How cocaine impairs flexible behavior:
A neuroscience perspective

By
Heather Ortega

Senior Honors Thesis
Psychology and Neuroscience
University of North Carolina at Chapel Hill

May 2018

Approved:

__________________
Dr. Regina Carelli, Thesis Advisor

Dr. Kathryn Reissner, Reader

Dr. Elizabeth West, Reader
Abstract

The inability to change behavior represents a loss of behavior flexibility. One way of testing this behavioral shift is using an animal model of reinforcer devaluation. A history of cocaine use makes rats unable to modify their behavior following the devaluation of the reward after several weeks of abstinence (i.e., behavior is inflexible or more “habitual”). This demonstrates long-term impairment in behavioral flexibility in cocaine-treated rats compared to control rats. Importantly, this is not due to a deficit in the ability of the rats to respond to the original conditioned stimulus to receive the reward. Rather, it impaired the ability of the rats to integrate the conditioned response with the new value of the reward impairing their ability to flexibly change their behavior post-devaluation (i.e., rats are responding using an inflexible, habitual strategy). One brain region that is critical for flexible behavior in this task is the prelimbic cortex (PrL). Therefore, we hypothesized that cocaine induced impairments may be due to alterations in the PrL. Here, we used in vivo electrophysiological methods to record from neurons in the prelimbic cortex to determine how a history of cocaine changes neural firing while animals are learning cue-outcome associations (Pavlovian conditioning) and during flexible behavior as measured by a devaluation task in both males and females. Since females self-administer more cocaine than males, this was a timely investigation to determine if a history of cocaine differently affects behavioral flexibility across sexes. We confirm previous work that females self-administer more cocaine than males, however, we found no differences in measures of behavioral flexibility between sexes.
Acknowledgements

I would like to thank the following people,

Dr. Regina Carelli, for her support and guidance over the past two years. She welcomed me into her lab when I had little research experience and no knowledge about working with animals. I worked with her as part of a research-intensive course, then as a McNair Scholar, and finally as an honors thesis student. She also provided wonderful resources for me to grow as a scientist. Without her I would not be a scholar ready for graduate school.

Dr. Elizabeth West, for her advice and mentorship. She taught me about animal research, scientific writing, presentation skills, and statistics. Liz was always available when I needed help and facilitated my transition from a research assistant to a researcher.

The Carelli Lab, for their welcoming atmosphere and constant help. I have only spent two short years with them but I have learned more about science and myself than all of the previous years combined.

My friends and family, for their unconditional love and support. I would not have made it through my four years as an undergraduate without them.

The Ronald. E. McNair Scholars Program, for the guidance and community they provided. Within the McNair program, I found a supportive family that drove me to become the scholar I am today.

This project was supported by a David Bray Peele Memorial Research Award from the Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill.
Introduction

Incessant drug-seeking behavior despite negative consequences (e.g., loss of job, family, etc.) characterizes drug addiction. During the early stages of substance use disorder, addicts seek drugs using goal-directed behavior (i.e., the individual takes the drugs in order to feel good). Once substance use becomes compulsive it is increasingly difficult to stop taking the drug because the behavior has transitioned to habitual. Habitual behavior is characterized as being routine, automatic, and not subject to executive control. It is also subject to much less top-down neural processing than goal-directed and, as such, it is more difficult to change. The inability to change habitual behavior represents a loss of behavioral flexibility. Behavioral flexibility is a vital skill that allows animals to shift behavior in response to the ever-changing environment. Following extended cocaine use, there is a shift in behavior from goal-directed to habitual which most likely results from alterations to the learning circuits in the brain (Everitt & Wolf, 2002).

One way of testing this shift from goal-directed to habitual behavior is using an animal model of reinforcer devaluation. Animals learn to associate specific cues with a reward, such as a flashing light with food pellets. When the reward is devalued through conditioned taste aversion (e.g., pairing food pellets with lithium chloride to induce illness), the animal can then be tested for behavioral flexibility. Animals that exhibit flexible behavior are able to integrate the devalued reward with the previously learned conditioned cue or action and appropriately avoid it (i.e., not going to the food cup during the cue, under extinction to avoid re-learning). Behavior is considered goal-directed, or flexible, if animals spend less time in the food cup during the cue following devaluation. Behavior is classified as habitual, or inflexible, if animals continue responding to the cue (going to the food cup) despite the devaluation of the food reward (i.e., they cannot flexibly change behavior post-devaluation).
A history of cocaine use makes rats unable to modify their behavior based on the devaluation of the reward. In other words, the behavior is inflexible and habitual (Schoenbaum & Setlow, 2004). This demonstrates long-term impairment in behavioral flexibility in cocaine-treated rats compared to control rats as all testing takes place after several weeks of abstinence. This was not due to a deficit in the ability of the rats to learn the original conditioned response to receive the reward, as history of cocaine did not impair the ability of the rats to preferentially approach the reward predictive cue. Instead, it impaired the ability of the rats to integrate the conditioned response with the new value of the reward, impairing their ability to flexibly change their behavior post-devaluation.

