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Neuronal Effects of Cocaine in an Animal Model of Social Stress: Analysis of Neuronal Recordings

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Dedication

This thesis is dedicated to my granny Helen Jones and my aunt Barbara “Bopsy” Jones. I hope that I have made you proud. Forever my guardian angels.

Acknowledgements

First and foremost, I would like to thank Dr. Del Arco for his instruction and guidance throughout the thesis process. I am forever grateful for the patience and kindness that he has shown me in the time I've spent in his lab. He has a true passion for neuroscience and it was a pleasure to learn from him. I would also like to thank my second and third readers, Dr. Willett and Dr. Loprinzi for being a part of my committee and providing valuable feedback. I am thankful to have had such an amazing team of mentors on this journey. Next, I would like to thank the Sally McDonnell Barksdale Honors College for the unwavering support throughout the thesis writing process and my 4 years at the University of Mississippi. Special thanks to Dean Scurlock and Dr. Williams who I can never repay for helping me get to where I am today. I will never forget the time that I have spent in or the lessons that I have learned as an Honors student. Finally, I would like to thank my family, friends, and sisters for always believing in me. Without your support and constant encouragement, I would not have made it to this point. I hope that I continue to make you all proud.

Abstract

Studies that use Intermittent (episodic) Social Defeat (ISD) in rats demonstrate that ISD increases cocaine-self administration several weeks after the end of the adverse experience and suggest that a history of social stress makes individuals more vulnerable to substance abuse in the long term. The medial prefrontal cortex (mPFC) plays a key role in regulating drug-seeking behavior. The present study investigates whether ISD enhances the response of mPFC neurons to cocaine. Male Long Evans rats (3-4 months) were implanted with electrode arrays in the mPFC (prelimbic area) and divided into two groups (Control, n= 4; Stress, n= 4). They were then exposed to ISD or handling (control group) once every three days for ten days (four stress episodes in total). Three weeks after the last stress episode, control and stressed rats were injected with either cocaine or saline (10 mg/kg, i.e.) and the resultant effects on mPFC neuronal activity and locomotion were assessed. The results show that cocaine injections increase locomotion in both groups. However, cocaine-induced locomotion was stronger in stressed rats compared to controls (data not shown). Our results also show that mPFC neurons decrease their activity in response to cocaine injections in both stressed and control rats. These results suggest that ISD does not change the effects of cocaine in the mPFC and therefore do not support a role of this area of the brain in stress-induced behavioral sensitization to cocaine. *Supported by NIGMS-NIH P30GM12273*

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List of Abbreviations

ISD - Intermittent Social Defeat

mPFC - Medial Prefrontal Cortex

PFC - Prefrontal Cortex

Introduction

A. Stress and Drug Abuse

The link between stress and drug abuse have long been investigated. Exposure to stressful stimuli not only increases the likelihood of drug use but increases vulnerability to drug addiction (Sinha 2009). The connection between the two are largely fueled by the ability of drugs to modulate neural pathways that are altered by stress. Stimulant drugs such as cocaine are widely used in particularly stressful environments because of their ability to modulate the brain's drug-reward pathway after it has been impacted by stress (Hollon et al 2015).

A variety of human and animal studies have investigated stressed-induced vulnerability to drug use (Miczek et al 2008). In human studies, the correlation between stress exposure and drug relapse has been shown in several classes of drug users including cocaine abusers (Sinha et al. 1999b). Even with psychological intervention, drug abusers that are exposed to stressful situations can lead to the reintroduction of drug coping strategies. Several animal

models also support the finding that both chronic and short-term stress exposures increase the risk of drug abuse (Adler et al. 1972, Ramsey and Van Ree 1993). One stress model that has been used to investigate drug vulnerability in animals (i.e., rodents) is Intermittent Social Defeat (ISD). ISD can be described as a short-term exposure to physical or emotional deterrents that initiates a physiological response. In this model, a test rat is introduced into the enclosure of an aggressive peer rat. This typically leads to violent interactions and these confrontations can cause the test individual to develop behavioral deficits associated with conditions from depression to substance abuse (Miczek et al 2011). In instances of social defeat, stressed individuals exhibit a higher frequency of drug dependence. This can be attributed to both the development of stress coping mechanisms (Cooper et al. 1992) and the prevalence of drug craving after repeated drug use (Dackis and Gold 1985). Previous ISD animal models support the notion that animals exposed to ISD self-administer cocaine at significantly higher rates than in non-stress controls (Covington and Miczek 2001). Stressed animals also exhibit behavioral sensitization to other psychostimulant drugs such as amphetamine (Covington and Miczek 2001). While these models provide strong evidence to support the connection between stress and drug abuse, there has not been extensive investigation at the neuronal level. It is not yet understood which specific brain mechanisms cause ISD exposed animals to seek out drugs.

