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Prelimbic Cortex Neuronal Assemblies In Behaviors Predictive Of Drug-Seeking Before And After Cocaine Self-Administration

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Dedication

I want to dedicate my thesis to my family, specifically to my mother who always supported me through this journey. Thank you for always been there and motivating me to keep going every time I doubted myself and wanted to give up. To Richard Doyle, a dear family friend who financed my tuition through the Dawn Doyle-Chilsom Memorial Scholarship that assists other students like me to further pursue their education and with his kindness and guidance had made this academic journey possible.

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by

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Abstract

Literature has established that there are certain behaviors that are predictive of drug seeking and relapse such as impulsivity, distress tolerance (DT), Pavlovian conditioned approach (PCA), anxiety and sensation-seeking. However, few studies have examined the behavioral interactions among these tasks and drug-seeking, and none have examined the neural interactions in the prelimbic cortex, which has an essential role in drug-seeking and is implicated in each of the aforementioned behaviors of interest.

We therefore hypothesized that certain behaviors including high impulsivity, low distress tolerance, high anxiety, and high locomotor activity would predict high cocaine-seeking. We further hypothesized that the neuronal activity in the prelimbic cortex during these predictive behaviors would also predict cocaine-seeking.

Sprague Dawley rats underwent a GCAMP6s viral infusion and lens surgery and performed various tasks upon recovery, and their prelimbic activity was recorded via calcium imaging. Afterwards, rats underwent cocaine or water self-administration for 2 weeks followed by an extinction task to measure drug-seeking, and the original behaviors were reassessed. Sex differences were observed, with females showing less anxiety and higher distress tolerance. Cocaine decreased distress tolerance.

Neuronal activity didn't significantly change following cocaine but correlated with self-administration behaviors. Notably, there were significant interactions between behaviors and their neuronal activity with self-administration and reward-seeking. Specifically, sensation seeking behavior predicted water and cocaine seeking, prelimbic activity during DT predicted cocaine and water self-administration, and prelimbic activity during PCA predicts cocaine and

water seeking. Overall, cocaine reduced distress tolerance but had limited effects elsewhere. However, behaviors and prelimbic activity significantly predicted drug intake and reward-seeking. Understanding these connections could inform targeted therapies for individuals prone to drug seeking and relapse.

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1.Introduction

1.1 Background

Certain behaviors are predictive of aspects of substance use disorders such as drug-seeking and relapse including distress tolerance (Moschak et al. 2018, Daughters et al. 2005, Koob et al. 2016, Gerra et al. 1999), incentive salience (Koob et al. 2016, Saunders et al.2010), impulsivity (Moschak et al. 2021, Gerra et al. 2004 and Economidou et al. 2009), anxiety (Pelloux et al. 2009), and novelty-seeking (Belin et al. 2008, Belin et al. 2016, Gerra et al. 2004).

Specifically, several studies have demonstrated that impulsivity predicts drug reinforcement and escalation of intake (Belin et al 2008; Carrol et al 2009; Dawley et al. 2007; and Diegaarde et al. 2008). Furthermore, distress tolerance (DT), defined as persistence in the engagement in a challenging task (Moschak et al. 2017), predicts the duration of drug abstinence (Daughters et al., 2005) as well as drug-seeking (Moschak et al. 2017). Other work has demonstrated that high incentive salience for cues, measured as a function of an individual's propensity to interact with cues paired with a reward over the area where a reward is delivered, is associated with higher drug-seeking behavior (Lovic et al. 2011). Additionally, rats with high anxiety-like behavior in the elevated plus maze (EPM) have increased cocaine intake in self-administration (Pelloux et al. 2009). Finally, locomotor activity in response to novel environment is higher in individuals with substance use disorder (Guerra et al. 2004) and predicts drug intake initiation (Nees et al. 2012; Sargent et al. 2010, Belin et al. 2008).

Multiple brain regions are involved in addiction (Koob et al. 2016). However, for the current study, we chose to investigate the prelimbic cortex because of its involvement in all our behaviors of interest. It is essential in the role of drug-seeking (McFarland et al. 2003, MacLaughlin et al. 2003), shows neuroplasticity after long periods of abstinence (West et al.

