., 2011) and are summarized here. Following locomotor assessment and surgery, animals were singly-housed within chambers outfitted for both housing and self-administration procedures so that animals remained in chambers for the duration of self-administration procedures. All self-administration procedures (9:00am – 3:00pm) occurred during the dark cycle. Each lever press resulted in the delivery of 1.5 mg/kg cocaine in 100 µl over 4 seconds. This dose was chosen for two reasons. First, 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 (Richardson and Roberts, 1996). Second, even though other studies exploring response to novelty as it corresponds to self-administration use lower unit doses of cocaine, we chose the current dose because our previous work on tolerance to the effects of cocaine used 1.5 mg/kg (Ferris et al., 2011; 2012). Therefore, we wanted to remain consistent with our previous work to avoid introducing additional variables. 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 responses produced no programmed consequence. The session was terminated after 40 injections or after 6 hours, whichever occurred first. Once the animals reached the maximum number of injections allowed in a single session (40), they were allowed to self-administer 40 injections per day for five consecutive days prior to the voltammetry experiment. Animals were limited to 40 injections per session once they acquired stable cocaine self-administration behavior in an effort to reduce variation in the amount of cocaine consumed over the entire experiment. Therefore, all animals, regardless of response to a novel environment, consumed the same amount of cocaine after acquisition (300 mg). The acquisition criterion was defined as the first of five consecutive days where the animals would lever press for 35–40 injections of cocaine on an FR1 schedule of reinforcement.

Fast-scan cyclic voltammetry

The procedures for voltammetry have been described previously (Ferris et al., 2012). All voltammetry experiments in self-administering animals were conducted 18h following (dark cycle) the final self-administration session. Voltammetry experiments in naïve animals were

conducted within one week of locomotor activity screening. Animals were placed in an induction chamber for approximately 2 minutes containing 5% isoflurane gas to induce anesthesia. Once under anesthesia, animals were decapitated and brains were rapidly removed. Multiple coronal slices (400 μ M) containing the NAc were prepared from each animal with a vibrating tissue slicer while immersed in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), CaCl_2 (2.4), MgCl_2 (1.2), NaHCO_3 (25), glucose (11), L-ascorbic acid (0.4), pH adjusted to 7.4. Once sliced, tissue was transferred to the testing chambers containing aCSF at 32° C which flowed at 1 mL/min. After a 30 minute equilibration period, a cylindrical carbon fiber microelectrode (100–200 μ M length, 7 μ M radius) and a bipolar stimulating electrode were placed into the NAc core. Specifically, recording and stimulating electrodes for each animal were placed + 1.5 mm anterior to bregma and dorsomedial to the anterior commissure; directly between the anterior commissure and the ventral tip of the lateral ventricle (Paxinos and Watson, 2007). Supplementary Figure 1 provides a schematic illustration of the recording area for all animals. We selected the NAc core because of the dense innervations of DA nerve terminals and because it is a critical locus for the reinforcing actions of cocaine. Our previous research has concentrated on plasticity of DATs in both the core and shell and demonstrated similar results (Mateo et al., 2005). DA was evoked by a single, rectangular, electrical pulse (300 μ A, 4 ms), applied every 5 min. DA was monitored at the carbon fiber electrode every 100 ms using fast-scan cyclic voltammetry (Kennedy et al., 1992) by applying a triangular waveform (– 0.4 to + 1.2 to – 0.4V vs. Ag/AgCl, 400 V/s). Once the DA response was stable (i.e., did not exceed 10 % variation in peak height for three successive stimulations), cocaine (0.3 – 30 μ M), was applied cumulatively to the brain slice. Immediately following each concentration-response curve, recording electrodes were calibrated by recording their response (in electrical current; nA) to a known concentration of DA in aCSF (3 μ M) using a flow-injection system. This value was then used to convert electrical current to DA concentration. To evaluate the effects of drugs, evoked levels of DA were modeled using Michaelis-Menten kinetics, as a balance between release and uptake (Wightman et al., 1988). Michaelis-Menten modeling provides parameters that describe the amount of DA released following stimulation, the maximal rate of DA uptake (V_{max}), and inhibition of the ability of DA to bind to the DAT, or apparent uptake inhibition (apparent K_m). For baseline modeling (pre-drug), we followed standard voltammetric modeling procedures by setting the apparent K_m to 160 nM based on well-established research on the affinity of DA for the DAT (Wu et al., 2001). Baseline V_{max} values were allowed to vary as the baseline measure of rate of DA uptake. Indeed, while the affinity of DA for the DAT does not substantially vary from animal to animal, chronic cocaine self-administration has been shown to alter the V_{max} by altering the number of DATs on the cell surface (Calipari et al., 2012). Following drug application, apparent K_m was then allowed to vary in order to account for changes in drug-induced DA uptake inhibition while the respective V_{max} value determined for that subject at baseline was held constant. The *apparent* K_m parameter models the amount of DA uptake inhibition following a particular dose of drug rather than the explicit affinity of DA for the DAT (i.e., K_m) *per se*. All voltammetry data were collected and modeled using Demon Voltammetry and Analysis Software (Yorgason et al., 2011). Data were compared using multiple regressions and correlations in addition to dividing animals according to the median split of their locomotor data. Grouped analyses included two-way ANOVA with experimental group and concentration of the drug as the factors. When significant main effects were obtained ($p < 0.05$), differences between groups at each dose were tested using Bonferroni post-hoc tests. All analyses were performed and graphs were created in GraphPad statistical and graphing software (version 5; La Jolla, CA).