B. Stress and the Reward System

Past substance abuse research has largely centered around social determinants of abuse comorbidity. Specifically, investigators have sought to determine what types of stressful stimuli induce substance use and results in increased instances of relapse. Now that there is conclusive evidence to support the neurobiological link between social stress and substance abuse (Koob and Schulkin 2019), the current research focuses on the underlying mechanisms involved in the connection. To gain a greater understanding of the stress-drug abuse connection, we must first examine neuronal pathways affected by stress exposure and drugs of abuse.

Both stress and drugs of abuse have been shown to alter the brain's reward system (Hollon et al. 2015). The reward system includes two key components, the mesocorticolimbic dopamine system and the prefrontal cortex (PFC). Previous research has been done to support the link between substance abuse and the mesocorticolimbic dopamine system. Dopamine neurons from the ventral tegmental area project to the PFC as well as the basal ganglia where motor and reward-based learning is regulated (Alarco 2007). When an individual is exposed to stress, the mesocortical dopaminergic pathway is negatively affected (Baik 2020). Studies show that dopamine release from these neurons can both increase and decrease under stressful conditions (Baik 2020). In the

case of acute stress, the release of dopamine in the PFC has been observed to increase (Arnsten 2009). While this response is evolutionarily related to maintaining homeostasis, its habituation can cause an individual to have an increased risk of drug abuse (Arnsten 2009). Stimulant drugs such as cocaine stimulate dopaminergic pathways and attenuate the negative effects associated with stress exposure, which ultimately can increase vulnerability in stressed individuals (Koob and Schulkin, 2019).

Additional studies have investigated links between exposure to stressful stimuli and the functioning of interneurons in the medial prefrontal cortex (mPFC) (Page and Coutellier 2019). Under physiological conditions, the mPFC is regulated by an intricate balance of excitatory and inhibitory signaling. The functioning of these signaling circuits relies on the integrity of the neurons and their respective receptors. In cases of chronic stress exposure, the integrity of these neurons is compromised by changes in glutamate activity (McKlveen 2019). Both human and animal models support that changes in receptor expression weaken the connection between the mPFC and the amygdala, the brain's key emotional processing center. Ultimately, these neuronal changes lead to the inability to properly regulate the negative emotions associated with stress exposure (McKlveen 2019). Without proper emotional regulation, stressed individuals are at increased risk for abusing unhealthy coping mechanisms such as stimulant drugs.

The current research aims to investigate the properties of neuronal changes in the mPFC under the interaction between stress and drugs of abuse. Previous studies suggest that ISD exposure induces effects on the mesocorticolimbic dopamine system and leads to increased cocaine-seeking behavior (Leonard, Miczek 2022). In fact, results have shown that cocaine use provides increased dopamine release in stressed animals when compared to controls (Leonard, Miczek 2022). While these findings provide evidence of the role of the brain's reward pathway in stress-abuse liability outcomes, it is not yet known whether ISD changes the effects of cocaine in the mPFC.

C. Hypothesis and Goals

The present study aimed to investigate whether exposure to ISD has effects on the activity of mPFC neurons following cocaine administration. The mPFC regulates drug-seeking behavior (Riaz et al 2019). Previous studies showed that ISD increases cocaine-self administration in rats several weeks after stress exposure has ceased. Based on this evidence, the working hypothesis of the current research was that ISD leads to an enhanced response of mPFC neurons to cocaine exposure. To test this hypothesis, we exposed rats to IDS and measured neuronal activity via *in vivo* electrophysiology following cocaine administration. A temporal map of mPFC neuronal activity was created to gauge the neuronal responses to cocaine (and saline) in stressed and control rats.

