Cocaine withdrawal alters the reward omission effect and enhances traits of negative urgency in rats across multiple days of testing

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Abstract

Background: The personality trait of negative urgency, characterized as behaving rashly when emotionally perturbed, is gaining attention as an indicator for susceptibility to problematic substance use. How this trait is influenced by exposure to drugs of abuse is still unclear. Using an animal model of binge cocaine consumption, we tested this relationship in a reward-omission task across multiple days.

Methods: Adult, male, Sprague-Dawley rats received seven daily (ip) injections of saline, cocaine (10–20 mg/kg), or cocaine (20–40 mg/kg). Cocaine doses increased linearly each day from the lower to the higher dose. A separate group received RTI-113 (3.0 mg/kg), a selective dopamine transporter inhibitor, for 7 days. Fifteen days after their final injection, rats were trained on a reward-omission task with an operant component to earn further rewards.

Results: Previous exposure to cocaine resulted in dose-dependent increases in negative urgency in separate behavioral variables across days of testing. The lower dose range increased negative urgency on the dimension of decreased reaction time to press a lever, while the higher dose range increased the rate of increase in lever presses made per trial. Rats receiving RTI-113 did not resemble either cocaine group and instead showed a decrease in lever pressing across days.

Conclusions: Our results indicate that previous binge cocaine consumption enhances behavioral markers of negative urgency in a dose-dependent, time-sensitive manner on discrete behavioral dimensions. The results with RTI-113 suggest the relationship between cocaine exposure and negative urgency is unlikely to be explained solely by inhibition of dopamine reuptake.

1. Introduction

Personality variables have long been associated with individual risk of substance abuse (Whiteside and Lynam, 2003). The trait of impulsiveness, once thought to be a well-defined construct, can be sub-factorized into the traits of disinhibition, sensation seeking, and urgency (Whiteside and Lynam, 2001). Each of these sub-factors interacts differentially with drug-consumption tendencies, with urgency showing the strongest relationship to developing problematic substance use (Bardo et al., 2007; Ersche et al., 2012; Gunn et al., 2013). An individual high in urgency is characterized as one who scores low in the personality trait of conscientiousness, high in impulsivity, and high in disagreeableness (Bardo et al., 2007; Ersche et al., 2012; Gunn et al., 2013). Urgency is distinct from general impulsivity by the inclusion of an emotional response component that goes beyond lack of behavioral inhibition or forethought (Settles et al., 2012); individuals with high degrees of urgency tend to behave impulsively when in emotionally elevated states, but are not otherwise volatile. Negative urgency is the tendency to make poor choices when in an emotionally distressed state, such as drinking to ameliorate stress (Settles et al., 2012; Whiteside and Lynam, 2001). This form of urgency is particularly interesting because it serves as a predictor of problem drinking and smoking in children as young as fifth grade (Settles et al., 2012), and is associated with cocaine dependence (Albein-Urrios et al., 2012; Verdejo-Garcia et al., 2008; Whiteside and Lynam, 2003). The neural mechanisms underlying negative urgency are purported to involve an imbalance of functional connectivity between cortical, striatal, and limbic areas (Cyders and Smith, 2008; Gipson et al., 2012; Judice-Daher and Bueno, 2013; Judice-Daher et al., 2012; Tavares et al., 2014). Repeated cocaine use impairs global functioning of frontal cortex, which manifests as hypofrontality, but also causes damage locally in orbito-frontal cortex, resulting in impairments in judgment and cognitive flexibility, as well as increased impulsivity.

References

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Considering the association of human cocaine abuse with negative urgency, we adopted a dosing schedule to mimic the binge consumption and dosage escalation common to human addicts (Ahmed and Koob, 1998; Ahmed et al., 2002, 2003). Each day for seven consecutive days of cocaine administration, the dosage was increased in a linear fashion so that the dose on the final day was twice the dose of the initial day. A similar dosage escalation is also seen in rats self-administering cocaine, and demonstrates the maintenance of a particular desired subjective state that requires an increasing amount of drug (Ahmed and Koob, 1998; Ahmed et al., 2002, 2003; Fischer et al., 2013; Knackstedt and Kalivas, 2007). After withdrawal, craving for the drug increases (Conrad et al., 2008; Grimm et al., 2001), marked by enhanced drug-seeking behavior, but also manifests at the cellular level as structural and functional changes largely in the pre-frontal cortex (PFC) and nucleus accumbens (NAcc) (Conrad et al., 2008; Fischer et al., 2013; Grimm et al., 2001; Sun and Rebec, 2006). The role of these mechanisms in re-establishing drug-seeking behavior and a subsequent loss of cognitive flexibility is well established. Here, we assessed the effects of binge cocaine withdrawal on negative urgency as manifested in a reward-omission task. Because cocaine inhibits dopamine reuptake, we also examined the effects of withdrawal from RTI-113, a highly-selective dopamine transporter (DAT) inhibitor (Carroll et al., 2004; Kuhar et al., 1999).