2014, Shin et al. 2018), and is implicated in the predictive behaviors of interest including incentive salience (Saddoris et al. 2016, and Haight et al. 2017), impulsivity (Moschak et al. 2021, Robbins et al. 2017 and Brevet-Aeby et al. 2016), distress tolerance (Moschak et al. 2023), anxiety (Hage et al 2012 and Lacroix et al. 1998) and locomotor activity (Lacroix et al. 1998 and Calipari et al. 2013). Understanding the relationship between behaviors and neuronal assemblies could serve as the underpinning of improved therapeutics for drug addiction.

Each behavior we selected to conduct our study had been previously established as a predictor of drug-seeking individually but neither it nor the underlying neural activity has been looked at in combination with other behaviors and drug-seeking.

1.2 Hypothesis

Previous studies established the relationship between individual behaviors and drug-seeking, but they have not investigated the role of the prelimbic cortex. Given the important role that the prelimbic hypothesis plays in each of the aforementioned behaviors and drug-seeking, we expect that neural activity during each of these behaviors will predict cocaine-seeking behavior. Specifically, we first hypothesize that increased excited activity during the impulsive task will predict high cocaine-seeking, since excited prelimbic activity predicts impulsive action (Moschak et al., 2021).

We postulate that during incentive salience increased excited activity like that seen in impulsive rats will also predict high cocaine-seeking given that rats considered as sign-trackers to also possess high impulsivity (Lovic et al. 2011; but see Haight et al. 2014). We further speculate that given that a history of cocaine administration reduces locomotion and functional activity in the prefrontal cortex (Calipari et al. 2013) that higher inhibition in the neuronal makeup of locomotor activity may predict drug-seeking. Additionally, since animals with higher

distress tolerance have higher phasic activity in the prelimbic cortex but those with low distress tolerance have higher drug-seeking (Moschak et al. 2023) we theorize that higher levels of nonphasic cells in the distress tolerance task to be predictive of drug-seeking. Lastly, given that lower activity in the prelimbic cortex is associated with higher anxiety (Jink et al 1997) and lower neuronal activity in the same brain region is associated with low drug-seeking (McLaughlin et al 2002) then we hypothesize that low prelimbic activity during high anxiety will be predictive of low drug-seeking.

1.3 Aims

Aim 1: Analyze both behavior and neuronal activity before and after cocaine or water self-administration

2. Research Strategy

We used female (n=11) and male (n=14) adult Sprague Dawley rats according to the following timeline (**Figure 1**).

2.1 Surgeries

Rats were subject to two distinct surgeries at different periods of the project's timeline. In the initial one, rats were subjected to a viral infusion of GCAMP6s and lens implantation in the prelimbic area (coordinates AP: +2.7 and ML:-/+ 0.6) at interchanging left and right sides from bregma. Animals received a ketamine and xylazine mixture as anesthesia and were allowed for a one-week post-surgery recovery period. Animals were trained in both the titration and PCA tasks (order counterbalanced) for approximately 2-3 weeks. After a 6-week viral infusion procedure, a cell check was conducted with the Miniscope software; rodents with a visual appearance of cells were installed with miniscope baseplates and proceed for intrajugular catheter implantation. Following recovery animals ran in the following tasks while neural activity was recorded using endoscopic in vivo calcium imaging.

2.2 Behavioral Tasks

Titration Task

The Titration Task (TT) is a behavioral measurement of impulsive action (Moschak et al. 2021). Animals were placed in the conditioning operant chamber where they must press the incoming lever after the cue light turns on for them to receive a reward (interchanging raspberry or peanut butter sugar pellet). Early errors are defined as lever presses before the appearance of the cue light. Impulsivity is calculated as the number of early errors divided by the total lever presses. Animals were food restricted (received 5- 25 grams of pellets depending on performance).

Distress Tolerance Task

Our Distress Tolerance (DT) task is based on the modeled rat version (Moschak et al. 2017) in which rats engaged in a structurally similar task to the TT with decreasing intervals of cue duration until it is impossible for the rats to have correct responses. DT is measured by the rat's ability to persist in engaging in the task while being distressed. Animals were food restricted (placed in free feed afterwards).