Results

Response to novelty predicts the ability of cocaine to inhibit DA uptake and increase locomotor activity

In order to determine whether locomotor response to novelty can predict subsecond DA release and uptake kinetics in the NAc, as well as the neurochemical and behavioral activating effects of acute cocaine, we assessed cocaine-induced locomotor activity and the ability of cocaine to inhibit DA uptake using voltammetry in brain slices in animals prescreened for their locomotor response to a novel environment.

Figure 1A and 1B show no relationship between response to novelty and DA release and the maximal rate of DA uptake ($V_{\max}$), respectively. We divided animals based on a median split of the locomotor responses to novelty. The average locomotor response (mean $\pm$ S.E.M.) for HR animals was 9698 ± 330 cm, while the average locomotor response for LR animals was 6689 ± 350 cm. When data are grouped by a median split of response to novelty (Figure 1C and 1D), there is no difference in baseline dopamine signaling between HR and LR rats. The ability of each cocaine dose to inhibit DA uptake is plotted against response to novelty in Figure 2A and demonstrates that cocaine is more effective at inhibiting DA uptake as response to novelty increases; an effect which is most pronounced at higher doses ($30\text{ }\mu\text{M}$; $r = 0.68$, $F_{(1, 10)} = 9.21$, $p < 0.01$). The main effect of response to novelty is apparent when data are grouped based on a median split of locomotor activity (Figure 2B), $F_{(1, 28)} = 2.19$, $p < 0.05$. Bonferroni post-hoc tests indicate that the ability of cocaine to inhibit DA uptake differs at the $30\text{ }\mu\text{M}$ dose. Furthermore, Figure 2C demonstrates that response to novelty also predicts the locomotor activating effects of cocaine, $r = 0.79$, $F_{(1, 9)} = 14.49$, $p < 0.01$.

Response to novelty predicts acquisition of cocaine self-administration

We were interested in studying the relationship between response to novelty and acquisition of cocaine self-administration. Animals were allowed 6 hours to self-administer cocaine per day with a maximum number of 40 injections. Acquisition was defined as the first of five consecutive days of 35–40 injections of cocaine on an FR1 schedule of reinforcement. These criteria led to substantial variability in the rate of cocaine acquisition. Figure 3A shows representative traces from three animals, one that immediately acquired cocaine self-administration, one that acquired at a moderate rate, and one that required a month of daily sessions before meeting criteria. For the vast majority of animals (75%), acquisition occurred within 10 days of placing them in testing chambers (Figure 3B). Figures 3C and 3D demonstrate that response to novelty predicted the rate at which animals would acquire cocaine self-administration ($r = -0.76$, $p < 0.01$), with higher responses to novelty predicting faster acquisition rates.

Response to novelty predicts tolerance of the DAT to cocaine following self-administration

In order to study possible differences between HR and LR animals in cocaine-induced changes in DA release and uptake kinetics, we sacrificed animals for slice voltammetry experiments 18 hours after their final cocaine self-administration session. The regressions in Supplementary Figure 2 show no relationship between response to novelty and stimulated DA release (Figure S2A) or $V_{\max}$ (Figure S2B) following a history of cocaine self-administration, similar to initial experiments in naïve animals. Animals were separated into two groups using a median split of their locomotor responses to novelty. The average locomotor response (mean $\pm$ S.E.M.) for HR animals was 8976 ± 816 cm, while the average locomotor response for LR animals was 4432 ± 599 cm. Cocaine self-administration did not change DA release, regardless of response to novelty (Figure S2C; all effects, $p > 0.05$). However, after cocaine self-administration, animals demonstrated an absolute shift to slower $V_{\max}$ values, as demonstrated by a main effect of treatment group, $F_{(1, 18)} = 9.43$, $p < 0.01$.