The current thesis is part of a bigger study that investigated additional behavioral and electrophysiological outcomes. The experimental part of this study was completed when I joined the laboratory and therefore my involvement was in the analysis of electrophysiological data related to cocaine and saline injections. In doing so, I learned to use analytical software (offline sorter) required for analyzing the neuronal recording channels corresponding to recording sessions in which cocaine and saline injections were administered

Methods

a. Experimental and stress protocol

Male Long Evans rats (3-4 months of age) were implanted with electrode arrays in the mPFC through stereotaxic surgery as previously described (Del Arco et al 2020). Two weeks after surgery, rats were split into two groups (control, n=4; stress n=4). Rats in the ISD group were exposed to 4 sessions of social defeat using the resident/intruder paradigm (Figure 1) (Lemon and Del Arco 2022). Intruder rats were introduced to the resident's cage and separated by a barrier for 10 minutes. After 10 minutes elapsed, the barrier was removed, and the rats were allowed to interact. Interaction continued until 6 attack incidents occurred, the intruder was in the supine position for 5 minutes or at the conclusion of 5 minutes. Thereafter, the barrier was returned to its original position and the intruder occupied the resident's cage for an additional 10 minutes. Control rats were moved to an alternative location during these interactions and handled.

Three weeks after the termination of the stress protocol, rats were placed in an operant box and connected to the recording system. After a habituation period (35 min), they were injected with cocaine (cocaine hydrochloride, Sigma-Aldrich) (10 mg/kg, i.e.) or saline (counterbalanced order). Motor and neuronal activity recordings began for 5 minutes before injections and continued for 15 minutes following injections. After the termination of recording sessions, animals were taken back to their home cages.

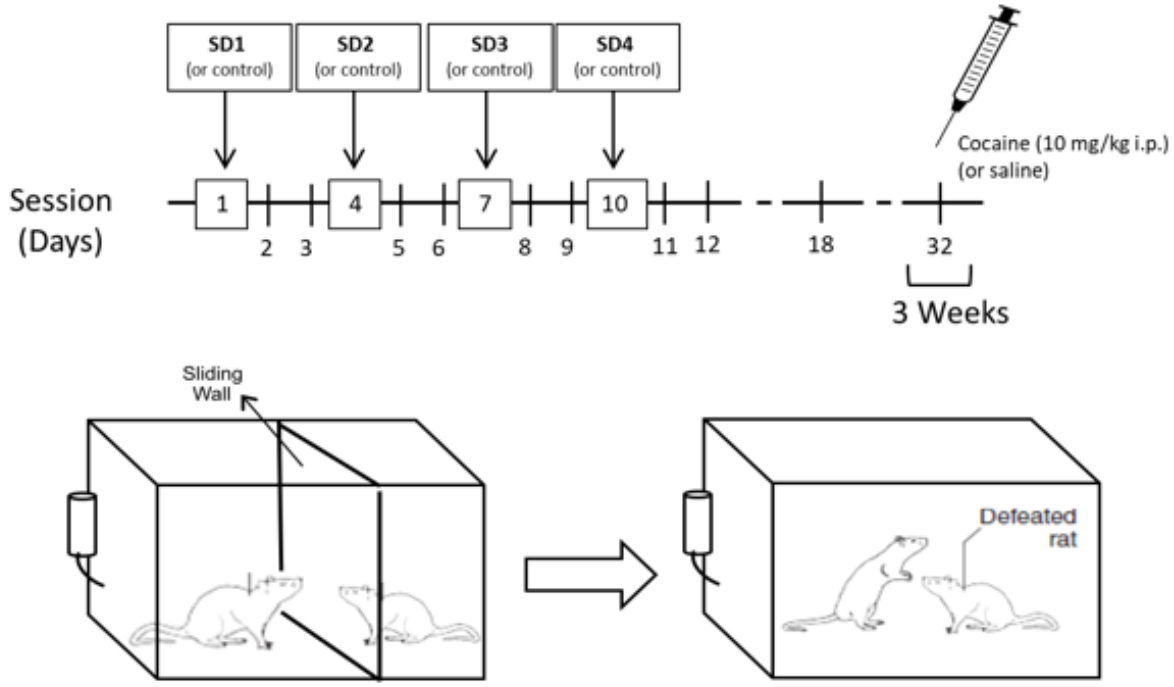

Figure 1

Diagram of the protocol (top) and experimental setup (bottom) for Intermittent Social Defeat (ISD) stress model.