2. Methods

2.1. Subjects

Adult, male, Sprague-Dawley rats, weighing approximately 300 g provided by Harlan Industries (Indianapolis, IN), were single-housed in clear plastic cages. Water was provided ad libitum throughout the course of study. During periods of training and testing, food intake was restricted to maintain 85% of free-feeding weight. Prior to training, during drug administration days and during the drug withdrawal portion of the experiment, food and water were available ad libitum. The rats were housed on a 12:12 light cycle, with all testing and drug administration conducted during the lights-on portion of the day. All housing and animal-use procedures followed NIH guidelines and were approved by the Indiana University Institutional Animal Care and Use Committee.

2.2. Drugs

The rats were divided into four treatment groups for injections, which occurred once daily for seven consecutive days: Saline, Cocaine (10–20 mg/kg), Cocaine (20–40 mg/kg), and because cocaine binds rather indiscriminately to monoamine transporters, we also assessed the behavioral effects produced by 2β-carbophenoxyl-3β-(4-chlorophenyl) tropane (RTI-113), an ultra-selective dopamine transporter inhibitor with similar behavioral effects to cocaine to help identify the relative contribution of dopamine uptake inhibition (Carroll et al., 2004; Kuhar et al., 1999). Rats in the RTI-113 group received 3.0 mg/kg, which is based on DAT binding affinities such that an equal saturation of DAT would occur with RTI-113 as in the Cocaine (20–40 mg/kg) group (Dworkin et al., 1998). USP grade cocaine HCl and RTI-113 provided by Research Triangle Institute, were dissolved in USP saline at a concentration that maintained a constant injection volume of 1.0 mL/kg. To simulate the escalating dosage schedule observed in human cocaine dependence, cocaine doses escalated each day in a linear fashion such that on the final day of injections the dose had doubled. Due to lack of safety data on high doses of RTI-113, this escalation of dose was limited to the two cocaine groups (see below for further discussion). Injections (ip) were administered once daily for seven days. After injection, subjects were returned to their home-cage for monitoring before returning to the colony room. Following the final day of injections, subjects remained in their home cages in the colony room with food and water available ad libitum for 15 days.

2.3. Apparatus

For training and data collection operant chambers were used. They were operated by a Med-PC SC-6510D controller using MED-PC IV (ver. 4.0.1.47) software from Med Associates, Inc. (St. Albans, VT). The boxes measure 31.75 cm W × 31.75 cm D × 41.9 cm H, and are equipped with a central food hopper with a lever and cue-light positioned to either side. Opposite the food-hopper is a house light and an embedded speaker. The food-hopper is attached to an external pellet dispenser that dispenses 48 mg TestDiet Sucrose Pellets (Richmond, Indiana).

2.4. Reward omission training

After their final day of injections, rats remained in their home cages for a 15-day withdrawal period before they began training on a reward omission test. Training consisted of three phases prior to data collection.

2.4.1. Phase I. Subjects are conditioned to anticipate a free-food pellet delivered immediately following the presentation of a cue light and tone. The session begins with illumination of the house light for 60 s. When the house light turns off, one of the two cue lights is illuminated on a randomly determined counterbalance for each light. The light is illuminated for 5 s, and is accompanied by a 400 Hz, 50 dB, 0.5 s tone along with the release of a single sucrose pellet into the food hopper. A 2 s dark period ensues followed by

![Fig. 1.](image-url)
illumination of the house light for a semi-random 45, 60, 75, or 90 s signaling the inter-trial period. Phase I consists of 32 trials and occurs once daily for 3 days. During Phase I training, animals are observed to ensure conditioning has taken place as indicated by hopper approach on presentation of cue.