(Figure S2D), with no difference between the slopes between naïve and cocaine SA animals (i.e., comparing Figure 1B and S2B; $p > 0.05$). Therefore, a history of cocaine self-administration slowed $V_{\max}$ for all animals, regardless of response to novelty.

Figure 4A demonstrates that following a history of cocaine self-administration, cocaine was less effective at inhibiting DA uptake in animals with greater response to the novel environment, an effect that was exacerbated as dose increased ($10 \mu\text{M}$, $r = -0.63$, $F_{(1, 10)} = 6.54$, $p < 0.05$; $30 \mu\text{M}$, $r = -0.76$, $F_{(1, 10)} = 13.71$, $p < 0.01$). Figure 4B extends this finding and illustrates that cocaine self-administration produced tolerance to DA uptake inhibition by cocaine only in HR (right panel), but not LR (left panel) animals. When HR and LR animals were analyzed separately and grouped according to whether they underwent a history of cocaine self-administration (Figures 4C and 4D), only the HR animals demonstrated a reduced ability of cocaine to inhibit DA uptake, $F_{(1, 40)} = 54.25$, $p < 0.0001$, that was significant at both the $10 \mu\text{M}$ and $30 \mu\text{M}$ doses.

Acquisition of cocaine self-administration predicts tolerance of the DAT to cocaine

There is a strong relationship between response to novelty and rate of cocaine acquisition demonstrated here (Figure 3C) and in the literature (Piazza et al., 1989; Davis et al., 2008; Mantsch et al., 2001). Therefore, using additional animals, we investigated whether the rate of cocaine self-administration acquisition, in a manner similar to response to novelty, could predict plasticity of the DAT. Similar to the inability of response to novelty to predict DA release and $V_{\max}$, the rate of cocaine acquisition did not significantly predict either of these variables, despite a trend toward faster $V_{\max}$ in faster acquiring animals (Figure 5A and 5B; $p > 0.05$). On the other hand, the rate of acquisition of cocaine self-administration significantly predicted changes in cocaine's ability to inhibit uptake. Namely, Figure 5C shows a reduced ability of cocaine to inhibit DA uptake in faster acquiring animals ($3 \mu\text{M}$, $r = 0.54$, $F_{(1, 19)} = 7.84$, $p < 0.05$; $10 \mu\text{M}$, $r = 0.59$, $F_{(1, 19)} = 9.78$, $p < 0.01$; $30 \mu\text{M}$, $r = 0.80$, $F_{(1, 20)} = 34.85$, $p < 0.0001$). These trends and effects are almost identical to the relationships exhibited between response to novelty and cocaine kinetic parameters. Therefore, this suggests that response to novelty and rate of cocaine acquisition have the same relationship to $V_{\max}$, DA release, and the ability of the DAT to be inhibited by cocaine following cocaine self-administration.

We wanted to investigate whether increased consumption of cocaine could lead to greater tolerance to cocaine's effect at the DAT. Therefore, we divided animals according to a median split of the number of days to acquire cocaine self-administration and examined total intake in the two groups. Figure 6A demonstrates significantly lower cocaine intake in fast acquiring animals relative to slow acquiring animals, $t_{20} = 3.59$, $p < 0.001$. Thus, the greater the number of days animals took to acquire cocaine self-administration, the greater the total amount of cocaine intake. It is unlikely, then, that greater intake leads to greater tolerance at the DAT. However, to empirically test this hypothesis, we correlated total cocaine intake and cocaine-induced DA uptake inhibition. Figure 6B shows a reduced ability of cocaine to inhibit DA uptake in animals that consumed less cocaine over the entire experiment, an effect that was exacerbated as dose increased ($3 \mu\text{M}$, $r = 0.58$, $F_{(1, 19)} = 9.54$, $p < 0.01$; $10 \mu\text{M}$, $r = 0.62$, $F_{(1, 19)} = 11.70$, $p < 0.01$; $30 \mu\text{M}$, $r = 0.78$, $F_{(1, 20)} = 30.27$, $p < 0.0001$). These trends and effects are almost identical to the relationships exhibited between days to acquire and cocaine kinetic parameters, and confirms that more cocaine does not produce greater tolerance at the DAT.

Discussion

The current study shows that animals most prone to acquire self-administration also show marked tolerance to cocaine inhibition of DA uptake after cocaine self-administration.