b. Analysis of electrophysiological data

The individual neuronal units were sorted through analysis by Offline Sorter (Plexon) technology. Figure 2 shows a summary of the neuronal recording set up and analysis as well as the anatomical location of the electrodes in the mPFC (Del Arco et al., 2020). Rats implanted with electrodes in the mPFC were placed in test chambers and connected to the acquisition system through a recording cable. The test chamber was connected to a computer where the *in vivo* neuronal activity was recorded throughout the completion of the task. The raw data for each neuron channel was saved onto a hard drive for further analysis using the Offline Sorter (Plexon) technology. Once imported into the Offline Sorter, we were able to visualize individual neuronal units (Yellow, green and blue: Figure 2). Using parameters based on action potential waveforms, we were able to isolate these units that were recorded during the task.

The firing rate of single units were normalized according to baseline (z score). Units were classified as activated or inhibited by cocaine (or saline) exposure if their average activity during the 10 min after injection was $Z > 2$ or $Z < 2$, respectively. A chi-square analysis was performed to compare the number of individual units that were activated or inhibited by cocaine or saline in both control and stressed rats. A $p < 0.05$ was considered statistically significant.

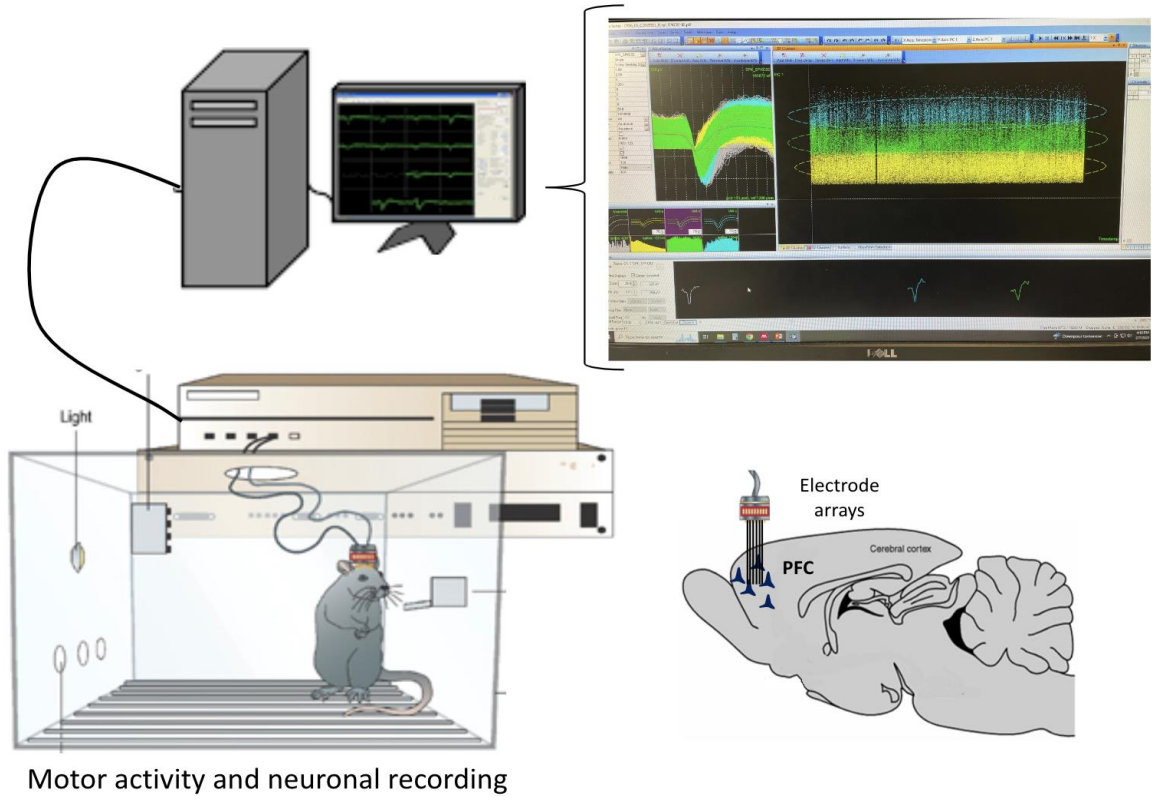

Figure 2

Diagram of the experimental set up for in vivo electrophysiology recording and offline sorting analysis of single neurons (see text for details).