Incentive Salience

Incentive Salience is measured through the Pavlovian Conditioned Approach Task. Rats were placed into the operant chamber where a lever extended for eight seconds. Then a sugar flavored (peanut butter or raspberry) pellet was delivered independently of the rat's response to the lever. Furthermore, the positioning of levers is vertical instead of horizontal to differentiate between this task and the titration task. To further distinguish the task, rats received different flavored pellets that they received in the TT and the DT. Animals were removed from food restriction the prior day.

Novelty-Seeking Task

Novelty-seeking is measured through locomotor activity. Subjects were placed inside a squared transparent box with no objects inside. Animals moved and explored their environment. While rodents explore the locomotor chamber, the program tracks the movements and positioning of the individual. Animals were removed from food restriction the prior day.

Anxiety Inducing Task

To measure anxiety, we utilized an elevated plus maze (44.5in x 44.5 in x 44 in Med Associates) that consists of a plus-shaped maze with two connected walled platforms and two connected platforms without walls. During this task, rats were placed in the middle of the elevated

plus maze and left alone in dim lighting in the room for 10 minutes. During the set time, the rodents are allowed to move anywhere they preferred in the maze. Rats that stay in closed arms more rather than open arms are considered more anxious (Montgomery et al. 1958).

Self-Administration

Drug self-administration is a task in which animals are allowed to self-administer a drug for a determined time. Each session took 6 hours to allow an escalation of cocaine intake (Ahmed et al. 1998) which may be predicted by behaviors such as high impulsivity (Badiani et al. 2013). During the task, the rat placed its nose inside an illuminated (cue) nose poke to either dispense water to the water dispenser or administer an intravenous dose of cocaine (reward). Doses of cocaine were based on the rat's weight before being introduced to the operant chamber. When dispensing the reward, the whole box illuminated, and a tone sounded. Rodents were not able to receive another dose until the cease of the sound and light turned off (20 s). Rats were water restricted. In the water groups the male received 20 ml meanwhile the female received 15 ml. On the other hand, the males in the cocaine group received 30 ml and females 25 ml.

Extinction

Individuals with Substance Use Disorder have the inability to refrain from drug seeking (Spanagel et al. 2017, Beckman et al. 2011) which can be observed in animals as well. Extinction is a task that we utilized to measure drug-seeking. After the 14th day of self-administration rats were placed in their boxes, and they received the tone and the light, which are the drug-paired cues, but neither cocaine nor water was dispensed. The program lasted 2 hours and the expected persistence in nose pokes to obtain a reward served to measure drug-seeking or water-seeking respectively. Rats remain in water restriction and are free water after task.

2.3 Endoscopic In Vivo Calcium Imaging

Imaging calcium signals allows the visualization of neuronal assemblies' activation in awake, behaving animals (Rusell 2011) that cannot be reproduced *in vitro*. Imaging was recorded by using a Miniscope system (UCLA Miniscope, Los Angeles, CA) with time-locked task events. Specifically, the Miniscope camera was attached to the implanted baseplate already on the rodent. It is connected via a tether to a commutator that avoids the tangling of the camera and the cable when the rat moved during the task.

Further, the other side of the tether was connected to a DAQ which received further signals from the MED-PC through a TTL and is connected directly to a laptop. On the laptop, we used the Miniscope software to track the neurons' activity in real time. The program recorded the neuronal assemblies and allowed us to see their activity while the rat performed the task of interest. The recording were fractionated into segments; for 10 minutes the LED blue light is on and imaging was recorded followed by a 5 min LED-off break to avoid photobleaching.

To ensure the plane of view is the same across different behaviors, an initial laptop screenshot prior to the start of every task was taken and was compared to previous images to ensure recordings showed the same cellular assemblies seen throughout all behaviors of interest.

3. Data Analysis

The behavioral analysis of the behaviors of interest was partly dependent on each task. For example, for the Impulsivity task, we calculated the early responses to the lever divided by the total responses; meanwhile, for the distress tolerance, we observed the time of how long it took the rat to commit five consecutive omission interactions with the lever. For the anxiety task, we measured the time spent on the open arms versus the closed arms in the elevated plus maze. Meanwhile, for novelty-seeking, we measured how much the rat moved in the locomotor activity chamber. For incentive salience, we utilized the PCA index, which is defined as the average of 1) the proportion of total lever presses over total nose pokes, 2) the probability of lever presses over the probability of nose pokes on a given trial, and 3) the proportion of trials where a lever press preceded a nose poke or vice versa. Once we obtained the desired data through MedPC (v.5) we used Microsoft Excel as well as IBM SPSS Statistics version 27 to conduct 2x2x2 mixed design ANOVAs (2, sex), (2, solution) and (2, session) to assess the effects of drug on these behaviors. In addition, we used Pearson correlations to see if each behavior predictor predicted self-administration, and water and cocaine EXT, furthermore, to see the effect of how cocaine affected behavior. Once we obtain all numerical data, we proceeded to utilize GraphPad Prism 10 to create our graphs and charts.