Tolerance to the effects of cocaine is one of the defining characteristics of cocaine addiction (DSM-IV, American Psychological Association, 2004). Previously, we found that neurochemical tolerance to cocaine's effects at the DAT is associated with tolerance to the locomotor activating effects of cocaine (Lack et al., 2008). The reduced effects of cocaine are consistent with clinical reports of reduced subjective and pharmacological effects of cocaine in addicts (Volkow et al., 1996; 1999; Wang et al., 1999). Therefore, while others have demonstrated clustering of an "addiction-prone" phenotype with DSM-IV criteria such as high motivation to use drugs and inability to reduce drug intake (Deroche-Gamonet et al., 2004; Belin et al., 2011), we now associate a "drug-use prone" phenotype with initial supersensitivity to cocaine-induced DA uptake inhibition and subsequent tolerance to cocaine's effects at the DAT after cocaine self-administration. Despite our earlier work showing locomotor tolerance following a history of cocaine self-administration (Lack et al., 2008), it remains unknown whether HR animals would be particularly susceptible to this locomotor tolerance. Thus, demonstrating individual differences in the reduced locomotor response to cocaine requires additional experimentation. This is particularly important given that the DSM-IV notion of tolerance primarily refers to behavioral outcomes, and not necessarily neurochemical outcomes.

High responders show increased locomotor and neurochemical responses to acute cocaine administration

The locomotor activating effects of acute cocaine as well as cocaine's ability to inhibit DA uptake were enhanced in drug-naïve HR rats relative to drug naïve LR rats. The enhanced potency of cocaine in HR animals is congruent with reports of greater cocaine-induced DA overflow in HR animals measured with microdialysis (Chefer et al., 2003). Note that DA overflow has been explicitly correlated to cocaine-induced uptake inhibition using voltammetry in previous work (Mateo et al., 2005; Ferris et al., 2011). Thus, it is likely that increased potency of cocaine at the DAT in HR animals would augment cocaine-induced DA overflow which, in turn, may underlie the increased locomotor activating effects of the drug.

The increased pharmacological effects of acute cocaine administration in HR animals may explain, at least in part, faster acquisition and more robust responding in these animals. Similar to how greater reward magnitude is associated with greater cue-induced DA release and greater appetitive responses (Beyene et al., 2010), it is possible that an enhanced pharmacological effect of cocaine in HR animals could lead to larger subsecond DA signals that underlie appetitive responding (Flagel et al., 2011). This could occur through greater cocaine-induced enhancement of the rapid DA signals/transients in HR animals, or through an enhanced discriminative stimulus effect of cocaine in HR animals. We chose a dose of cocaine that is at the top of the dose-response curve in measures of reinforcing efficacy, and preferred over lower doses in choice studies (Richardson and Roberts, 1996), indicating a greater appetitive value of this dose over lower doses.

Factors other than increased pharmacological effects at the DAT should be considered as well, because this is the only study, to our knowledge, that has shown a distinction in rate of acquisition between HR and LR animals at this high of a cocaine dose (c.f., Mantsch et al., 2001). It is known, for example, that higher doses of cocaine produce anxiogenic effects, and it is possible that LR animals are more sensitive to the anxiogenic effects of high dose cocaine, which may cause a delay in their rate of acquisition. LR rats (when compared to HRs) are less likely to explore anxiety-inducing environments such as open arms in mazes or the lighted side of light-dark boxes (Kabbaj et al., 2000; White et al., 2007). Therefore, at the high dose used in our studies, faster acquisition in HR animals may be a result of increased pharmacological effects of cocaine at the DAT combined with a relative insensitivity to anxiogenic effects of high dose cocaine in HR animals. Additionally, there

are important procedural differences such as acquisition criteria, days spent administering cocaine, days allowed to acquire, and even locomotor screening methods between our study and many others that do not detect HR and LR differences in acquisition of cocaine self-administration at high doses (e.g., Mantsch et al., 2001, Deroche-Gamonet et al., 2004, McCutcheon et al., 2009). A small percentage of animals exhibit much slower rates of acquisition than is typically encountered under extended access conditions. However, our animals live within their testing chambers 24 hours/day, and self-administer cocaine during the dark cycle. In addition, these animals received no prior training in operant behavior prior to cocaine self-administration. Typically, animals in studies using extended access cocaine self-administration begin with training in short access conditions, or with food self-administration for a number of weeks in order to learn operant behavior (Ahmed and Koob, 1998, 2004; Ahmed et al., 2003). Therefore, relatively unique aspects of our self-administration procedure may explain the discrepancy between slower acquiring animals in our study and those in previous work.