Results

a. mPFC neuronal response to cocaine and saline injections

In vivo neuronal recordings were used to assess changes in the activity of single neurons isolated by offline sorting techniques. Figure 3 is a heat map that shows the changes in activity of isolated mPFC units. The plot revealed that cocaine injections decreased the activity of mPFC neurons in both stressed and control individuals compared to saline injections. These effects were stronger during the first 10 min after cocaine injections. Interestingly, the results of Figure 3 also show a clear increase in the firing rates of mPFC neurons following the injection of saline in stressed rats compared to control rats.

Figure 4 quantifies the neuronal activity depicted in Figure 3. Specifically, Figure 4 shows the number of units that were significantly activated or inhibited by cocaine or saline injections. As shown, cocaine increased the number of units inhibited in control [$\chi^2 = 11.57$, $p < 0.01$] and stressed [$\chi^2 = 4.79$, $p < 0.09$] rats, although in this last case this effect did not reach statistical significance. There were no significant differences in the number of units inhibited by cocaine

between control and stressed rats [χ^2=0.34, p> 0.1]. In contrast, there were more units activated by saline in stressed compared to control rats [χ^2=6.62, p< 0.05].

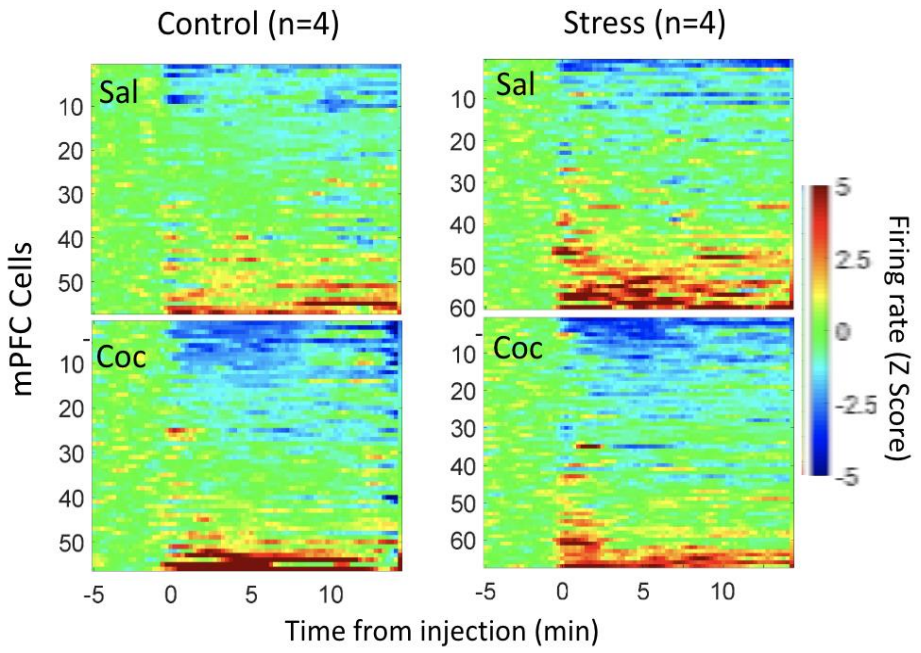

Figure 3

Heat plots show the normalized firing rate of mPFC neurons before and after cocaine injections (Coc), compared to saline (Sal), in control (left column) and stressed (right column) rats. Every row represents one single neuron. Red colors and blue colors represent increases and decreases in firing rate, respectively. The results of this Figure are quantified in Figure 4.