2.4.2. Phase II. Rats are trained to press a lever for food pellets on an FR10 schedule. Rats begin by autoshaping overnight in the operant chamber with an available lever that dispenses a single 48 mg sucrose pellet reward on an FR1 schedule of reinforcement with 100 rewards possible. Each subsequent training session is conducted during daylight hours. After earning all 100 available awards within 3 h at the FR1 lever, the rats progress to FR3 the next day, followed by FR5, and finally FR10. Animals continue to press at FR10 each day until 3 consecutive days of consistent performance is observed, at which time Phase II is complete.

2.4.3. Phase III. The two previous phases are combined into a single session. Rats are placed into the operant box and a 60 s pre-session begins with the house light illuminated. The house light shuts off, and one of the two cue lights switch on in a counter-balanced manner for 5 s. At the offset of the cue light, a 500 ms tone is emitted along with delivery of a pellet. This is followed by a 2 s dark period, at the end of which the right-hand lever is presented. The lever is present for 45 s, during which time the rat can earn as many food pellets as possible on an FR10 schedule. After 45 s, the levers retracted and the house light is illuminated for a semi-random 45, 60, 75, or 90 s inter-trial interval. Each session is composed of 32 trials, with one session per day for 3 consecutive days.

2.4.4. Phase IV. Reward omission is introduced. Phase IV is identical to Phase III with the exception that on a random 33% of the trials the free-food pellet that follows the cue lights + tone is omitted. Data are collected on the following parameters: latency to lever-press after cue presentation, rate of lever pressing (presses/s), and total number of lever presses. Phase IV data are collected for 4 days with 3 days of Phase III inserted between each day of Phase IV to decrease the frequency of omission sessions overall and maintain their “surprising” nature.

2.5. Data analysis

Trials with no response were excluded from analysis. The data were then analyzed in SPSS22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) using a generalized linear model of fixed effects. Such an analysis allows subject to be accounted for as a random variable and day treated as a numerical rather than ordinal variable to allow for slope rather than means comparison across days.

3. Results

Group sizes after training and data collection were as follows: Saline (n = 16), Cocaine (10–20 mg/kg) (n = 10), Cocaine (20–40 mg/kg) (n = 10), and RTI-113 (3.0 mg/kg) (n = 16). Responses on the inactive lever were recorded and constituted less than 1% of responding for all rats. Trials in which no response occurred were less than 1% of all trials.

3.1. Omission versus reward

To confirm the presence of negative urgency, we monitored Phase IV behavior on omission trials compared to rewarded trials across days for all groups. To fit a normal distribution, latency and rate data underwent a log transformation. The lever press data similarly underwent a square root transformation for it to fit a normal distribution.

Our fixed-effects model revealed a trial-type-by-day interaction in latency to lever press. (F(6,306.40) = 24.87, p < 0.05). On rewarded trials, latency was consistently higher than on omission trials. Additionally, omission trials displayed a consistent decline in reaction time across days while rewarded trials remained relatively constant. These data are summarized in Fig. 1a. Our lever-pressing data (number of lever presses and rate of lever pressing) did not show a trial-type-by-day interaction, and are presented in Fig. 1b and c. Collectively, these data demonstrate latency to lever press as a reliable marker of negative urgency.

3.2. Group analysis

Next we examined pairwise comparisons. Consistent with our expectations, pairwise comparisons collapsed across days revealed that all groups displayed shorter reaction times on omission trials compared to reward trials. Lever-pressing data displayed a trial-type difference for the RTI-113 condition, but the others remained relatively constant. Pairwise comparison data are presented in Table 1.

3.3. Group-by-day analysis

A slope analysis based on a fixed-effects linear model was used to analyze differences between groups. This allowed day to be a categorical variable, which fits a slope to the linear trajectory of adaptation to the test across days.