High-, but not low-, responders become tolerant to cocaine-induced DA uptake inhibition following self-administration

We have previously found that high-dose cocaine self-administration (1.5 mg/kg/infusion) can reduce the ability of cocaine to inhibit DA uptake at the DAT, independent of the DAT's ability to transport substrate (Ferris et al., 2011) and regardless of whether $V_{\max}$ increases or decreases (Mateo et al., 2005; Ferris et al., 2011; 2012). Indeed, the current investigation showed that HR animals, while more sensitive to acute cocaine effects, are particularly susceptible to development of tolerance to cocaine's effects at the DAT following high-dose cocaine self-administration. Alternatively, LRs remain at the same, albeit lower, initial DAT sensitivity to cocaine after self-administration.

Earlier work established that the reduced effects of cocaine on the DAT resulted in reduced cocaine-induced DA overflow as measured by microdialysis (Mateo et al., 2005; Ferris et al., 2011). This suggests that HR, but not LR, animals would show blunted DA overflow to cocaine following self-administration. This is similar to reports in the literature of reduced DA overflow and reduced conditioned-place preference (CPP) in high cocaine responding animals only after repeated experimenter-delivered cocaine administration (Allen et al., 2007; Nelson et al., 2009). Note, however, that these studies (Allen et al., 2007; Nelson et al., 2009) do not show a relationship between acute cocaine response and response to novelty and therefore may be investigating a slightly different phenotype than the one in the current investigation.

Future experimentation is needed to fully elucidate why HRs, and not LRs, show tolerance to cocaine inhibition of the DAT after a history of cocaine self-administration. Consistent with our previous findings, it is clear that altered $V_{\max}$ for DA uptake is not a mechanism for the reduction in cocaine's ability to inhibit the DAT (Ferris et al., 2011; 2012; Calipari et al., 2012) because there was no difference before self-administration and there was an absolute shift to slower $V_{\max}$ in all animals after self-administration. In addition, increased total cocaine intake cannot explain tolerance. LR animals took longer to reach acquisition criteria and as a result had a higher total cocaine intake than HR animals. Thus, HR animals actually took less cocaine overall and exhibited more robust tolerance. We are currently testing two competing hypotheses to explain the differences in cocaine's effect at the DAT. One hypothesis is that initial supersensitivity of cocaine at the DAT in HR animals predisposes the animals to fundamentally different (possibly faster, binge-like) patterns of intake, which in turn causes tolerance to cocaine at the DAT. The alternative hypothesis is that the same inherent differences in DAT function that causes initial supersensitivity of cocaine at the DAT also mediate the reduced effects after cocaine experience, regardless of intake.

For example, we are currently exploring the possibility that cocaine possesses greater affinity for the DAT in HR animals. This putative difference in affinity may be the result of an underlying physiological difference in DAT protein conformation which, in turn, might also make HR DAT proteins susceptible to allosteric alteration in DAT function following cocaine self-administration. DATs are known to undergo allosteric modifications that lead to functional changes in DA uptake through phosphorylation or protein-protein interactions with other presynaptic proteins such as DAT, D2 receptors, and vesicular monoamine transporters (Chen et al., 2005; Egana et al., 2009; Chen and Reith, 2010), although this has yet to be tested in our model. Ultimately, the differences between HR and LR animals in both their acute response to cocaine and cocaine-induced plasticity of the DAT could lead to valuable information for targeted pharmacotherapy for cocaine addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH grants R01 DA024095, R01 DA03016 (SRJ), K99 DA031791 (MJF), K01 DA025279 (RAE), T32 DA007246 (MJF & ESC) and F31 DA031533 (ESC), R01 DA14030 (DCSR), P50 DA006634 (SRJ and DCSR).

References

- Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. *Science*. 1998; 282:298–300. [PubMed: 9765157]
- Ahmed SH, Koob GF. Changes in response to dopamine receptor antagonist in rats with escalating cocaine intake. *Psychopharmacology*. 2004; 172:450–454. [PubMed: 14647962]
- Ahmed SH, Lin D, Koob GF, Parsons LH. Escalation of cocaine self-administration does not depend on altered cocaine-induced nucleus accumbens dopamine levels. *Journal of Neurochemistry*. 2003; 86:102–113. [PubMed: 12807430]
- Allen RM, Everett CV, Nelson AM, Gulley JM, Zahniser NR. Low and high locomotor responsiveness to cocaine predicts intravenous cocaine conditioned place preference in male Sprague-Dawley rats. *Pharmacology Biochemistry and Behavior*. 2007; 86:37–44.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4. American Psychiatric Press; Washington DC: 1994.
- Antoniou K, Papathanasiou G, Papalexi E, Hyphantis T, Nomikos GG, Spyraiki C, Papadopoulou-Daifoti Z. Individual responses to novelty are associated with differences in behavioral and neurochemical profiles. *Behavioural Brain Research*. 2008; 187:462–472. [PubMed: 18036673]
- Beckmann JS, Marusich JA, Gipson CD, Bardo MT. Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. *Behavioural Brain Research*. 2011; 216:159–165. [PubMed: 20655954]
- Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V. High-Novelty-Preference Rats are Predisposed to Compulsive Cocaine Self-administration. *Neuropsychopharmacology*. 2011; 36:569–579. [PubMed: 20980989]
- Beyene M, Carelli RM, Wightman RM. Cue-Evoked Dopamine Release in the Nucleus Accumbens Shell Tracks Reinforcer Magnitude During Intracranial Self-Stimulation. *Neuroscience*. 2010; 169:1682–1688. [PubMed: 20600644]
- Blanchard MM, Mendelsohn D, Stamp JA. The HR/LR model: Further evidence as an animal model of sensation seeking. *Neuroscience and Biobehavioral Reviews*. 2009; 33:1145–1154. [PubMed: 19497336]
- Chefer VI, Zakharova I, Shippenberg TS. Enhanced responsiveness to novelty and cocaine is associated with decreased basal dopamine uptake and release in the nucleus accumbens:

