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THE INFLUENCE OF LONG-ACCESS COCAINE SELF-ADMINISTRATION ON BEHAVIORAL
MEASURES OF ANXIETY AND CRAVING IN RATS

by

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for graduation with Honors in the Health and Human Physiology

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Thesis Mentor

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All requirements for graduation with Honors in the
Health and Human Physiology have been completed.

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The influence of long-access cocaine self-administration on behavioral measures of anxiety and craving in rats

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Abstract

Cocaine addiction is a prevalent and substantial problem. It is very difficult for cocaine-addicted individuals to receive treatment, avoid relapse, and go into full remission. Animal models of cocaine taking can be used to study different facets of this disease. One such model is cocaine self-administration. Although self-administration is a widely used model in the field of drug-addiction biology (Müller Ewald V., Lalumiere R., 2017), its influence on rat-specific characteristics such as anxiety and craving is not clear. In this experiment, we sought to correlate drug-taking levels during self-administration with measures of anxiety-like behaviors and cocaine craving. Male Sprague-Dawley rats (n=21) were implanted with intrajugular catheters and underwent cocaine self-administration. Rats then underwent two tests, before and after withdrawal, to serve as a measure of cocaine craving. Our results suggest that levels of cocaine taking during cocaine self-administration does not appear to impact levels of cocaine craving during periods of withdrawal or levels of anxiety-like behaviors of rats during experimental studies. Overall, this strengthens the self-administration model and its ability to study the neurobiology of addiction.

Introduction

Cocaine addiction is a prevalent and substantial problem. According to the White House Drug Policy, 36.8 million Americans aged 12 and older have used cocaine at least once in their lives; furthermore, cocaine use and related complications result in the death of approximately 15,000 Americans annually (Cocaine Statistics, 2017). Cocaine addiction can lead to various health problems, impact the individual's social connections and interactions, create significant stressors, and greatly affect the individual's quality of life. Despite these pervasive issues, it is very difficult for cocaine-addicted individuals to receive the necessary treatment, avoid relapse, and go into full remission. Indeed, evidence indicates that approximately 25% of the individuals who seek treatment for cocaine addiction will relapse within one year (Tackett B., 2017). Through studies of cocaine-addicted individuals, we have gained significant knowledge pertaining to the neurobiology of this disease and its impacts, but we still have much to learn. Animal models of cocaine taking can be used to

study different facets of this disease, such as neurobiological changes that occur as a result of drug-taking, or different factors that induce drug-seeking behavior. One such model commonly used in research laboratories is cocaine self-administration, which is a form of operant conditioning, in which an animal, often a rat, is placed in an operant chamber and rewarded with an infusion of cocaine following a lever press. Rats can readily learn this behavioral task, which can then be used to examine the basic neurobiology underlying drug-taking, craving, and relapse. Although self-administration is a widely used model in the field of drug-addiction biology (Müller Ewald V., Lalumiere R., 2017), its influence on rat-specific characteristics such as anxiety and craving, and the impact such interactions can have on resulting experimental paradigms, is not clear. Thus, to inform this issue, we sought to correlate drug-taking levels during self-administration with measures of anxiety-like behavioral phenotypes and cocaine craving following self-administration.

Prior studies have demonstrated that cocaine seeking is increased after a period of drug abstinence (Tran-Nguyen L. et al., 1998), a phenomenon called incubation of cocaine craving. This phenomenon is reliably seen in rodents (Grimm J. et al., 2001), and evidence suggests that a phenomenon similar to incubation of craving is also seen in human drug users (Bedi et al., 2011; Wang et al., 2013; Li et al., 2014). However, it is yet to be determined whether levels of drug-taking during periods where animals have access to the drug has an impact on the level of drug craving during a period of withdrawal, where animals do not have access to the drug. Thus, one focus of this study is to examine whether there is a correlation between cocaine taking during self-administration and cocaine craving during withdrawal. If we find a positive correlation between these variables, we can utilize this information to determine if the results of an experiment involving cocaine self-administration are impacted depending on the levels of cocaine taking and craving exhibited by the rats.