On the other hand, for the neuronal activity data, the recordings were collected and paired with the behavioral data for each task. Once recordings and files were paired and videos were verified for quality, the recordings proceeded to be downsampled to facilitate analysis by other software. Once downsampling was completed, we used the program CalmAn's Constrained NonNegative Matrix Factorization - microEndoscope (CNMF-E) to establish fluorescent activity across time for individual neurons (Giovannucci et al. 2019). This was converted into estimated

spiking activity (arbitrary deconvolved units) using CaImAn's deconvolution algorithm. Then we used a script for Multisession Registration to align individual neurons across multiple videos and behaviors. CaImAn produced a set of text files containing the deconvolved spike train for each neuron, whether the neuron is valid, and the alignment of each neuron across multiple behavioral sessions. This data was organized with a Matlab script and exported to another software known as NeuroExplorer.

This program allows us to observe each neuron's activity during task trials through PeriEvent Histograms and provides us numerical data to calculate the statistical relevance of the neuronal assemblies' excitation and inhibition between different behaviors. In the impulsivity, distress tolerance, incentive salience (PCA) locomotor, and extinction tasks, a within-subjects T-test compared a 10 s baseline before lever extension to the 600 ms time following lever extension to determine if there is a significant increase or decrease in activity. For the elevated plus maze test, the T-test compared relative overall neural activity in the open arms vs. the closed arms. Neurons with a significant increase in activity was classified as "Excited", those with a significant decrease in activity were classified as "Inhibited", and all others were classified as "Nonphasic" (see **Figure 2**). All behaviors except drug acquisition were repeated after post-self-administration to measure any neuronal population change before and after drug intake.

4. Histology

On completion of the experiment, animals were euthanized with an overdose of isoflurane (Covetrus, ANADA), the brain was removed, and it was left overnight in 4 % paraformaldehyde and transferred to 30% sucrose the following day. After that, brains were sectioned at 30µm by a cryostat and mounted on slides to verify lens placement verification with a fluorescent microscope (**Figure 3**) and it was later aligned according to an atlas (Paxinos and Watson, 1998. See **Figure 4**)

5. Results

We collected a series of both behavioral and neuronal outcomes in impulsivity, distress tolerance, incentive salience, anxiety, and sensation-seeking in adult males and females. We did not observe any significant sex difference during the impulsivity task (**Figure 5A**) as well as in the incentive salience task (**Figure 5E**). On the other hand, we observed significant sex differences in the remaining behaviors. Regarding the distress tolerance task (**Figure 5B**), we observed that females had significant higher ($F(1,23) = 16.307$, $p = <0.001$) distress tolerance than males. Regarding the sensation seeking task (**Figure 5C**), females had higher locomotor activity ($F(1,18) = 17.930$, $p = <0.001$). Lastly, regarding anxiety (**Figure 5D**), females spent more time on the open arm of the maze ($F(1,23) = 7.137$, $p = 0.014$).

Regarding the behavioral impact and changes by cocaine usage we observed that in the impulsivity task (**Figure 6A**) there was no change in impulsivity in our control group and unexpectedly there was no significant increase in impulsivity after cocaine intake either (Drug x Session interaction: $F(1,23) = 0.114$, $p = 0.739$). On the other hand, for the distress tolerance task (**Figure 6B**) we observed a significant (Drug x Session interaction: $F(1,23) = 6.060$, $p = 0.022$; Bonferonni post hoc test was significant comparing naïve to abstinent cocaine) decrease in the persistence to participate following a history of cocaine. Further, for the sensation seeking task (**Figure 6C**) there was no change in ambulation in our control group and no significance in the experimental group (Drug x Session interaction: $F(1,18) = 2.924$, $p = 0.104$). For anxiety (**Figure 6D**) there was no change after self-administration (Drug x Session interaction: $F(1,23) = 0.833$, $p = 0.371$). Interestingly, regarding incentive salience (**Figure 6E**) there was a change in behavior (Drug x Session interaction: $F(1,20) = 9.161$, $p = 0.007$), animals became more sign-trackers after

self-administration regardless of if they received water or cocaine (Drug x Session interaction: $F(1,20) = 0.002$, $p=0.963$).