Psychostimulants, such as cocaine, only show this effect when administered before learning, suggesting that the drugs change how the association is encoded (Schoenbaum & Setlow, 2004; Nelson & Killcross, 2006). If rats receive psychostimulants after learning, they show normal behavioral flexibility by using associations encoded before cocaine use (Nelson & Killcross, 2006). One brain region implicated in the ability to shift behavior is the prelimbic cortex (PrL). If the PrL is lesioned or inactivated before learning, rats are impaired in flexible behavior (Ostlund & Balleine, 2005, Tran-Tu-Yen et al., 2009). However, if the PrL is lesioned or inactivated after learning, then the rat’s behavior is unimpaired (Ostlund & Balleine, 2005, Tran-Tu-Yen et al., 2009). This is similar to the effects of psychostimulants on flexible behavior. Thus, we hypothesize that cocaine induced impairments may be due to alterations in the PrL. We will use electrophysiology methods to record from neurons in the PrL to see how a history of cocaine changes neural firing while animals are learning cue-outcome associations (Pavlovian conditioning) and during flexible behavior as measured by a devaluation task.
While both sexes readily self-administer cocaine (Becker, 2016), learn reward-associated cues (Ostlund & Balleine, 2005, Tran-Tu-Yen et al., 2009), and successfully devalue rewards (Sengstake, 1991); females self-administer higher doses of cocaine (Becker, 2016, Sanchis-Segura & Becker, 2016), learn faster and respond more strongly in reward-related tasks (Dalla & Shors, 2009), and return to baseline behavior following conditioned taste aversion induced-devaluation more rapidly (Sengstake, 1991). Because of these known differences, it is important to consider potential sex differences in behavioral flexibility.

**Methods**

**Subjects**

Long-Evans rats (Charles River), 90-120 days old were used. The males (n=28) and females (n=17) weighed 300-350 g and 200-250 g at start, respectively. They were individually housed in reverse 12-hour light/dark cycle with lights off at 7:00 am. After arrival, they were given *ad libitum* food and water until behavioral training began (1 week after surgical procedures). During the self-administration sessions, they were mildly water restricted (30 ml/day for males and 25 ml/day for females) throughout the 2-week duration. Following self-administration, they were placed into their home cages for 3 weeks where food and water were available *ad libitum*. During Pavlovian training, devaluation, and post-devaluation tests, they were fed 20-25 g standard rat chow (Purina RMH3000) per day for males, 15-20 mg per day for females and kept at no less than 90% preoperative body weight. All procedures are approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.
Procedure

**Surgery.** The rats were anesthetized with a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) cocktail intramuscularly. Eight microwire electrodes (50 micrometer diameter) were placed into the prelimbic cortex (AP: +2.6, ML: +/- 0.6, DV: -4.0 from skull) and a catheter was placed into the jugular vein as previously described (West et al., 2014).

**Self-administration.** After 7 days of recovery from surgery, behavioral training began in standard operant chambers (Med Associates, Inc). Rats were mildly water deprived (20-30 ml/day) and then trained to nosepoke to self-administer cocaine (0.33 mg/press) or saline during 2 hour daily sessions for 14 consecutive days as previously described (Saddoris & Carelli, 2014). Following each nosepoke, a tone–houselight conditioned stimulus turned on for 30 s (additional nosepokes were unrewarded during this period). Water was paired with saline-infusions to ensure similar operant nosepoke experience in the control group.