- Quantitative microdialysis in rats under transient conditions. *Journal of Neuroscience*. 2003; 23:3076–3084. [PubMed: 12684494]
- Chen NH, Reith MEA. Substrates dissociate dopamine transporter oligomers. *Journal of Neurochemistry*. 2008; 105:910–920. [PubMed: 18088380]
- Chen R, Han DD, Gu HH. A triple mutation in the second transmembrane domain of mouse dopamine transporter markedly decreases sensitivity to cocaine and methylphenidate. *Journal of Neurochemistry*. 2005; 94:352–359. [PubMed: 15998286]
- Davis BA, Clinton SM, Akil H, Becker JB. The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats. *Pharmacology Biochemistry and Behavior*. 2008; 90:331–338.
- del Castillo M, Palmero DJ, Lopez B, Paul R, Ritacco V, Bonvehi P, Clara L, Ambroggi M, Barrera L, Vay C. Mesotherapy-associated Outbreak Caused by *Mycobacterium immunogenum*. *Emerging Infectious Diseases*. 2009; 15:357–359. [PubMed: 19193300]
- Dellu F, Piazza PV, Mayo W, Lemoal M, Simon H. Novelty-seeking in rats - Biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. *Neuropsychobiology*. 1996; 34:136–145. [PubMed: 8916071]
- Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science*. 2004; 305:1014–1017. [PubMed: 15310906]
- Deroche-Gamonet V. Psychobiological features associated with cocaine addiction-like behavior in rats. *International Journal of Psychology*. 2008; 43:181.
- Deroche V, Piazza PV, Deminiere JM, Lemoal M, Simon H. Rats Orally Self-Administer Corticosterone. *Brain Research*. 1993; 622:315–320. [PubMed: 8242374]
- Deroche V, Marinelli M, Maccari S, Lemoal M, Simon H, Piazza PV. Stress-Induced Sensitization and Glucocorticoids 1. Sensitization of Dopamine-Dependent Locomotor Effects of Amphetamine and Morphine Depends on Stress-Induced Corticosterone Secretion. *Journal of Neuroscience*. 1995; 15:7181–7188. [PubMed: 7472472]
- Duclot F, Hollis F, Darcy MJ, Kabbaj M. Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders. *Physiology & Behavior*. 2011; 104:296–305. [PubMed: 21172365]
- Egana LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Pena K, Quiroz M, Hong WC, Dorostkar MM, Janz R, Sitte HH, Torres GE. Physical and Functional Interaction between the Dopamine Transporter and the Synaptic Vesicle Protein Synaptogyrin-3. *Journal of Neuroscience*. 2009; 29:4592–4604. [PubMed: 19357284]
- Ferris MJ, Mateo Y, Roberts DCS, Jones SR. Cocaine-Insensitive Dopamine Transporters with Intact Substrate Transport Produced by Self-Administration. *Biological Psychiatry*. 2011; 69:201–207. [PubMed: 20801429]
- Ferris MJ, Calipari ES, Mateo Y, Melchior JR, Roberts DCS, Jones SR. Cocaine Self-Administration Produces Pharmacodynamic Tolerance: Differential Effects on the Potency of Dopamine Transporter Blockers, Releasers, and Methylphenidate. *Neuropsychopharmacology*. 2012; 37:1708–1716. [PubMed: 22395730]
- Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB. Response to Novelty Predicts the Locomotor and Nucleus-Accumbens Dopamine Response to Cocaine. *Synapse*. 1991; 9:121–128. [PubMed: 1821483]
- Hooks MS, Colvin AC, Juncos JL, Justice JB. Individual-Differences in Basal and Cocaine-Stimulated Extracellular Dopamine in the Nucleus-Accumbens Using Quantitative Microdialysis. *Brain Research*. 1992; 587:306–312. [PubMed: 1525663]
- Jerzemska G, Plucinska K, Kulikowski M, Trojnar W, Wrona D. Locomotor response to novelty correlates with the number of midbrain tyrosine hydroxylase positive cells in rats. *Brain Research Bulletin*. 2012; 87:94–102. [PubMed: 22056623]
- Kennedy RT, Jones SR, Wightman RM. Dynamic Observation of Dopamine Autoreceptor Effects in Rat Striatal Slices. *Journal of Neurochemistry*. 1992; 59:449–455. [PubMed: 1352798]
- Lack CM, Jones SR, Roberts DCS. Increased breakpoints on a progressive ratio schedule reinforced by IV cocaine are associated with reduced locomotor activation and reduced dopamine efflux in nucleus accumbens shell in rats. *Psychopharmacology*. 2008; 195:517–525. [PubMed: 17879088]