Previous studies also suggest that anxiety may lead to a vulnerability to cocaine addiction in human populations (Dilleen R. et al., 2012). Similarly, studies in animal models have demonstrated that high levels of anxiety-like behaviors, as measured by performance on an elevated plus maze, are related to high levels of cocaine taking during self-administration (Dilleen R. et al., 2012). The measure of anxiety-like behaviors utilized in our laboratory is an open field test, which is argued to be virtually interchangeable with elevated plus maze measures in terms of observing an animal's relative level of anxiety-like behaviors (Carola V. et al., 2002). Thus, the second focus of this study is to determine whether there is a correlation between cocaine self-

administration and level of anxiety-like behaviors as measured by the open field test. If we find a positive correlation between cocaine taking and anxiety-like behaviors, we can determine if experiments involving cocaine self-administration impact a rat's level of anxiety-like behaviors, thus potentially introducing a confounding variable that differs from animal to animal.

We hypothesize that there will be a positive correlation between cocaine taking and cocaine craving of the drug during the withdrawal period, in addition to a positive correlation between cocaine taking and levels of anxiety-like behavior as observed in an open field test.

Methods

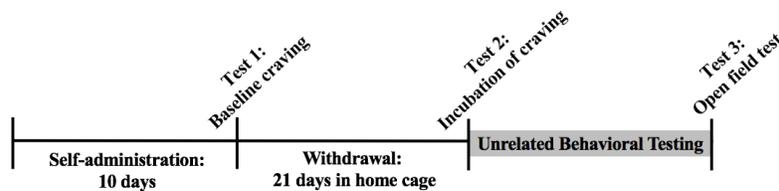


Figure 1. Experimental timeline for proposed experiments

Surgery

Male Sprague-Dawley rats (~300g at the time of the surgery, n=21) were implanted with intrajugular catheters used for cocaine delivery during self-administration.

Cocaine self-administration

Rats underwent a minimum of 10 days of cocaine self-administration. Animals were placed in an operant chamber with an inactive and active lever for 6 hours a day, 6 days a week. Active lever presses were rewarded with a 0.05 ml infusion of 2mg/ml cocaine dissolved in saline directly into the blood stream via catheter, as well as a light and tone cue. Each infusion was followed by a 20 second timeout to avoid risk of overdose—for 20 seconds following a lever press, the lever remains extended, although lever presses have no consequence. Inactive lever presses did not produce a reinforcing stimulus but were collected to assess motor behavior.

Homecage withdrawal

Rats underwent 21 days of homecage withdrawal, in which they remained in their homecages and did not engage in self-administration. During this time, they did not have access to the drug or drug-taking environment.

Baseline/Incubation of craving tests

Homecage withdrawal was flanked by two tests that were used to calculate a measure of cocaine craving. Each test lasted an hour and were separated by 21 days of homecage withdrawal. In these tests, lever presses yielded cocaine-associated light and tone cues, but not the drug itself. In this way, we were able to assess the rat's motivation to drug seek.

Open field test

After a period of unrelated behavioral testing, rats were allowed to explore the open field chamber for 15 minutes. The open field chamber is an empty, open top, 40cmx40cmx40cm box. Rats were given 7 minutes to acclimate to the chamber, returned to their homecage for one minute, and then returned back to the chamber for 15 minutes. A video camera is placed above the open field chamber, and Ethovision hardware and software (Noldus, Leesburg, WV) is used to measure total distance traveled. Total distance traveled was used as a measure of anxiety-like behaviors, such that the greater the distance traveled during the rats' time in the chamber, the lower their level of anxiety.

Statistical analyses

We analyzed many correlations based on different measures of cocaine taking, cocaine craving, and anxiety-like behaviors. Since this study was exploratory, and there were no previously established measures of cocaine taking, craving, and anxiety-like behaviors, we developed our own measures based on the literature and our own expertise. There were two measures of cocaine taking. One measure was calculated based on the average of the active lever presses during the last two days of self-administration (Self-administration score). The second measure was calculated in terms of the average number of the infusions of cocaine during the last two days of self-administration (Self-administration infusion score).

We used three measures of cocaine craving. The first measure of the level of craving was determined based on the difference between active lever presses of the baseline craving test and the incubation of craving test (Craving level). The second measure of craving was determined based on the number of active lever presses during the final incubation of craving test (Incubation of craving). The third measure of the level of craving was determined based on the percent change from active lever presses on the fourth day of self-administration (when the rat begins to figure out how the operant chamber works, and the initial level of active lever presses stabilizes) to the last day of self-administration (Escalation of intake).

We analyzed correlations demonstrating the relationship between cocaine taking and cocaine craving comparing all of the different measures of both, such as the relationship between the SA score and escalation of craving. Pearson correlational statistics were used to determine the relationship between cocaine taking and cocaine craving.