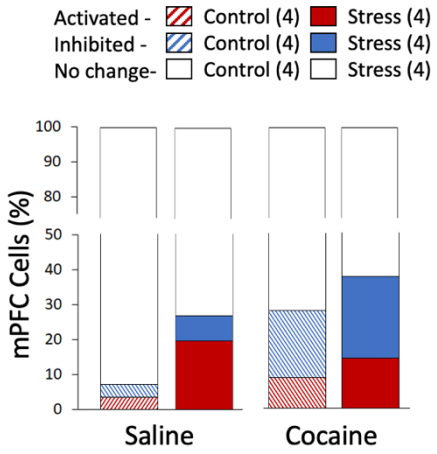

Figure 4

Number of mPFC cells (%) that increase (red) and decrease (blue) their firing rate after saline and cocaine injections in control and stressed rats. Cocaine decreases neuronal activity in both groups of rats. Saline increases neuronal activity in stressed compared to control rats.

Discussion

a. Summary of Findings

Previous animal models have supported the link between stress exposure and drug administration. ISD exposure increases drug self-administration and locomotion compared to controls (Miczek et al., 2011). With the mPFC housing a brain's key hub for regulating reward-seeking behavior it was hypothesized that cocaine would produce different effects in the neuronal activity of the mPFC neurons of stressed compared to control subjects. However, the results of the current study do not support this hypothesis. When individual neuronal units were analyzed, both control and stressed rats that received cocaine injections showed a decrease in the neuronal activity in the mPFC. There was no significant difference in this effect across both groups which suggests that exposure to ISD does not change the neuronal response to cocaine in the mPFC.

b. Electrophysiological Sorting

My primary role in the current study was sorting the electrophysiological data into individual neuronal units. Isolating these units allowed us to locate the temporal dynamics of activation/inhibition after cocaine injections within the mPFC. In sorting the neuronal units, a semi-automatic approach (i.e., manual and template) was utilized. The same process was realized for both the control and stressed animals. This methodology is standard and utilized by us as well as many other laboratories (Del Arco et al., 2020; Narayanan and Laubach, 2009). However, new studies are applying full automatic approaches to sort out neurons that are more accurate and faster (Chung et al., 2017).

Briefly, the neuronal channels were first evaluated to eliminate the channels that did not contain cells. There were 16 channels per rat and each was analyzed. After selecting the correct channel, the threshold was set to -0.7 and the magnification was set to 25x for action potential detection. Channels were cleaned using parameters based on the action potential waveform. A principal component analysis (PCA) was performed to remove noise and isolate single units. The principal components included measurements of peaks and valleys, various energy forms, and the time-stamp before and after injection. Most channels only contained one cell however there were instances of up to 3 cells being present (see Figure 2, for example). After manually locating a cell, the template was run to create clusters corresponding to the isolated cells that

contained at least 85% of the signal. Once the units were isolated in a channel, the data were saved and imported to matlab for further analysis and quantifications.

c. Cocaine and the mPFC

The mPFC plays an important role in goal-directed behavior. Goal-directed behavior consists of tasks that involve some kind of emotional component. These tasks usually involve an individual weighing the risks of the tasks with the potential reward (Gazit et al. 2020). ISD exposed animals show behavioral sensitivity to cocaine as well as increased self-administration of cocaine. Given that the mPFC regulates drug-seeking behavior, we hypothesized that cocaine would produce stronger effects on the mPFC of stressed compared to control animals.

We found the opposite effect that cocaine reduces neuronal activity in both control and stressed animals. Previous studies have shown that both acute and chronic cocaine administration reduce cortical neuronal activity (Chen et al., 2019; Chen et al., 2013). This result could be due to the fact that the activity could be mediated by dopamine release in the mPFC. Cocaine increases the release of dopamine in the mPFC which could play a role in the observed behavior (Han et. al 2014). Indirectly, these results suggest that the release of

dopamine in the mPFC is not changed in stressed individuals compared to controls. Further studies would be needed to substantiate this speculation.