**Pavlovian Training.** Pavlovian conditioning occurred in chambers distinct from the self-administration chambers after 3 weeks of abstinence. Rats underwent magazine training for two days in which they received a pellet every 45 seconds (variable interval-45) including 20 trials of food pellets and 20 trials of sugar pellets each day. They received food (Purina lab diet) and sucrose (Purina lab diet) pellets for 10 days throughout daily conditioning sessions. During conditioning sessions, two conditioned stimuli (CS+) were presented (one at a time) for 10 seconds followed by a distinct counter-balanced reward (food or sucrose). Two conditioned stimuli (CS-) were not predictive of reward. Presentation of stimuli lasted 10 seconds and the order was pseudorandomized between solid CS+, flashing CS+, solid CS-, flashing CS-. Inter-trial intervals were pseudorandomized between 75, 90, 105, and 120 seconds and varied each day. Three sessions contained the same order of cue presentations: day 1, day 10, and test day.
Devaluation Procedure (LiCl). During the last 2 days of Pavlovian training, rats were habituated in a standard cage. Following habituation, rats were allowed to consume sucrose pellets ad libitum for 30 minutes followed by an injection of lithium chloride (LiCl, 0.3 M, 10 ml/kg). On the following day, they were allowed to consume food pellets followed by an injection with saline (0.9% solution, 10 ml/kg). Forty-eight hours later (including a rest day), the 2-day procedure was repeated.

Post-Devaluation Testing. At least 48 hours after completion of the devaluation procedure, rats experienced the same Pavlovian paradigm as during training but under extinction (i.e. no rewards were given).

Devaluation Food Choice Test. In the same cage and using the same food/sugar as during the devaluation procedure, each rat was allowed to freely consume sugar and food pellets for 30 minutes. The amount of food and sugar pellets consumed was recorded.

Measures

Electrophysiological Recordings. Before the start of each session, the subject was connected to a flexible recording cable attached to a commutator (Med Associates, Inc), which allowed virtually unrestrained movement within the chamber. Online isolation and discrimination of neuronal activity was accomplished using a commercially available neurophysiological system (OmniPlex system, Plexon). Continuous recordings from each electrode were virtually referenced (PlexControl, Plexon) and fed into a Pentium computer. Continuous signals were high-pass filtered (300Hz) to identify individual spike events. Discrimination of individual waveforms began by setting a threshold level (3.5 above background noise) for each wire. Individual waveforms corresponding to a single cell were discriminated using template analysis procedures and time-
voltage boxes provided by the neurophysiological software system. Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon, Inc). This allowed neuronal data to be further assessed based on the principle component analysis of the waveforms, cell firing characteristics such as autocorrelograms and interspike interval distribution. It also ensured that putative cells showed biologically appropriate firing refractory periods, and cross-correlograms to ensure that multiple cells recorded on the same wires showed firing independently of each other. Finally, an additional computer processed operant chamber input and output (Med Associates, Inc) and sent digital outputs corresponding to each event into system to be time stamped along with the neural data.

**Data Analysis**

**Behavioral**

Sex-differences in cocaine consumption were analyzed by a t-test comparing average cocaine consumption over the 14 days of self-administration. Percent of time spent in food cup for each stimulus (solid CS+, flashing CS+, solid CS-, flashing CS-) was calculated through video recordings in day 1, day 10, and test day following devaluation. A two-way ANOVA was done to compare percent of time spent in food cup for Day (1 and 10) and stimulus (solid CS+, flashing CS+, solid CS-, flashing CS-). Sex-differences in learning on day 10 were examined with three-way ANOVA with sex (male vs female), cue type (CS+, CS+2, CS-, CS-2), and group (cocaine vs control) as factors to compare the amount of time spent in the food cup for each cue type. On the devaluation test day, we calculated a devaluation index (DI) as previously described (West et al., 2012, West & Carelli, 2016). The DI is calculated as the amount of time spent in the food cup after
predictive cues for the nondevalued (food) and devalued (sugar) rewards. 

\[ DI = \frac{ND - D}{ND + D} \]

This allowed us to compare the degree and direction of each rat’s response to the cues (CS+1 and CS+2). A devaluation index of 1 indicates that all of the time spent in the food cup were under the NonDevalued condition (flexible behavior), 0 represents equal amount of time in the food cup to the CS+ that is paired with the NonDevalued reward (i.e., food) and CS+ paired with the Devalued reward (i.e., sugar). Devaluation indices were analyzed with a two-way ANOVA comparing devaluation indices with factors (cocaine vs control) and sex (male vs female). Devaluation indices were also analyzed for each group (male-control, male-cocaine, female-control, female-cocaine) using a one-sample t-test (West et al., 2012) in order to determine if the devaluation index for each group was different from the theoretical value of 0 (i.e., numerical representation of equal responding to both cues). Thus, scores that are significantly higher than zero indicated that the group showed a devaluation effect. To confirm successful devaluation, a two-way ANOVA was used to compare amount of food consumed through pairing (first or second) and food choice (food or sucrose). Behavioral data analyses were performed using GraphPad Prism (Graphpad Software, Inc. La Jolla, CA) or SPSS.