To correlate self-administration performance and the level of anxiety-like behaviors, the above data concerning self-administration, including all measures of cocaine taking and cocaine craving, were compared to the measure of anxiety-like behaviors—the total distance each rat traveled during their time spent in the chamber. Pearson correlational statistics were used to determine the relationship between self-administration and anxiety-like behaviors.

Results

First, we examined if there was a correlation between self-administration score (the average of the active lever presses during the last two days of self-administration) and craving level (the difference between active lever presses of the baseline craving test and the incubation of craving test). We found no statistically significant correlation between the self-administration score and craving level (**Figure 2**, $r = 0.1715$, $P = 0.4963$). We further correlated craving level to self-administration infusion score (the average number of the infusions of cocaine during the last two days of self-administration) and escalation of intake (the percent change of active lever presses on the fourth day of self-administration to the last day of self-administration). We found no statistically significant correlation between craving level and self-administration infusion score (**Figure 3**, $r = 0.2331$, $P = 0.3227$), and no statistically significant correlation between craving level and escalation of intake (**Figure 4**, $r = -0.2329$, $P = 0.3683$).

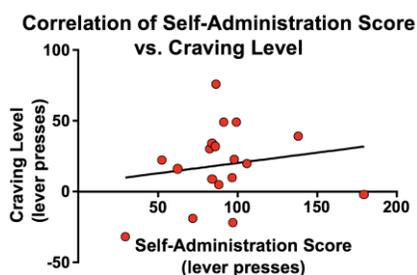


Figure 2. Correlation of self-administration score and craving level. The average of active lever presses during the last two days of self-administration was calculated and correlated to the

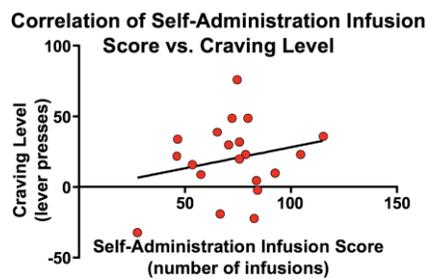


Figure 3. Correlation of self-administration infusion score and craving level. The average number of the infusions of cocaine during the last two days of self-administration was calculated and

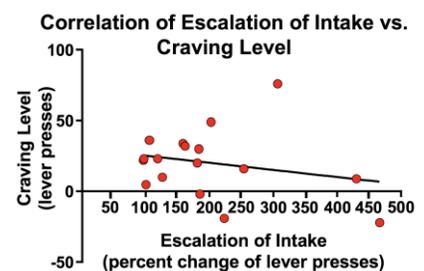


Figure 4. Correlation of escalation of intake and the craving level. The percent change from active lever presses on the fourth day of self-administration to the last day of self-administration was

difference between active lever presses of the baseline craving test and the incubation of craving test. No statistically significant correlation was found ($r= 0.1715$, $P= 0.4963$, $n= 18$).

correlated to the difference between active lever presses of the baseline craving test and the incubation of craving test. No statistically significant correlation was found ($r= 0.2331$, $P= 0.3227$, $n= 20$).

calculated and correlated to the difference between active lever presses of the baseline craving test and the incubation of craving test. No statistically significant correlation was found ($r= -0.2329$, $P= 0.3683$, $n= 17$)

We next determined the correlation between incubation of craving (the number of active lever presses during the final incubation of craving test) and the self-administration score. We found no statistically significant correlation between incubation of craving and the self-administration score (**Figure 5**, $r= 0.1864$, $P= 0.4589$). Then, we correlated incubation of craving and the self-administration infusion score, in addition to incubation of craving and escalation of intake. We found no statistically significant correlation between incubation of craving and the self-administration infusion score (**Figure 6**, $r= 0.014$, $P= 0.9533$), and no statistically significant correlation between incubation of craving and escalation of intake (**Figure 7**, $r= -0.01791$, $P= 0.9456$).

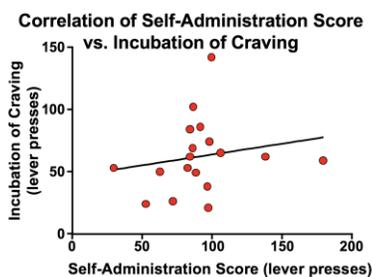


Figure 5. Correlation of self-administration score and incubation of craving. The average of active lever presses during the last two days of self-administration was calculated and correlated to the number of active lever presses during the final incubation of craving test. No statistically significant correlation was found ($r= 0.1864$, $P= 0.4589$, $n= 18$).

