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This paper received a QuaesitUM outstanding paper award.

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The Effects of Chronic Cocaine Exposure and Withdrawal on Dopamine Functioning

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Abstract

The mesolimbic dopamine pathway, often referred to as the reward pathway, consists of cell bodies in the ventral tegmental area (VTA) that project to limbic regions, particularly the nucleus accumbens (NAc). Cocaine increases dopamine activity by blocking dopamine transporters (DATs), consequently inhibiting dopamine reuptake. This study aimed to determine the effects of chronic cocaine exposure and withdrawal on dopamine functioning. Mice were divided into four groups: chronic cocaine exposure, chronic saline exposure, chronic cocaine exposure plus a withdrawal period, and chronic saline exposure plus a withdrawal period. In vivo fixed amperometry was used to measure VTA stimulation-evoked dopamine release and reuptake in the NAc of anesthetized mice before and after an in-test cocaine injection. Cocaine exposure and withdrawal seemed to have the greatest effect on variables related to DAT functioning rather than dopamine release. Studies such as these may be useful in improving treatments for substance use disorders.

Introduction

The 2019 National Survey on Drug Use and Health (NSDUH) reported that 5.5% of Americans between ages 18 and 25 used cocaine that year. The misuse of cocaine can lead to health conditions and even overdose causing death. According to the National Institute on Drug Abuse (NIDA, 2021), there were more drug-related overdose deaths in 2020 than in any other one-year period in history; this was an increase of 30 percent from the previous year. Surveys also report that relapse is common for those who attempt to quit cocaine, with drug addiction treatment resulting in a rate of relapse between 40% to 60% of the cases (Wagener & Thomas, 2021). There are different types of behavioral therapies that are offered to those who are wanting to stop using cocaine; however, there are currently no FDA-approved pharmaceuticals for stimulant use disorder. To work towards this pharmaceutical and improving treatment for stimulant use disorder, a better understanding of neural changes following chronic cocaine use is necessary.

Cocaine prolongs the activity of dopamine in the synapse by blocking the dopamine reuptake mechanism, dopamine transporters (Wise, 1984). The mesolimbic dopamine pathway, often referred to as the reward pathway, consists of dopamine cell bodies in the ventral tegmental area (VTA) that project to limbic regions, particularly the nucleus accumbens (NAc). When the NAc is stimulated by extracellular dopamine, the cells in the NAc further ignite neural circuits that produce reinforcing effects. Thus, cocaine's ability to greatly increase dopamine activity in the NAc drives the reinforcing properties of the drug and the continued use of the drug even while facing negative consequences (Nestler, 2005).

Previous research showed that repeated cocaine use alters the functioning of DAT, potentially altering the way brains respond to rewarding stimuli. For example, Mash et al. (2002) found that DAT function was severely elevated following chronic cocaine use in baboons compared to age-matched drug-free controls (Mash et al., 2002), and Daws et al. (2001) found that cocaine increases the cell surface distribution of DAT in rodents. Furthermore, abstinence from chronic cocaine use is known to induce withdrawal effects, such as cravings, anxiety, and anhedonia (Ball et al., 2018; Mendez & Salazar-Juarez, 2019; Stoker & Markou, 2011). Many of these withdrawal behaviors are thought to be driven by drug-induced dopaminergic changes. In a study by Cerruti et al. (1993), a 10 day withdrawal period from cocaine lead to a long-lasting decrease in DAT binding. In other words, the gene expression was altered many days after withdrawal from cocaine. Cerruti et al. (1993) concluded that this altered DAT could be a significant factor

underlying behavioral and biochemical changes during cocaine withdrawal. Altogether these studies suggest that chronic cocaine use increases the number of DAT, which is then decreased following a withdrawal period. DAT fluctuations greatly control the amount of extracellular dopamine available in the synapse.