We further pursued the analysis of the correlation between the amount of cocaine and water intake on the change in behavior. Regarding change in impulsivity, there was no correlation between the water group ($r= 0.137$, $p=0.641$) nor the cocaine group ($r=0.1794$, $p=0.557$). For the distress tolerance group there was no correlation between change in distress tolerance and amount of cocaine intake ($r=-0.091$, $p=0.769$) nor water water intake ($r=-0.236$, $p=0.417$). In the incentive salience task, there was no correlation between change in incentive salience and amount of cocaine intake ($r=0.008$, $p=0.977$) nor water intake ($r=0.074$, $p=0.802$). Furthermore, there was no correlation between change in sensation seeking and cocaine intake ($r=0.235$, $p=0.439$) nor water intake ($r=-0.214$, $p=0.465$). Lastly, there was no correlation between change in anxiety and the amount of cocaine intake ($r=-0.025$, $p=0.935$) nor water intake ($r=-0.389$, $p=0.169$)

Self-administration and reward-seeking were used as behavioral measures to study drug-addiction. Self-administration was analyzed separately according to the solution administered. The experimental group (**Figure 7A**) and the control group (**Figure 7B**) did not show significant differences by sex in their respective self-administration (Sex x Solution interaction: $F(1,23) = 3.968$, $p= 0.058$). However, there were significant changes in the levels of self-administration throughout the days. In cocaine group (**Figure 7A)** we can observed the escalation of cocaine intake (Days x Solution interaction: $F(13, 143) = 3.616$, $p<0.001$) meanwhile, in the water group (**Figure 7B**) we can observe the decrease in self-administration (Days x Solution interaction: $F(13, 156) = 2.352$, $p=0.007$). On the other hand, upon observing sex differences in the extinction

task (**Figure 7C**), we observed that females possess high reward-seeking (Session x Sex interaction: F (1,25) =8.758, p=0.007).

To further understand the mechanisms and the predictability of the behaviors regarding drug-, water-seeking and drug/water intake, we conducted correlations to observe the relationship between the behaviors. We observed that among all behaviors only animals in the water group who performed the anxiety task (**Figure 8A** r= 0.66, p=0.01) and the sensation seeking task (**Figure 8B** r= 0.801, p<0.05) had a significant positive correlation with water self-administration.

Regarding drug- and water-seeking we observed that during the naïve stages (i.e. before self-administration), sensation seeking (**Figure 9A** r=0.577, p=0.030), distress tolerance (**Figure 9B** r=0.548, p=0.042) and anxiety (**Figure 9C** r=0.568, p=0.030) had a positive correlation between their behaviors and water-seeking only. On the other hand, sensation seeking at the abstinence stage (i.e. after self-administration) had a positive correlation with water-seeking for the control group (**Figure 9D** r=0.695, p=0.005) and a significant positive correlation with drug-seeking (**Figure 9E** r=0.686, p=0.009) in the experimental group.

To analyze the effects of cocaine on neuronal activity in the prelimbic cortex, we classified the cells as excited, inhibited or nonphasic during the performance of the behavioral tasks (see **Figure 2**). For the cells determined as excited during impulsivity (**Figure 10A**) there were no effects (Drug x Session interaction: F (1,17) =0985, p=0.335). There was also no effect in the distress tolerance task (**Figure 10B** Drug x Session interaction: F (1,16) = 0.152, p=0.702), sensation seeking task (**Figure 10C** Drug x Session interaction: F (1,14) = 1.447, p=0.249), or incentive salience task (**Figure 10D** Drug x Session interaction: F (1,15) = 0.737, p=0.404).