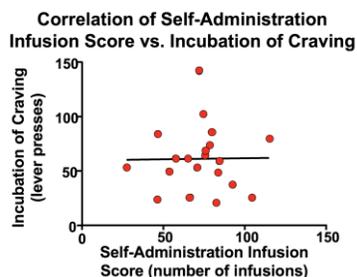


Figure 6. Correlation of self-administration infusion score and incubation of craving. The average number of the infusions of cocaine during the last two days of self-administration was calculated and correlated to the number of active lever presses during the final incubation of craving test. No statistically significant correlation was found ($r= 0.014$, $P= 0.9533$, $n= 20$).

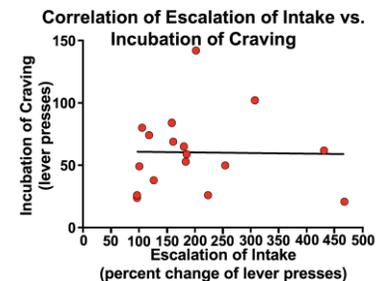


Figure 7. Correlation of escalation of intake and incubation of craving. The percent change from active lever presses on the fourth day of self-administration to the last day of self-administration was calculated and correlated to the number of active lever presses during the final incubation of craving test. No statistically significant correlation was found ($r= -0.01791$, $P= 0.9456$, $n= 17$).

We investigated the correlations between the anxiety score (the distance traveled during an open field test) and the self-administration score, the self-administration infusion score, escalation of intake, craving level, and incubation of craving. We found no statistically significant correlation between the anxiety score and the self-administration score (**Figure 8**, $r= -0.01255$, $P= 0.9646$). Additionally, we found no statistically significant correlation between the anxiety score and the self-administration infusion score (**Figure 9**, $r= -0.1764$, $P= 0.4983$), and no statistically significant correlation between the anxiety score and escalation of intake (**Figure**

10, $r = -0.08732$, $P = 0.7666$). Finally, there was no statistically significant correlation between the anxiety score and craving level (**Figure 11**, $r = -0.4122$, $P = 0.1002$), and no statistically significant correlation between the anxiety score and incubation of craving (**Figure 12**, $r = -0.05948$, $P = 0.8206$).

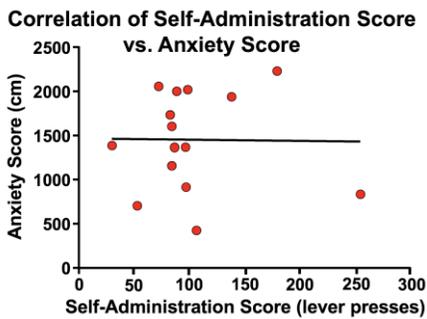


Figure 8. Correlation of self-administration score and anxiety score. The average of active lever presses during the last two days of self-administration was calculated and correlated to the total distance traveled during an open field test. No statistically significant correlation was found ($r = -0.01255$, $P = 0.9646$, $n = 15$).

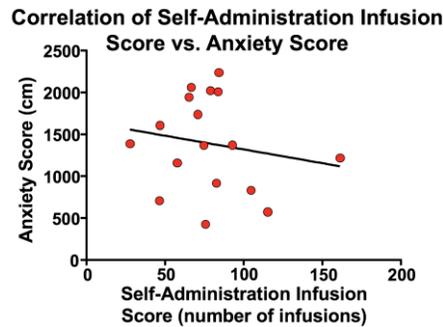


Figure 9. Correlation of self-administration infusion score and anxiety score. The average number of the infusions of cocaine during the last two days of self-administration was calculated and correlated to the total distance traveled during an open field test. No statistically significant correlation was found ($r = -0.1764$, $P = 0.4983$, $n = 17$).

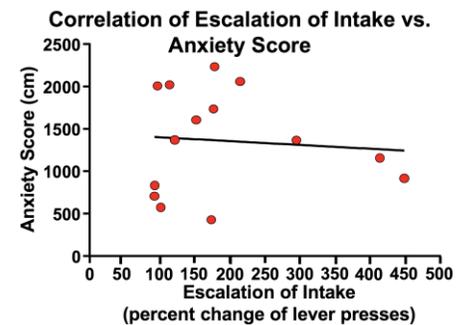


Figure 10. Correlation of escalation of intake and anxiety score. The percent change from active lever presses on the fourth day of self-administration to the last day of self-administration was calculated and correlated to the total distance traveled during an open field test. No statistically significant correlation was found ($r = -0.08732$, $P = 0.7666$, $n = 14$).