Here, we are addressing the research question of how chronic cocaine plus withdrawal affects functional dopamine release. A better understanding of the neurochemical effects of cocaine use and abstinence may lead to improved treatments for cocaine use disorder. We used fixed potential amperometry in vivo to measure VTA stimulation-evoked dopamine release and reuptake in four groups mice: chronic cocaine exposure, chronic saline exposure (control), chronic cocaine exposure plus a withdrawal period, and chronic saline exposure plus a withdrawal period (control). Dopamine release was determined by measuring the magnitude of the stimulated response, and dopamine reuptake was quantified by measuring the synaptic half-life of dopamine. The synaptic half-life of dopamine is an indication of how quickly dopamine is being cleared from the synapse. The synaptic half-life of dopamine is dependent on DAT functioning, with a faster synaptic half-life indicating more efficient DAT functioning (Holloway, 2018).

To further test DAT functioning and the way the mesolimbic dopamine system response to a dopaminergic drug, all mice received an in-test cocaine challenge during dopamine recordings. Therefore, dopamine release and reuptake (synaptic half-life) were quantified before and after the in-test cocaine challenge. It was hypothesized that chronic cocaine would increase DAT functioning compared to saline, resulting in a shorter baseline dopamine half-life and a reduced dopaminergic response to the in-test cocaine challenge. It was also hypothesized that chronic cocaine plus a withdrawal period would decrease DAT functioning compared to saline, resulting in a longer baseline dopamine half-life and an increased dopaminergic response to cocaine. The findings of the current study will further the understanding of neural changes following chronic cocaine administration and potentially further the research in addiction treatments.

Methods

The proposed experiment was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Memphis and were also aligned with those outlined in The Public Health and Service Policy on Humane Care and Use of Laboratory Animals (National Institutes of Health 2012) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2015).

Subjects

Twenty-seven male C57BL/6J mice were acquired from Jackson Laboratory (Bar Harbor, ME, USA) at 3 weeks of age. Upon arrival, mice were immediately and randomly separated into separate cages with 2 to 4 mice per cage. Cages were kept in a temperature-controlled room (21 ± 1° C) with a 12-hour circadian cycle. Food and water were available ad libitum. Mice remained in the animal colony until time of experiments. Mice were adults at the time of experiments (3-5 months old).

Procedures

Drug pretreatments consisted of the mice being intraperitoneally (ip) injected with cocaine (20 mg/kg) or saline every day for seven days. Some of the mice (n = 16, 8 cocaine-exposed and 8 saline-exposed) were tested on the eighth day, which is 24 hours after the last injection. The other mice were exposed to a one week withdrawal period (n = 11, 5 cocaine-exposed and 6 saline-exposed), meaning that the testing occurred on day 15. See Table 1 for a list of experimental groups and Figure 1 for a depiction of the experimental timeline. On the day of dopamine measurements, the mice were permanently anesthetized using two urethane injections (totaling 1.5 g/kg, ip). Foot and tail pinch and eye blink induced reflexes fifteen minutes after second injection guaranteed proper anesthetization. They were then mounted in a stereotaxic frame, while maintaining a body temperature of approximately 37°C. Stereotaxic carriers were used to guide all electrodes.

A stimulating electrode was placed into the left VTA (coordinates: AP -3.3 mm from bregma, ML +0.3 mm from midline, and DV -4.0 mm from dura; Paxinos & Franklin, 2001). A reference and auxiliary electrode combination was placed in contact with cortical tissue contralateral to the stimulating electrode (-3.0 mm from bregma). Lastly, a carbon fiber recording electrode was implanted into the left NAc (AP +1.5 mm from bregma, ML +1.0 mm from midline, and DV -4.0 mm from dura). See Figure 2 for a depiction of the surgical setup. A fixed current of +0.8 V was applied, with dopamine oxidation currents being continuously monitored (10K samples/sec) by an electrometer (ED401 e-corder 401 and EA162 Picostat, eDAQ Inc). Stimulation parameters consisted of 20 pulses at 50 Hz every 30 sec. Baseline measurements were recorded for 5 minutes, then mice received a cocaine challenge (20 mg/kg, ip). The same stimulation parameters continued, and dopamine recordings continued 1-hour post-cocaine injection. Immediately following data collection, direct anodic current of 100 mAmps was applied for 10 seconds to mark the electrode positions. Mice were then euthanized by intracardiac injection of an overdose of urethane (0.345 g/mL)