In addition to dopamine release, the fact that the effects of cocaine are not different in stressed animals suggest that other areas of the brain are involved. These other areas such as the basal ganglia may account for the stronger behavioral effects (i.e., behavioral sensitization) following ISD as opposed to the mPFC (Miczek 2011).

d. Saline injection as an aversive experience

An unexpected result of the present study was that saline injection increased neuronal activity in the mPFC in stressed individuals when compared to controls. This effect could be due to developed hypersensitivity to aversive stimuli in stressed individuals compared to control individuals. Previous studies have noted aversive stimuli produce stronger behavioral and physiological effects in stressed animals compared to controls (Ulrich-Lai and Herman, 2009). This hypersensitivity following physiologically stressful experiences has also been noted to lead to anxiety and burnout in human studies (Boogert et al. 2022). While this doesn't reveal insight into the connection between drug sensitivity and stress, it does confirm that ISD alters the brain processing of aversive stimuli and also gives us a better idea of the role stress plays in behavior. The increased sensitivity to stimuli in general can lead to the development of secondary

conditions and noting these connections can help us potentially prevent them. In addition, an enhanced sensitivity to aversive stimuli has been associated with substance use disorders as well as other psychiatric disorders (Thibeault et al., 2019).

e. Limitations

Though the study did produce valuable findings, there are limitations that could affect the interpretation of the results. First, there were only 4 rats analyzed for this thesis project. The analysis of the additional 4 rats in the study may have changed the activity observed. Additionally, only one dose of cocaine was administered in the study. Without the administration of other doses of cocaine, we do not know if the same results will appear in different concentrations. There might not have been a distinction between the activity saline and cocaine in the dose used but a higher or lower dose could have produced alternative results. Finally, cocaine was injected into the rats as opposed to self-administered. There are clear behavior differences noted between injection and self-administration so this could have affected the results.

Conclusion

In vivo neuronal recordings assess changes in the activity of single neurons that can be isolated by offline sorting techniques. Cocaine injections decrease neuronal activity in the mPFC of control and stressed rats. This effect was not different between both groups which suggests that ISD does not change the neuronal response to cocaine in the mPFC. These results suggest that the mPFC does not contribute to the stronger behavioral effects of cocaine after social stress found in previous studies.

References

Adler, M. W., Bendotti, C., Ghezzi, D., Samanin, R., & Valzelli, L. (1975). Dependence to morphine in differentially housed rats. *Psychopharmacologia*, *41*(1), 15–18. https://doi.org/10.1007/BF00421299

Alcaro, A., Huber, R., & Panksepp, J. (2007). Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective. *Brain research reviews*, *56*(2), 283–321. https://doi.org/10.1016/j.brainresrev.2007.07.014

Arnsten A. F. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature reviews. Neuroscience*, *10*(6), 410–422. https://doi.org/10.1038/nrn2648

Baik J. H. (2020). Stress and the dopaminergic reward system. *Experimental & molecular medicine*, *52*(12), 1879–1890. https://doi.org/10.1038/s12276-020-00532-4

Chen, B. T., Yau, H. J., Hatch, C., Kusumoto-Yoshida, I., Cho, S. L., Hopf, F. W., & Bonci, A. (2013). Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. *Nature*, *496*(7445), 359–362. https://doi.org/10.1038/nature12024

Chen, W., Volkow, N. D., Li, J., Pan, Y., & Du, C. (2019). Cocaine Decreases Spontaneous Neuronal Activity and Increases Low-Frequency Neuronal and Hemodynamic Cortical Oscillations. *Cerebral cortex (New York, N.Y. : 1991)*, *29*(4), 1594–1606. https://doi.org/10.1093/cercor/bhy05

Chung, J. E., Magland, J. F., Barnett, A. H., Tolosa, V. M., Tooker, A. C., Lee, K. Y., Shah, K. G., Felix, S. H., Frank, L. M., & Greengard, L. F. (2017). A Fully Automated Approach to Spike Sorting. *Neuron*, *95*(6), 1381–1394.e6. https://doi.org/10.1016/j.neuron.2017.08.030

Cooper, M. L., Russell, M., Skinner, J. B., & Windle, M. (1992). Development and validation of a three-dimensional measure of drinking motives. Psychological Assessment, 4(2), 123–132. https://doi.org/10.1037/1040-3590.4.2.123,

Covington, H. E., 3rd, & Miczek, K. A. (2001). Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology*, *158*(4), 388–398. https://doi.org/10.1007/s002130100858