Analysis of reward trials during Phase IV testing indicated nearly flat slopes for latency across days in all groups. Slopes for lever presses on reward trials also failed to show a cocaine effect. In omission trials analyzed for latency, we detected a condition-by-day interaction (F(1793.28) = 2.89, p < 0.05) that shows how each of the groups adapt to the omission trials across days of Phase IV testing. Post hoc analysis revealed that the cocaine (10–20 mg/kg) group had a significantly different slope (m = −0.09) than saline (m = −0.05; t(1808.83) = −2.95, p < 0.05) and RTI-113 (m = −0.05, t(6316.49) = −4.19, p < 0.05). As shown in Fig. 2a, these data demonstrate a time-based behavioral shift toward greater negative urgency in the low-dose binge cocaine group. Interestingly, the highest dose binge cocaine (20–40 mg/kg) group (m = −0.07) showed a trend toward greater negative urgency with

<p>| Table 1 | Mean (±SEM) rate of lever pressing, latency for reaction time on the lever after presentation, and mean (±SEM) number of presses. Data are cumulative across four days of collection. Analysis was within groups comparisons of reward to omission trials. |</p>
<table>
<thead>
<tr>
<th>Rate (±SEM)</th>
<th>Reaction time (s ± SEM)</th>
<th>Lever presses (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.04(±0.02)</td>
<td>1.03(±0.01)</td>
</tr>
<tr>
<td>Cocaine (10–20 mg/kg)</td>
<td>1.03(±0.02)</td>
<td>1.03(±0.01)</td>
</tr>
<tr>
<td>Cocaine (20–40 mg/kg)</td>
<td>1.05(±0.02)</td>
<td>1.05(±0.01)</td>
</tr>
<tr>
<td>RTI-113</td>
<td>1.11(±0.02)</td>
<td>1.12(±0.01)</td>
</tr>
</tbody>
</table>

*p < 0.05.
Fig. 2. (A) Mean (±SEM) latency for reaction time on the lever during omission trials across days. Trends across days are depicted as a slope analysis using a fixed-effects linear model with day as a numerical variable. The cocaine (10–20 mg/kg) group compared to all others has a more negative slope (t(1800.83) = 2.95, p < 0.05). This suggests an increase in negative urgency in the low-dose cocaine groups compared to the others on the variable of latency. (B) Mean (±SEM) number of lever presses during omission trials across days. Trends across days are depicted as a slope analysis using a fixed-effects linear model with day as a numerical variable. The cocaine (20–40 mg/kg) group displays a steeper slope compared to the other groups (t(1801.10) = 2.51, p < 0.05), suggesting a growth in negative urgency on the variable of lever pressing. The RTI-113 group, however, shows an opposite effect with a shallower slope than the others, suggesting a reduction in negative urgency. (C) Mean (±SEM) rate of lever pressing (LP) during omission trials across days. The cocaine (10–20 mg/kg) group has a more negative slope than saline; (t(1801.04) = −2.04).

4. Discussion

Our results indicate that an increase in negative urgency following withdrawal from binge cocaine is dependent on adaptation across multiple days of testing. Thus, although the groups appear similar when days are pooled together, a clear emergence of an omission effect is manifested across days in Fig. 1a. When each group is represented across all 4 days of Phase IV testing, differences appear in the rate of adaptation to the testing. Interestingly, each cocaine group differed on either lever pressing or latency to press the lever. Low-dose binge cocaine resulted in a greater rate of decrease in reaction time on the lever as days progressed, while all other groups were unaffected. Similarly, high-dose binge cocaine increased the rate of increase in number of lever presses as days progressed. These findings demonstrate an increase in negative urgency in both cocaine groups by the escalation of behavior prompted by surprise omission of an expected reward (Amsel and Ward, 1965; Dudley and Papini, 1995). The cocaine (10–20 mg/kg) group increased responding with a faster reduction in reaction time, while the cocaine (20–40 mg/kg) group increased the number of presses per trial more quickly. The RTI-113 group, which we expected to resemble either one or both of the cocaine groups because of DAT inhibition, actually showed opposite effects on lever pressing than both cocaine groups.

Our extended period of withdrawal ensured that no drug was on board during behavioral testing. Additionally, analysis of inactive lever presses during Phases III–IV showed the rats unambiguously chose the active lever throughout, arguing against a cocaine-induced generalized increase in behavioral activation.