Data Analysis

There were two independent variables for this project: chronic drug exposure (cocaine or saline) and withdrawal period (no withdrawal period or plus withdrawal period). There were four dependent variables: (1) baseline dopamine release (nAmp), (2) baseline dopamine half-life (sec), (3) dopamine release following cocaine administration (% change from baseline, with baseline set at 100%), and (4) dopamine half-life following cocaine administration (% change from baseline, with baseline set at 100%). Dopamine release and half-life measurements were compared at the peak effect time of cocaine (which was 20 min following the in-test cocaine challenge). Two-way between-subject ANOVAs were used to determine the effect of drug exposure and withdrawal on each of the 4 dependent variables. When appropriate, one-way ANOVAs with Tukey HSD post hoc analyses were used to determine specific group differences.

Table 1. Experimental Groups

Drug Exposure	Withdrawal	*N*
Cocaine	None	8
	Withdrawal	5
Saline	None	8
	Withdrawal	6
	TOTAL	**27**

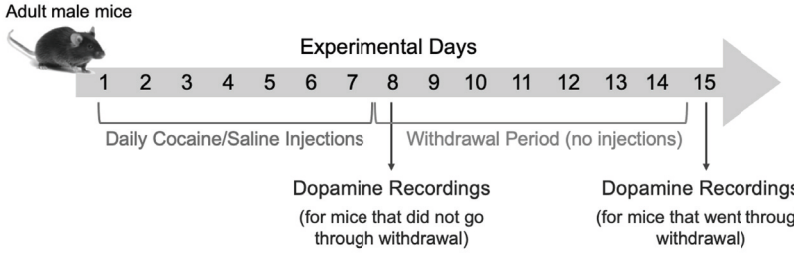

Figure 1. Experimental Timeline

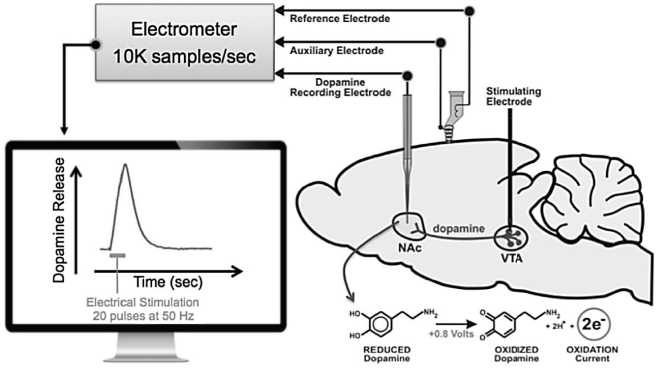

Figure 2. Surgical Set-up for Amperometric Recordings. In vivo fixed amperometry with carbon fiber recording electrodes quantified nucleus accumbens (NAc) dopamine release and half-life evoked by stimulation of dopamine cell bodies within the ventral tegmental area (VTA).

Results

Baseline Dopamine Functioning

Regarding the baseline dopamine release, a two-way between subjects ANOVA was used to compare mean dopamine release before the in-test cocaine administration. There was not a significant main effect of drug (chronic cocaine or saline) or withdrawal on baseline dopamine release, [drug: $F(1,1) = 2.2$, $p = 0.152$; withdrawal: $F(1,1) = 0.481$, $p = 0.495$], and no significant interaction was observed between the effects of drug and withdrawal on baseline dopamine release, [$F(1,1) = 0.007$, $p = 0.936$] (Figure 1).