When analyzing the inhibited neurons on the behavioral tasks we could observe that there were no effects in the impulsive task (**Figure 11A** Drug x Session interaction: F (1,17) = 0.321, p=0.578). In the distress tolerance group (**Figure 11B**) we observed a trend for a decrease in the proportional inhibited neurons (Drug x Session interaction F (1,16) =3.228, p=0.091) but it was not significant. In the sensation seeking task (**Figure 11C** Drug x Session interaction: F (1,14) = 0.222, p=0.645) there was no change in the proportional inhibited cells in either the water or cocaine group. Regarding the incentive salience (**Figure 11D**) and the proportional inhibited cells there was a significant interaction (Drug x Session interaction: F (1,15) = 5.385, p=0.035) suggesting that water rats increased the proportion of inhibited neurons following abstinence, but cocaine rats decreased the proportion of inhibited neurons following abstinence. However, these comparisons were not significant following Bonferonni correction.

Finally, cells that show neither excited nor inhibited behavior were classified as nonphasic and were analyzed regarding the behaviors of interest. In the impulsivity task (**Figure 12A**), distress tolerance task (**Figure 12B**), sensation seeking task (**Figure 12C**), and incentive salience task (**Figure 12D**) there were no effects (Impulsivity-Drug x Session: F (1,17) = 0.003, p=0.956, Distress Tolerance- Drug x Session: F (1,16) = 0.501, p=0.489, Sensation-Seeking- Drug x Session: F (1,14) = 0.01, p=0.923, Incentive Salience-Drug x Session: F(1,15)= 6.348, p=0.024).

Neural analysis in the anxiety task was different because animals often had too few open arm entries to statistically classify neurons. Therefore, we instead analyzed the relative activity rate in each arm compared to the overall activity rate. Neuronal activity was analyzed during the elevated plus maze while the animal spent time in the open arm (**Figure 13A** Drug x Session: F (1,9) = 2.691, p=0.135) and spent time in the closed arm (**Figure 13B** Drug x Session: F (1,9)

=1.262, p=0.29). There was no significant effect of cocaine in the activity that occurred while the animal was at the open arm nor the closed arm.

Given that there is no significant impact of cocaine over the cellular activity in individual behaviors, we sought to investigate the relationship between neuronal activity during the behaviors of interest with drug-, water-seeking and drug/water intake. Regarding the behaviors during the naïve stage, we observed a significant negative correlation (**Figure 14A** r= -0.808, p=0.001) between proportional inhibited neurons in distress tolerance and cocaine self-administration. Further, there was a positive correlation (**Figure 14B** r= 0.671, p=0.03) between the proportional excited cells in incentive salience with self-administration in the water group. With respect to the behaviors during the abstinence period we found a negative correlation (**Figure 14C** r=-0.8, p=0.005) between the inhibited cells in the distress tolerance and self-administration in the cocaine group. On the other hand, we found a significant positive correlation (**Figure 14D** r=0.841, p=0.002) between the nonphasic neurons in the distress tolerance with self-administration in the water group.

Regarding the neuronal activity of the behaviors in relationship with drug- and water-seeking, we found that there were only significant correlations in the behaviors during the abstinence period. We found a negative correlation (**Figure 15A** r=-0.794, p=0.006) between the relative activity of neurons in the open arm in the anxiety task with water seeking. On the other hand, we found a positive correlation (**Figure 15B** r=0.8, p=0.005) between the inhibited cells in the incentive salience task and water seeking. Also in the incentive salience task, we found a negative correlation (**Figure 15C** r=-0.707, p=0.01) between the nonphasic cells and cocaine-seeking and a positive correlation (**Figure 15D** r=0.726, p=0.007) between the excited cells and cocaine-seeking.

6. Discussion

The goal of the project was to analyze the influence of cocaine intake on both behavior and prelimbic neural activity as well as assess the ability of behavior and prelimbic activity to predict reward-seeking. We noticed several sex differences in the behaviors independently of drug intake history but no significant influence on behavior with exception of distress tolerance. Additionally, no significant influence was observed on the neuronal activity in any behavior. However, upon observing the interactions between the behaviors, we observed many significant correlations with self-administration and reward-seeking for both the control and experimental group.