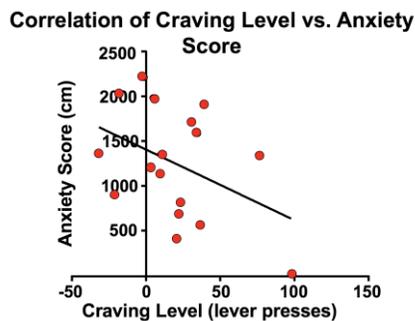


Figure 11. Correlation of craving level and anxiety score. The difference between active lever presses of the baseline craving test and the incubation of craving test was calculated and correlated to the total distance traveled during an open field test. No statistically significant correlation was found ($r = -0.4122$, $P = 0.1002$, $n = 17$).

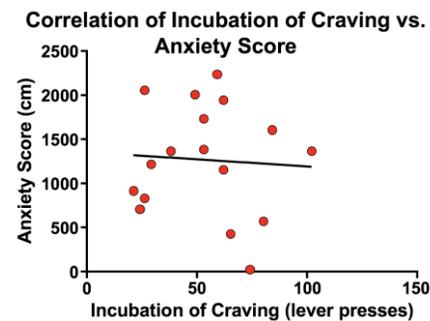


Figure 12. Correlation of incubation of craving and anxiety score. The number of active lever presses during the final incubation of craving test was calculated and correlated to the total distance traveled during an open field test. No statistically significant correlation was found ($r = -0.05948$, $P = 0.8206$, $n = 17$).

Discussion

Based on the data collected in this experiment, levels of cocaine taking during cocaine self-administration does not appear to impact levels of cocaine craving during periods of withdrawal. Additionally, cocaine self-administration does not appear to impact levels of anxiety-like behaviors (measured via open field test). Overall, this is good news for researchers utilizing the cocaine self-administration model to study the

neurobiology of addiction. Differences in rat-specific characteristics (such as anxiety and craving) could be indicative of confounding variables in drug addiction experiments and potentially skew the data gathered from the studies.

The self-administration model of drug addiction has undergone over 30 years of research experience and has clearly demonstrated its value in these studies. The results of this experiment further indicate the robustness of this model and its ability to produce data and information to help researchers gain insight into the neurobiology of addiction. Although it would be ideal to study human drug addiction in human subjects, it is necessary to conduct experiments on laboratory animals, specifically rats, since they allow invasive neurobiological studies not feasible in humans, amongst other limitations (Ahmed S.H., 2012). This approach additionally has the benefit of providing each animal with equal access and exposure to the drug, a strength that would be difficult to accomplish in a human study (Ahmed S.H., 2012).

There may be some limitations to this study. In this experiment, levels of anxiety were determined after self-administration of cocaine rather than before. Perhaps rats that demonstrated anxiety-like behaviors at the beginning of the experiment were never able to learn self-administration due to their anxious behavior and were excluded from the study. Anxiety-like behaviors would consequently have an impact on cocaine taking. On this same note, rats that never reached the “threshold of addiction” (the minimum level of drug exposure required for inducing addiction (Benowitz and Henningfield, 1994)) during cocaine self-administration may have never developed addictive-like behaviors to cocaine and consequently were removed from the study. We may have seen an impact on the level of cocaine craving during withdrawal if these rats were included in the study.

In another study, rats with low levels of anxiety were found to be more sensitive to amphetamine compared to rats with higher levels of anxiety (Lehner M.H. et al., 2017). Although there are differences in the duration of side effects and the origin of the drugs, cocaine and amphetamines are both stimulants and produce similar effects on the brain. (The Difference Between Amphetamines and Cocaine, 2018). This experiment utilized a conditioned place preference test and the two-injection protocol of sensitization (TIPS) to determine the impact of anxiety. As a result, further studies can take a similar approach to the Lehner M.H. et al. study to determine if these different measures of anxiety-like behaviors in rats have an impact on cocaine self-administration. Additionally, further studies can investigate the elevated plus maze as a measure of

anxiety-like behaviors, in addition to dividing the open field chamber into center and surround to determine levels of anxiety-like behaviors.

In addition to the behavioral measures analyzed in this study, there are potentially other rat-specific characteristics that could influence a drug addiction experiment, such as level of curiosity or intelligence. Also, it is possible that the behavioral measures studied in this experiment may impact the cocaine self-administration model, but further research into this topic is necessary to determine other measures of anxiety-like behaviors and craving of cocaine, if there are individual differences between rats, and how these differences may impact the cocaine self-administration model of addiction.

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