Regarding the baseline dopamine half life, a two-way between-subjects ANOVA was used to compare the mean synaptic half-life of dopamine before the in-test cocaine administration. Similarly to that observed in baseline dopamine release, there was not a significant main effect of drug or withdrawal on baseline dopamine half-life, [drug: $F(1,1) = 0.193$, $p = 0.664$; withdrawal: $F(1,1) = 0.057$, $p = 0.814$]. However, there was a statistically significant interaction between drug and withdrawal on baseline dopamine half-life, [$F(1,1) = 8.618$, $p = 0.007$] (Figure 2), indicating that the synaptic half-life of dopamine was affected by the withdrawal period differently depending on drug exposure (cocaine or saline). A follow up one-way ANOVA was used to compare group differences in baseline dopamine half-life. The ANOVA neared significance, but did not reach significance, [$F(3,23) = 2.880$, $p = 0.058$]; therefore, post hoc analyses were not conducted.

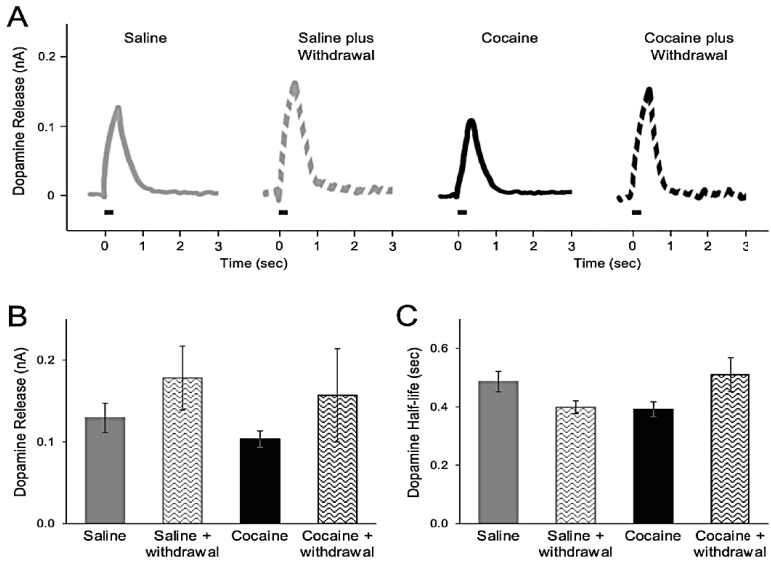

Figure 3. Baseline Dopamine Responses. Profiles indicate representative responses from each group (A). No significant effects were observed in mean (± SEM) dopamine release between chronic drug or withdrawal groups (B). There was a significant interaction between drug and with-drawal on baseline dopamine half-life, but no significant differences were observed between groups (C).

Dopamine Functioning Following the Cocaine Challenge

Regarding the dopamine release following the cocaine challenge, a two-way ANOVA was used to compare percent change in dopamine release following the in-test cocaine administration. There was not a significant main effect of drug or withdrawal on cocaine-induced changes in dopamine release, [drug: $F(1,1) = 0.354$, $p = 0.558$; withdrawal: $F(1,1) = 1.408$, $p = 0.248$], and no significant interaction was observed between drug and withdrawal on percent change in dopamine release following cocaine, [$F(1,1) = 0.334$, $p = 0.569$] (Figure 3).

Regarding the dopamine half-life following the cocaine challenge, a two-way ANOVA was used to compare mean dopamine half-life after cocaine administration. There was a near significant main effect of drug on dopamine half-life, [$F(1,1) = 3.412$, $p = 0.078$], but no follow-up analyses were conducted. There was a statistically significant main effect of withdrawal on dopamine half-life, [$F(1,1) = 5.603$, $p = 0.034$]. A follow up independent samples t-test showed that there was a statistically significant difference in dopamine half-life post cocaine when comparing the no-withdrawal group to the withdrawal group, $t(25) = 2.213$, $p = 0.036$. These results indi-

cate that the mice that experienced a withdrawal period (1 week without injections) displayed an increased percent change in dopamine half-life following cocaine. No significant interaction was observed between drug and withdrawal on dopamine half-life post cocaine challenge, [$F(1,1) = 0.346$, $p = 0.592$] (Figure 4)