**Electrophysiology**

Differences were calculated in the proportion of phasic neuronal responses to the cue (i.e., excitation vs. inhibition) on the last day of Pavlovian conditioning to compare the control group and cocaine group using a Fisher’s exact test. Neurons are phasic if they respond with an increase (excitation) or decrease (inhibition) from basal firing during cue presentation.
Histology

Upon completion of the experiment, rats were deeply anesthetized with an intraperitoneal injection of a ketamine and xylazine mixture (100 and 10 mg/kg, respectively). A 15-μA current was passed through each microwire electrode for 5 seconds to mark the placement of electrode tips. Transcardial perfusions were then performed using physiological saline and 3% potassium ferricyanide in 10% formalin, and brains were removed. After post-fixing and freezing, 40-μm coronal brain sections were mounted. The addition of potassium ferricyanide allowed for a blue reaction corresponding to the location of the electrode tip which was viewed under a 1X microscope lens. Placement of an electrode tip within the PrL was determined by examining the relative position of observable reaction product to visual landmarks and anatomical organization of the NAc and PrL represented in a stereotaxic atlas.

Results

Self-Administration

As shown in Figure 1, female rats (n=7) consumed significantly more cocaine for their weight (mg/kg) than males (n=16). Specifically, females consumed more cocaine (mg/kg) (M=27.39, +/- 1.8) over the 14 days of self-administration (averaged) compared to males (M=20.41, +/- 1.151) as shown by a student’s unpaired t-test (t=3.271, p<0.05).
Pavlovian Conditioning

Controls

After 10 days of conditioning, controls rats (female n=7, male n=9) spent more time in the food cup for cues that predicted rewards (CS+1 and CS+2) than cues that did not predict rewards (CS-1 and CS-2). CS+1 predicts the food reward while CS+2 predicts the sugar reward. Two-way repeated measures ANOVAs with day (Day 1 vs Day 10) and cue presentation (CS+1, CS+2, CS-1, CS-2) as factors were performed for each sex (male and female, see details below). Comparing Day 1 to Day 10, the control rats (both male and female) responded preferentially to the different types of cues (CS+ v. CS-) on Day 10 (Figure 2, A and C).

Male Rats. Analysis of the male control group revealed a significant main effect of day (F_{1,8}=33.83, p<0.05), a significant main effect of cue presentation (F_{3,24} = 16.77, p<0.05), and a significant interaction (F_{3,24}=16.47, p<0.05). Post-hoc analysis revealed no difference in the percent of time spent in the food cup to any of the cues before learning on Day 1 (p >0.10). After learning (on Day 10), post-hoc analysis of male-control rats showed that they spent significantly more time in the food cup during CS+1 compared to both CS-1 (t=6.376, p<0.05) and CS-2 (t=5.971, p<0.05) and more time in CS+2 compared to both CS-1 (t=7.884, p<0.05) and CS-2 (t=7.478, p<0.05). There was no significant difference between CS+1 and CS+2 (t=0.1028, p>0.1) and for CS-1 and CS-2 (t=0.4058, p>0.1). Analysis shows that the male control group preferentially responds to reward predictive cues on day 10.

Female Rats. Analysis of the female control group revealed a significant main effect of day (F_{1,6}=89.57, p<0.01), a significant main effect of cue presentation (F_{3,18} = 3.562, p<0.05), and a trend for interactions (F_{3,18}=2.753, p=0.0726). For both control groups (male and female), post-
hoc analysis revealed no difference in the percent of time spent in the food cup to any of the cues before learning on Day 1 (p >0.10). After learning (on Day 10), post-hoc analysis of female-control rats showed that they spent significantly more time in the food cup during CS+1 compared to both CS-1 (t=3.46, p<0.05) and CS-2 (t=3.101, p<0.05) and more time in CS+2 compared to CS-1 (t=3.049, p<0.05). Females showed a trend towards differentiating between CS+2 and CS-2 (t=2.69, p=0.0899). There was no significant difference between CS+1 and CS+2 (t=0.4111, p>0.1) and for CS-1 and CS-2 (t=0.3597, p>0.1). Analysis shows that the female control group preferentially responds to reward predictive cues on day 10.