Other than negative urgency, one possible interpretation of the findings for the low-binge cocaine (10–20 mg/kg) group could relate to adaptive learning processes rather than frustration. The decrease in reaction times was present in all groups, but disproportionately so in the cocaine (10–20 mg/kg) group perhaps because the rats were less tolerant for delay once the lever was presented, but they could also be adaptively moving toward the lever once they detected no reward was delivered. If that is the case, then a shorter delay on the lever is beneficial and would suggest that the low-dose range of binge cocaine promotes reward-related learning. The lack of similar effects with the high-dose range may be due to the neurotoxic effects of high doses of cocaine, which fall into our 20–40 mg/kg dosage range (Stalnaker et al., 2007a).

This interpretation is consistent with the ability of cocaine to enhance activation of dorsal striatum (White et al., 1998), which mediates stimulus-response learning (Takahashi et al., 2007), and induce neurotoxicity to the orbitofrontal cortex which impairs associative learning (Stalnaker et al., 2007a). Thus, our dose range of 10–20 mg/kg may have been sufficient to generate greater plasticity in associative structures while still low enough to not cause functional damage in frontal cortex. Interpreting the data within the context of a learning paradigm could also help account for the day effect that we observed such that the curve across days could be viewed as learning the associations of reward versus no-reward in an attempt to minimize delay between rewards. This may explain well why the cocaine (10–20 mg/kg) group was the only group showing differences in rate of pressing across days. The change in rate for this group was actually a decrease, which is inconsistent with an explanation of negative urgency, but would still fit this explanation if these rats are learning to press more quickly but do not earn a larger number of rewards, which in fact is what we observed.

The decrease in lever pressing in the RTI-113 group is suggestive of a decrease in negative urgency, but could also be interpreted as an increase in reward sensitivity, which would be consistent with low responding on both reward and omission trials. This effect is interesting because RTI-113 was chosen as an active psychostimulant that isolates the relative contribution of DAT alone on behavior with a chosen dose that simulates the degree of DAT occupancy seen with the cocaine (20–40 mg/kg) dose range. If DAT inhibition...
is a primary mechanism for our results, we would expect the RTI-113 and cocaine (20–40 mg/kg) to appear similar. That they did not suggests that DAT is not a primary or sole mediator of the ability of cocaine withdrawal to enhance negative urgency. Another possibility relates to pharmacodynamic differences between cocaine and RTI-113. Cocaine has a relatively quick rise and fall in plasma concentration with subsequent brevity of reinforcing effects, and greater number of self-administrations (Kimmel et al., 2008). RTI-113, on the other hand, has a rather slow rise and fall of plasma concentration with psychostimulant effects lasting over 12 h. In fact, this is part of its attraction for use as an agonist-substitute therapy for cocaine dependence (Kimmel et al., 2008). The extensive half-life of RTI-113 precluded an escalating dosage-schedule in our study. The dosage used was calculated to have equivalent DAT occupancy as the cocaine (20–40 mg/kg) group, which placed our dosage at the higher end of known safety data. With such a lengthy half-life coupled with daily injections, there is a real possibility of a growing accumulation of the drug, producing a de facto escalating dosage schedule. Additionally, we decided not to include a non-escalating schedule of cocaine because it would provide a rather poor comparison to the effects of RTI-113 due to the rapid metabolism and clearance of cocaine and rapid development of tolerance.

Although we confirmed Pavlovian conditioning in Phase I training by visual observation of approach to the food hopper on cue presentation, our methodology was not quantified and is thus susceptible to subjectivity. Failure to condition would preclude a reward omission effect on subsequent testing. Our evidence of a latency effect on reward omission trials argues against this outcome, but quantification of cue-induced food hopper entries, as in calculation of an elevation score (Bouton and Sainsbury, 2003), would provide definitive support for successful Phase I training.

In summary, our results demonstrate the ability of withdrawal from binge cocaine to increase negative urgency in a dose-dependent manner on discrete behavioral variables in a reward omission task. Our low-dose range of binge cocaine resulted in a greater rate of decline in reaction time on the lever across days, while the high-dose range produced a greater rate of increase in lever pressing across days. The RTI-113 group in contrast had lower levels of lever pressing compared to all other groups, suggesting that DAT inhibition is not a primary mechanism mediating the changes seen with binge cocaine.

Conflict of interest
No conflict declared.

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Contributors
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G.V. Rebec: Experimental design, data analysis, author.
Both authors have read and approved of this submission.

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References