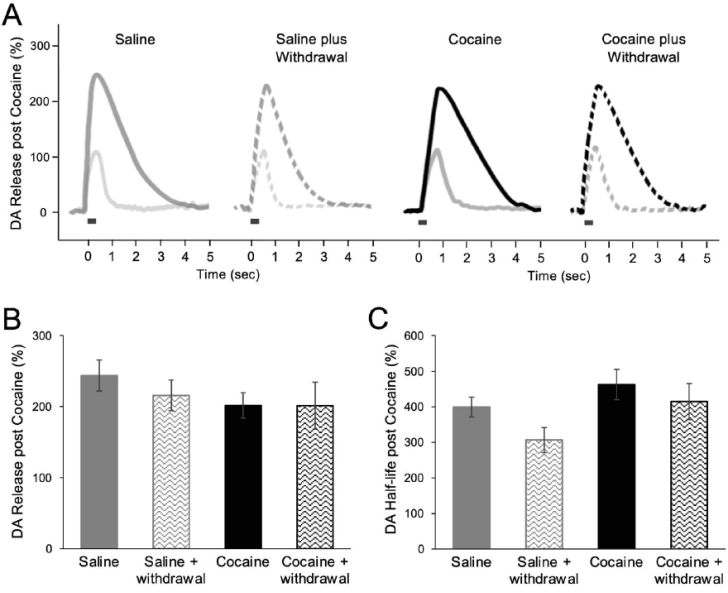

Figure 4. Dopamine Responses post Cocaine Administration. Profiles indicate representative responses from each group at 20 min post injection (A). No significant effects were observed in mean (± SEM) percent change in dopamine release following cocaine (B). There was a near significant main effect of drug and a significant main effect of withdrawal on percent change in dopamine half-life follow-ing cocaine, but no interactive effect between drug and withdrawal (C).

Discussion

The purpose of the current study was to examine the effects of cocaine exposure and withdrawal on NAc dopamine functioning. This study used four groups depending on the changes of the two variables, which are drug exposure and withdrawal, resulting in 4 experimental groups: chronic cocaine exposure, chronic saline exposure (control), chronic cocaine exposure plus a withdrawal period, and chronic saline exposure plus a withdrawal period (control). We used fixed potential amperometry to measure dopamine

release before and after the cocaine challenge. The dependent variables were baseline dopamine release, baseline dopamine half-life, dopamine release post cocaine challenge, and dopamine half-life post cocaine challenge. Overall, cocaine exposure and withdrawal seemed to have the greatest effect on variables related to dopamine transporter (DAT) functioning rather than dopamine release.

Effects of Chronic Cocaine

All mice in this project were chronically exposed with daily injections to either cocaine or saline. Regarding baseline dopamine functioning, which was before the in-test cocaine challenge, no main effect of cocaine exposure was observed on either dopamine release or synaptic half-life. Given that cocaine does not directly alter dopamine release mechanisms, it was expected that cocaine exposure would not alter baseline dopamine release. These results fit with a previous study (Pattison et al., 2012). However, unexpected results showed that cocaine exposure did not alter baseline dopamine half-life. Given that DAT are responsible for clearing dopamine from the synapse, dopamine half-life is a measurement of DAT function. Daws et al. (2001) found that cocaine exposure increases the cell surface redistribution and expression of dopamine transporters, which should lead to faster synaptic clearance. A direct visual comparison of the cocaine-exposed mice and the saline-exposed mice, without including the withdrawal groups, does indicate a reduced dopamine half-life, which means faster clearance following cocaine exposure, but these direct comparisons were not statistically analyzed given the initial plan of the project.