**Cocaine**

After 10 days of conditioning, cocaine (female n=7, male n=6) exposed rats spent more time in the food cup for cues that predicted rewards (CS+1 and CS+2) than cues that did not predict any rewards (CS-1 and CS-2). Two-way repeated measures ANOVAs with day (Day 1 vs Day 10) and cue presentation (CS+1, CS+2, CS-1, CS-2) as factors were performed for each sex (male and female, see details below). For cocaine-exposed rats (male and female), post-hoc analysis revealed no difference in the percent of time spent in the food cup to any of the cues before learning on Day 1 (p >0.10). The rats (both male and female) responded preferentially to the different types of cues (CS+ and CS-) by spending more time in the food cup when cues are predictive of a reward (both sugar and food) (Figure 2, B and D).

**Male Rats.** Analysis of the male cocaine group revealed a significant main effect of day (F₁,₅=8.387, p<0.05), a significant main effect of cue presentation (F₃,₁₅ = 14.55, p<0.0001) and a significant interaction (F₃,₁₂=12.02, p<0.05). After learning (on Day 10), post-hoc analysis of cocaine-exposed male rats showed that they spent significantly more time in the food cup during CS+1 compared to both CS-1 (t=8.006, p<0.0001) and CS-2 (t=6.294, p<0.0001) and more time
in CS+2 compared to both CS-1 (t=6.948, p<0.0001) and CS-2 (t=5.236, p<0.01). There was no significant difference between CS+1 and CS+2 (t=1.057 p>0.1) or CS-1 and CS-2 (t=1.712, p>0.1). Analysis shows that the male cocaine group preferentially responds to reward predictive cues on day 10.

**Female Rats.** Analysis of the female cocaine group revealed a significant main effect of day (F\(_{1,6} = 39.44, p<0.05\)), a significant main effect of cue presentation (F\(_{3,18} = 8.299, p<0.05\)) and a significant interaction effect (F\(_{3,18} = 4.542, p<0.05\)). After learning (on Day 10), post-hoc analysis of cocaine-exposed female rats showed that rats spent significantly more time in the food cup during CS+1 compared to both CS-1 (t=5.1, p<0.05) and CS-2 (t=3.14, p<0.05) and more time in CS+2 compared to the CS-1 (t=4.62, p<0.05). There was a trend towards differentiating between CS+2 and CS-2 in the females (t=2.66, p=0.0957). The trend shows a preference for CS+2 over CS-2. There was no significant difference between CS+1 and CS+2 (t=0.48, p>0.1) or CS-1 and CS-2 (t=1.96, p>0.1). Analysis shows that the female cocaine group preferentially responds to reward predictive cues on day 10.

**Comparing Learning Across Groups**

No sex (male and female) or group (cocaine and control) differences were observed on Day 10 of learning, but there were differences observed across cue types. For Day 10, a three-way repeated measures ANOVA with cue presentation (CS+1, CS+2, CS-1, CS-2), sex (male or female), and group (cocaine or control) as factors were performed. Analysis revealed no significant main effect of sex (F\(_{1,25} = 2.702, p>0.1\)), no significant main effect of group (F\(_{1,25} =1.296, p>0.1\)), a significant main effect of cue presentation (F\(_{1,25} =27.492, p<0.001\)) and no significant interaction effect between group and sex (F\(_{1,25} = 0.917, p>0.1\)). Both groups and sexes responded preferentially to the reward predictive cues on Day 10 of Pavlovian conditioning.
Post-Devaluation Food Choice Test

Both the control and cocaine groups demonstrated successful devaluation of the sugar by not consuming it and consuming the nondevalued food instead, during the free choice test (Figure 3, A and B). Using group (cocaine vs control) and devaluation status (devalued vs nondevalued) as factors, two-way repeated measures ANOVAs were performed for each sex (male and female). Analysis of male rats (control n=6, cocaine n=5) revealed no significant main effect of group (F_{1,6} =1.722, p>0.1), a significant main effect of devaluation status (F_{1,6} = 233.6, p<0.05) and no significant interaction (F_{1,6}=2.042, p>0.1). Post-hoc analysis showed rats ate significantly more nondevalued food than devalued sugar in both control (t=9.748, p<0.0001) and cocaine (t=8.131, p<0.0001) groups. Similarly, analysis of female rats (control n=8, cocaine n=7) revealed no significant main effect of group (F_{1,13} = 4.305, p=0.0584), a significant main effect of devaluation status (F_{1,13} = 43.93, p<0.0001) and a significant interaction (F_{1,13} = 6.021, p<0.05). Post-hoc analysis showed rats ate significantly more nondevalued food than devalued sugar in both control (t=3.055, p<0.05) and cocaine (t=6.218, p<0.0001) groups.