- Mantsch JR, Ho A, Schlussman SD, Kreek MJ. Predictable individual differences in the initiation of cocaine self-administration by rats under extended-access conditions are dose-dependent. *Psychopharmacology*. 2001; 157:31–39. [PubMed: 11512040]
- Mateo Y, Lack CM, Morgan D, Roberts DCS, Jones SR. Reduced dopamine terminal function and insensitivity to cocaine following cocaine binge self-administration and deprivation. *Neuropsychopharmacology*. 2005; 30:1455–1463. [PubMed: 15702135]
- McCutcheon JE, White FJ, Marinelli M. Individual Differences in Dopamine Cell Neuroadaptations Following Cocaine Self-Administration. *Biological Psychiatry*. 2009; 66:801–803. [PubMed: 19539267]
- Nelson AM, Larson GA, Zahniser NR. Low or High Cocaine Responding Rats Differ in Striatal Extracellular Dopamine Levels and Dopamine Transporter Number. *Journal of Pharmacology and Experimental Therapeutics*. 2009; 331:985–997. [PubMed: 19729579]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 6. Academic Press; 2007.
- Piazza PV, Deminiere JM, Lemoal M, Simon H. Factors That Predict Individual Vulnerability to Amphetamine Self-Administration. *Science*. 1989; 245:1511–1513. [PubMed: 2781295]
- Piazza PV, Rougepont F, Deminiere JM, Kharoubi M, Lemoal M, Simon H. Dopaminergic Activity Is Reduced in the Prefrontal Cortex and Increased in the Nucleus-Accumbens of Rats Predisposed to Develop Amphetamine Self-Administration. *Brain Research*. 1991; 567:169–174. [PubMed: 1726140]
- Richardson NR, Roberts DCS. Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*. 1996; 66:1–11. [PubMed: 8794935]
- Rouge-Pont F, Deroche V, Le Moal M, Piazza PV. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *European Journal of Neuroscience*. 1998; 10:3903–3907. [PubMed: 9875367]
- Substance Abuse and Mental Health Services Administration (SAMHSA). [Accessed: 01 October 2012.] National Survey on Drug Use and Health. 2008. Retrieved at <http://www.oas.samhsa.gov/nhsda.htm>
- Verheij MMM, de Mulder ELW, De Leonibus E, van Loo KMJ, Cools AR. Rats that differentially respond to cocaine differ in their dopaminergic storage capacity of the nucleus accumbens. *Journal of Neurochemistry*. 2008; 105:2122–2133. [PubMed: 18315567]
- Verheij MMM, Cools AR. Reserpine differentially affects cocaine-induced behavior in low and high responders to novelty. *Pharmacology Biochemistry and Behavior*. 2011; 98:43–53.
- Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Ding YS, Logan J, Dewey SL, Hitzemann R, Lieberman J. Relationship between psychostimulant-induced “high” and dopamine transporter occupancy. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:10388–10392. [PubMed: 8816810]
- Volkow ND, Fowler JS, Wang GJ. Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Journal of Psychopharmacology*. 1999; 13:337–345. [PubMed: 10667609]
- Wang GJ, Volkow ND, Fowler JS, Logan J, Pappas NR, Wong CT, Hitzemann RJ, Netusil N. Reproducibility of repeated measures of endogenous dopamine competition with [¹¹C]-raclopride in the human brain in response to methylphenidate. *Journal of Nuclear Medicine*. 1999; 40:1285–1291. [PubMed: 10450679]
- Wightman RM, Amatore C, Engstrom RC, Hale PD, Kristensen EW, Kuhr WG, May LJ. Real-Time Characterization of Dopamine Overflow and Uptake in the Rat Striatum. *Neuroscience*. 1988; 25:513–523. [PubMed: 3399057]
- Wills TA, Windle M, Cleary SD. Temperament and novelty seeking in adolescent substance use: Convergence of dimensions of temperament with constructs from Cloninger’s theory. *Journal of Personality and Social Psychology*. 1998; 74:387–406. [PubMed: 9491584]
- Wu Q, Reith MEA, Wightman RM, Kawagoe KT, Garriss PA. Determination of release and uptake parameters from electrically evoked dopamine dynamics measured by real-time voltammetry. *Journal of Neuroscience Methods*. 2001; 112:119–133. [PubMed: 11716947]