Regarding the dopaminergic response to the in-test cocaine challenge, cocaine increased both dopamine release and half-life in all mice, as expected. Chronic cocaine exposure did not alter the percent change in dopamine release following the cocaine challenge. However, there was a near significant main effect of drug exposure on percent change in dopamine half-life following the cocaine challenge. Although not to a significant extent, the time dopamine remained in the synapse was increased by cocaine to a greater degree in cocaine-exposed mice compared to saline-exposed mice. These differences may have been driven by the surprisingly low responses observed in the saline-withdrawal group, which is discussed below. We expected a decreased dopaminergic response to the cocaine challenge following chronic cocaine exposure, as observed in previous studies (Francis et al., 2019; Lopez-Arnau et al., 2019). Our results may be related to drug exposure periods; however, we chose this dosing regimen as others have shown it to alter dopamine-related cellular functioning and behaviors (Feng et al., 2014; Hiroi et al., 1997)

Effects of Chronic Cocaine Plus Withdrawal

Roughly half of the mice in this project were subjected to a 7-day withdrawal period with no injections before dopamine recordings. Some of the mice were going through withdrawal following chronic cocaine exposure and some following chronic saline exposure. It was important to have a saline withdrawal group to serve as a control for the cocaine withdrawal group in order to determine whether the 7-day absence of the injection protocol made a difference. It has been shown that animals can experience injection stress with any kind of injection (Baik, 2020), and stress has been shown to affect dopamine levels and neuronal activity in the mesolimbic dopamine system (Baik, 2020). Thus, removal of daily injections may have altered dopamine functioning, regardless of the drug. Withdrawal, without taking the cocaine/saline exposure into consideration, did not alter baseline dopamine release or half-life, but it did alter the dopaminergic response to the in-test cocaine challenge.

Specifically, a main effect of withdrawal was observed on the percent change in dopamine half-life following cocaine. Mice that went through withdrawal displayed a decreased dopaminergic response to cocaine. Mice in an active state of stress have been shown to have increased dopaminergic responses to psychostimulants (Baik, 2020). Thus, animals that did not go through the withdrawal period (7 days of no injections) may have been in an active state of stress, especially if they were receiving saline injections). Future studies could monitor stress levels, such as through hormone measurements or behavioral assays, to answer these questions. Future studies could also use a different route of drug administration, such as consumption through food or drink, in order to subject the animals to less stress. Future studies could also lengthen the withdrawal period to better understand the development and timing of withdrawal-related effects.

Examining the interactions between the drug exposure and withdrawal is the important component in determining whether cocaine withdrawal resulted in differences in dopamine functioning relative to saline withdrawal. There was no interaction observed between drug exposure and withdrawal on baseline dopamine release. There was, however, a significant interaction observed in baseline dopamine half-life. It looked as though withdrawal following cocaine exposure lead to an increased dopamine half-life compared to the cocaine exposed mice, indicating that some DAT compensation occurred during the cocaine withdrawal, as the dopamine half-life of the cocaine withdrawal mice more closely resembled that of the saline exposed controls.

Saline withdrawal, however, seemed to result in a reduced dopamine half-life (increased DAT function) relative to the saline exposed. The follow-up one way ANOVA analysis was not significant ($p = .058$). Adding a few mice per group may reduce the observed error and clarify these results. No significant interaction was observed between drug and withdrawal on the dopaminergic response to cocaine (in either percent change-in-release or half-life).

Conclusion

The present study indicates that cocaine exposure and withdrawal alter dopamine functioning, specifically dopamine transmission related to DAT function. These findings are not surprising given that cocaine's mechanism of action is to inhibit DAT and have shown DAT expression to fluctuate based on cocaine administration. DAT functioning seems to be restored to some degree by the withdrawal period, but more research is needed given that the withdrawal period also altered dopamine functioning in the saline-exposed mice. These findings are limited by a few aspects of the experimental design.

Sex has been shown to affect dopamine release and responses to psychostimulants (Perez et al., 2018), and the current study was only conducted on males. Future studies should include females to determine whether these findings are generalizable to females. Also, as mentioned above, altering the dosing regimen and length of the withdrawal period could provide useful information about the timing of compensatory mechanisms. Furthermore, some limitations could be due to the age of the mice.

Future studies could include a wider variety of age in subjects to examine the differences in adolescent metabolism versus adult metabolism. This would be a further investigation into dopaminergic response, as there may be a cocaine dopamine sensitization difference across ages. Overall, the current study further adds to the understanding of neural changes following chronic cocaine administration and withdrawal. Such studies are needed to improve treatments for substance use disorders.

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