Dackis, C. A., & Gold, M. S. (1985). New concepts in cocaine addiction: the dopamine depletion hypothesis. *Neuroscience and biobehavioral reviews*, *9*(3), 469–477. https://doi.org/10.1016/0149-7634(85)90022-3

Del Arco, A., Park, J., & Moghaddam, B. (2020). Unanticipated Stressful and Rewarding Experiences Engage the Same Prefrontal Cortex and Ventral Tegmental Area Neuronal Populations. *eNeuro*, *7*(3), ENEURO.0029-20.2020. https://doi.org/10.1523/ENEURO.0029-20.2020

Hollon, N. G., Burgeno, L. M., & Phillips, P. E. (2015). Stress effects on the neural substrates of motivated behavior. *Nature neuroscience*, *18*(10), 1405–1412. https://doi.org/10.1038/nn.4114

Koob, G. F., & Schulkin, J. (2019). Addiction and stress: An allostatic view. *Neuroscience and biobehavioral reviews*, *106*, 245–262. https://doi.org/10.1016/j.neubiorev.2018.09.008

Lemon, C., & Del Arco, A. (2022). Intermittent social stress produces different short- and long-term effects on effort-based reward-seeking behavior. *Behavioral brain research*, *417*, 113613. https://doi.org/10.1016/j.bbr.2021.113613

Leonard, M.Z. and Miczek, K.A. (2022). Cues Associated with Social Defeat Stress Recruit CRF in the Nucleus Accumbens and Invigorate Drug-Seeking following Abstinence from Cocaine. The FASEB Journal, 36:. https://doi.org/10.1096/fasebj.2022.36.S1.L7914,

McKlveen, J. M., Moloney, R. D., Scheimann, J. R., Myers, B., & Herman, J. P. (2019). "Braking" the Prefrontal Cortex: The Role of Glucocorticoids and Interneurons in Stress Adaptation and Pathology. *Biological psychiatry*, *86*(9), 669–681. https://doi.org/10.1016/j.biopsych.2019.04.032

Miczek, K. A., Nikulina, E. M., Shimamoto, A., & Covington, H. E., 3rd (2011). Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *31*(27), 9848–9857.

Miczek, K. A., Yap, J. J., & Covington, H. E., 3rd (2008). Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacology & therapeutics*, *120*(2), 102–128. https://doi.org/10.1016/j.pharmthera.2008.07.006

Narayanan, N. S., & Laubach, M. (2009). Methods for studying functional interactions among neuronal populations. *Methods in molecular biology (Clifton, N.J.)*, *489*, 135–165. https://doi.org/10.1007/978-1-59745-543-5_7

Ramsey, N. F., & Van Ree, J. M. (1993). Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. *Brain research*, *608*(2), 216–222. https://doi.org/10.1016/0006-8993(93)91461-z

Page, C. E., Shepard, R., Heslin, K., & Coutellier, L. (2019). Prefrontal parvalbumin cells are sensitive to stress and mediate anxiety-related behaviors in female mice. *Scientific reports*, *9*(1), 19772. https://doi.org/10.1038/s41598-019-56424-9

Riaz, S., Puveendrakumaran, P., Khan, D., Yoon, S., Hamel, L., & Ito, R. (2019). Prelimbic and infralimbic cortical inactivations attenuate contextually driven discriminative responding for reward. *Scientific reports*, *9*(1), 3982. https://doi.org/10.1038/s41598-019-40532-7

Sinha R. (2008). Chronic stress, drug use, and vulnerability to addiction. *Annals of the New York Academy of Sciences*, *1141*, 105–130. https://doi.org/10.1196/annals.1441.030

Sinha, R., Catapano, D., & O'Malley, S. (1999). Stress-induced craving and stress response in cocaine dependent individuals. *Psychopharmacology*, *142*(4), 343–351. https://doi.org/10.1007/s002130050898

Thibeault, K. C., Kutlu, M. G., Sanders, C., & Calipari, E. S. (2019). Cell-type and projection-specific dopaminergic encoding of aversive stimuli in addiction. *Brain research*, *1713*, 1–15. https://doi.org/10.1016/j.brainres.2018.12.024

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature reviews. Neuroscience*, *10*(6), 397–409. https://doi.org/10.1038/nrn2647