Yorgason JT, Espana RA, Jones SR. Demon Voltammetry and Analysis software: Analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *Journal of Neuroscience Methods*. 2011; 202:158–164. [PubMed: 21392532]

Figure 1.

No relationship between electrically-stimulated dopamine release (A) or the maximal rate of dopamine uptake (B; V_{max}) and response to a novel environment ($n = 11$). Performing a median split on the response to novel environment demonstrates no differences in dopamine release (C) or maximal rate of dopamine uptake (D) between high responders and low responders.

Figure 2.

Cocaine is more effective at inhibiting dopamine uptake as behavioral response to novel environment increases, an effect exacerbated as the dose of cocaine increases (A; $n = 9-12$). Plotting each dose of cocaine according to median split of response to novel environment demonstrates that higher doses of cocaine are more effective at inhibiting dopamine uptake in high responders relative to low responders (B). The locomotor activating effects of cocaine are greater in rats with higher response to novel environment (C, $n = 11$). $**p < 0.01$, $*p < 0.05$.

Figure 3.

There is significant variability in the acquisition rate of cocaine self-administration. (A) Representative self-administration activity across days in fast (dark), medium (dark gray) and slow (light gray) acquiring animals. (B) Most animals (75%) acquire within 10 days of self-administration with the remaining animals take between 10 – 30 days to reach criterion ($n = 22$). The locomotor response to a novel environment for a subset of animals (C, $n = 12$) or when grouped according to median split (D) predicts the number of days for animals to acquire cocaine self-administration ($r=0.76$), with higher locomotor response predicting faster acquisition. $^{**}p < 0.01$

Figure 4.

A history of cocaine self-administration (SA) reduces the ability of cocaine to inhibit dopamine uptake in animals with high responders (HR), but not in low responders (LR). (A) Following a history of cocaine self-administration, cocaine is less effective at inhibiting dopamine uptake (y-axis) as animals' initial response to novel environment increases (x-axis). This effect becomes more robust as the dose of cocaine increases. * $p < 0.05$, $n = 12$. Representative dopamine traces (B) and grouped data (C, D) demonstrate no difference in the ability of cocaine to inhibit dopamine uptake between LR naïve (green traces) and LR cocaine SA (blue traces) animals (B left panel, C). HR animals with a history of cocaine SA, however, demonstrated a leftward shift in the descending limb of the dopamine curve when cocaine is applied, indicating a reduced ability of cocaine to inhibit dopamine uptake (B right panel). This is also shown in HR data when naïve and cocaine SA animals are compared (D). *** $p < 0.001$, $n = 6$ HR and 6 LR per group.

Figure 5.

The relationship between dopamine kinetics (y-axes) and days to acquire cocaine self-administration (SA, x-axes) is similar to the relationship between dopamine kinetics and response to a novel environment. There is no relationship between electrically-stimulated dopamine release (A) or the maximal rate of dopamine uptake (B) and the number of days to acquire cocaine self-administration (SA). Cocaine is less effective at inhibiting dopamine uptake in animals that acquire cocaine SA more rapidly (C). *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, $n = 19-22$.

A.

B.

Figure 6.

Faster acquisition of cocaine self-administration is associated with less cocaine intake, and less cocaine intake is associated with greater tolerance to the ability of cocaine to inhibit dopamine uptake. (A) There is less total cocaine intake in animals that acquire cocaine self-administration more rapidly, based on median split on the number of days to acquisition. The red, dotted line represents the amount of cocaine consumed during the five days of 40 injections/day, (i.e., following acquisition; 300 mg). Total intake above the red, dotted line represents amount of cocaine consumed during the acquisition phase. (B) The relationship between cocaine uptake inhibition (y-axis) and total intake of cocaine (x-axis) is similar to the relationship between cocaine uptake inhibition and days to acquire cocaine self-administration (Figure 5C). Cocaine is less effective at inhibiting dopamine uptake in animals that consumed less cocaine in the experiment. *** $p < 0.0001$, ** $p < 0.01$, $n = 19-22$.