Post-Devaluation Extinction Test

The cocaine groups’ (male and female) mean devaluation indices were not significantly different from zero, meaning they did not differentiate between the cues for the devalued (sugar) and nondevalued (food) rewards (Figure 4). In contrast, the control groups’ (male and female) means were positive and significantly different from 0, indicating they responded preferentially to the cues for the nondevalued (food) rewards (Figure 4) as analyzed using a one-sample t-test comparing the scores to zero. Specifically, the cocaine (male n=7, female n=6) exposed groups showed a mean devaluation index that was not significantly different from 0 (male, M=0.002951, +/- 0.1451; female, M=0.04698, +/- 0.2754) as shown by one sample t-tests (male, t=0.04981,
p>0.1; female, t=0.4178, p>0.1). The cocaine-exposed rats responded equally to both cues. The control groups (male n=6, female n=6) showed a mean devaluation index that was positive and significantly different from zero (male, M=0.3329, +/- 0.2987; female, M=0.1977, +/- 0.1533) as one sample t-tests (male, t=4.246, p<0.01; female, t=3.158, p<0.05). This result indicates that the control rats responded preferentially to the food predictive cues over the sugar predictive cues.

There were no sex-differences in the devaluation indices. Using group (cocaine vs control) and sex (male vs female) as factors, a two-way repeated measures ANOVA revealed a trend towards a main effect of group (F_{1,20} = 4.289, p = 0.0512), no significant main effect of sex (F_{1,20} = 0.3487, p>0.1) and no significant interaction (F_{1,20} = 0.0001282, p>0.1).

**Electrophysiology**

Figure 5 shows representative PrL neurons in male rats described above on Day 10 of Pavlovian conditioning. Distinct populations of neurons in the PrL are excited (Figure 5A) or inhibited (Figure 5B) during cue presentations and classified as “phasic” to the CS+ (both CS+ included, 10 trials each). In controls (left pie chart, n=6 rats), phasic PrL neurons were predominately excited by the CS+ presentation (17 of 22 total phasic cells). In contrast, in rats with a history of cocaine (right pie chart, n=4); phasic PrL neurons were predominately inhibited during CS+ presentation (n= 9 of 15 cells). The population distribution (i.e., excited vs inhibited) in the control group and the cocaine group was significantly different (control neurons more excited) as shown by a Fisher’s Exact Test. Future studies are needed to increase sample sizes and to determine if similar neural profiles are evident in female rats.
Based on the results from Pavlovian conditioning (Figure 2), both groups (cocaine and control) and sexes (male and female) preferentially responded to the CS+ cues that predicted rewards more than the CS- cues that do not predict rewards. One day 1 the rats spent the same amount of time in the food cup for all cues. On day 10 they differentiated between the cues and spent significantly more time in the food cup following CS+ compared to CS- cues. A history of cocaine use does not impair the ability of the rats to determine which cues predict rewards and which do not across sexes. There were fewer female rats in the group, so more rats will be needed to explore potential sex differences during learning.

However, a history of cocaine did impair behavioral flexibility after reward devaluation as evidenced by the cocaine rats’ continuation of responding to the cue predictive of the devalued reward by going to the food cup, while the control rats did not respond to the cue predicting the devalued reward (Figure 4). There are no sex differences in the devaluation index, even though we observed differences in the amount of cocaine the two sexes consumed (Figure 1). Both control groups continued responding to the nondevalued reward predictive cue, and decreased responding to the devalued reward predictive cue, indicating that the control rats were able to successful shift behavior while the cocaine group did not (they continued responding to both nondevalued and devalued reward predictive cues equally). All groups (male, female, cocaine, and control) successfully learned the devaluation of the sugar, demonstrated by a free-choice test where the rats preferentially ate the food and not the sugar (Figure 3). This suggests that the cocaine groups continue to respond to the devalued reward predictive cues because they are responding habitually rather in a goal-directed manner (Smith et al., 2014). In this experiment, the rats learn that when the cue light comes on they go to the food cup (a habitual response). The cocaine groups (both
males and females) continues to respond in this way despite the cue representing the delivery of the sugar pellets that made them sick previously. Alternatively, the control group is able to employ goal-directed behavior and successfully avoid the cue that predicts the pellets that made them sick.

Electrophysiology recordings in the PrL show that in male-control animals, neurons that are phasic to the cue are predominantly excitatory (77%) (Figure 5C). In the male-cocaine group, about half (60%) of the phasic neurons are excitatory while the others are inhibitory (Figure 5D). This hypoactivity in the PrL is consistent with findings in over trained rats. When animals are over trained and thus displaying habit-like behavior they show impaired behavioral flexibility and hypoactivity in the PrL (Smith et al., 2014). These animals will continue responding to cues predictive of the devalued reward, similar to the cocaine animals. Those with a history of cocaine use display behavior similar to the over trained animals even though they have the same amount of training as control animals that are not responding habitually. It appears that cocaine use predisposes the rat to habit formation. Thus, the history of cocaine leads to habit-like behavior sooner (with less training) than in control animals. In humans, those who recreationally use cocaine may be predisposed to become compulsive (habitual) users.

The PrL projects to several subcortical regions including the nucleus accumbens (NAc), and these projections may be driving some of our behavioral findings. The NAc is also involved in flexible behavior (West & Carelli, 2016). In the NAc core of cocaine animals, phasic neurons show no response to a learned cue following Pavlovian conditioning and are unable to learn higher order processing as a result (Saddoris & Carelli, 2014). As there are direct connections between the PrL and NAc, the hypoactivity we observe in the PrL may drive this dampening effect in the NAc. Future studies may employ optical stimulation to reverse the inhibition in the PrL-NAc circuit and reinstate flexible behavior.
Both sexes learned the Pavlovian conditioning task (shown by preferential responding to reward predictive cues). Both cocaine-exposed groups (males and females) demonstrated impaired behavioral flexibility compared to control groups (male and female). The inability of the cocaine group to use new information (devaluation of the reward) to change habitual behavior connects with instances of relapse. Analysis of the male’s electrophysiology data showed a dampening of the PrL in the cocaine group compared to control. Patients with history of cocaine also show hypofrontality (Goldstein & Yolkow, 2011). The prefrontal cortex in humans and the prelimbic cortex in rats serve similar functions. Impaired flexibility may prevent patients from employing cognitive behavioral therapies to overcome their addiction. This critical knowledge can be used to adapt current therapies and increase the rates for successful recovery.
References


HOW COCAINE IMPAIRS FLEXIBLE BEHAVIOR

encoding in the nucleus accumbens necessary for higher-order learning. *Biol Psychiatry*, 75(2), 156-64.


Appendix

![Cocaine Consumption Chart](image)

Figure 1: Average amount of cocaine (mg/kg) self-administered per day by males and females. The females consumed significantly more cocaine per body weight than males. * p < 0.05.
Figure 2: Amount of time spent in food cup for each cue type on days 1 and 10 for each group (cocaine or control) and sex. On day 1, both male and female rats did not differentiate between the different cue types and spent similar amounts of time in the food cup for each cue. On day 10 the both sexes spent significantly more time in the food cup when the cue predicted a reward. There is no significant different between sexes or groups. * p<.05.
Figure 3: Effect of sugar devaluation on free food choice. Both groups consumed little to no sugar when presented with the option of consuming food or sugar, demonstrating successful devaluation of the sugar. This remains true for both sexes (males left, females right).

Figure 4: Devaluation index for control and cocaine groups. The control group did not respond (by going to the food cup) when the cue predicted the devalued reward (sugar) while responding appropriately to the nondevalued (food) predictive cues. The cocaine group responded similarly to cues for both the devalued and nondevalued rewards. There are no sex differences. * p<.05 for one sample t-test.
Figure 5: PrL neurons shift population response profiles to cues following devaluation. A) demonstrates a representative excitatory neuron phasic to the cue. B) demonstrates a representative inhibitory neuron, phasic to the cue (onset represented by black line). The control group contained predominately excitatory phasic neurons while the cocaine group showed predominately inhibited neurons.