Our first primary finding was that we saw several sex differences. Males gave up sooner in the distress tolerance task compared to females, contrary to that described by Ali et al (2015) and Moschak et al (2023). Although females in our study had comparably higher distress tolerance, they also had significantly higher reward-seeking in both the control and experimental groups. This suggests that there could be other variables besides behavioral factors that explain the direction of distress tolerance to predict reward-seeking. As one possible difference, in the previous studies (Ali et al. 2015, Moschak et al. 2023, Moschak et al. 2017, Daughters et al. 2005), they used either Long Evans rats or humans instead of the Sprague Dawley rats that we used in our experiments. In addition to distress tolerance, we also found that females had higher levels of nosepokes in cocaine- and water-seeking, replicating findings from Moschak et al. (2021). No sex differences were visualized in the incentive salience task in either the control or the experimental groups. However, in the sensation seeking task females had higher ambulation than males in both water and cocaine groups. Our study therefore replicates previous findings that females traveled greater distance in the open field (Bishnoi et al. 2020 and Knight et al.

2021). During the anxiety task, females presented less anxiety than males, also replicating previous literature (Knight et al. 2021; but see Bishnoi et al. 2020).

Regarding the behavioral impact after cocaine intake, we expected to observe an increase in impulsivity after self-administration as described in Moschak et al. (2021) and a decrease in locomotor activity as described in Calipari et al. (2013). However, we did not find any significant change in these or in the other behaviors apart from distress tolerance. For distress tolerance, we observed a significant decrease after a history of cocaine usage in both males and females. This suggests that cocaine access is responsible for the decrease in distress tolerance, replicating what was observed in Moschak et al. (2017). Previous literature (Moschak et al. 2021) established that there is an increase in impulsivity after cocaine self-administration. However, they also found that cocaine intake decreased prelimbic activity and that this decreased activity correlated with their rats increased impulsivity. It is plausible that since cocaine had no significant effect on prelimbic activity in our animals then no impulsivity was modified. Differences in neuronal activity during these behaviors could the result of using Sprague Dawley rats instead of the Long Evans rats used in other studies. The same can be argued with respect to our results in locomotor activity compared to other literature (Calipari et al. 2013) given that we did not observe significant changes in the neuronal activity in our cocaine group. Furthermore, we did tend to see an impact of cocaine on locomotor activity in the expected direction ($p=0.104$). A stronger effect may have been obscured because cocaine rats tended to have lower locomotor activity at baseline, an accidental side effect of random assignment.

We next analyzed the relationship between the behavioral predictors, cocaine-intake, and cocaine-seeking which is an indicator of drug-addiction (Belin et al. 2012). We observed that high locomotor activity predicted high reward-seeking behaviors in both water and cocaine

groups as well as high water self-administration. Other experiments (Beckman et al. 2011; see Belin et al. 2012) showed similar trends with self-administration. Meanwhile, high distress tolerance had a significant positive correlation with water-seeking only. This finding failed to replicate previous findings (Ali et al. 2015, Moschak et al. 2023, Moschak et al. 2017, Daughters et al. 2005) where low distress tolerance predicted heightened drug-seeking. This may be the result of differences in abstinence duration.

Specifically, in Moschak et al. (2017) they observed a relationship between distress tolerance and cocaine seeking after a month of abstinence, meanwhile, in our study we assessed this relationship within a week of abstinence. Finally, low anxiety before self-administration predicted subsequent high water-seeking. This finding contrasts with the ones of Dillen et al. (2012) who found a positive correlation between anxiety and cocaine self-administration. The discrepancy may be because of the different amount of time in the elevated plus maze. Their rats spent 5 minutes in the maze while ours spent 10 minutes. Importantly, according to Montgomery et al. (1958), anxiety decreases towards the end of a 10-minute period.

Regarding the influence of cocaine with the activity in the prelimbic cortex, we observed no significant impact with any of the behaviors. This is contradictory with multiple previous studies (Hage et al. 2012, Moschak et al. 2023, Moschak et al. 2021, Dark et al. 2022, Lovic et al. 2011; but see Haight et al 2014) such as in impulsivity (Moschak et. al 2021) in where after cocaine self-administration it decreased neuronal activity in the prelimbic cortex in male rats. Furthermore, regarding distress tolerance (Moschak et al. 2023) found that males possessed increased phasic activity in the PrL during high distress tolerance but upon access to cocaine self-administration both the distress tolerance and the activity decreased. Differences in neuronal

activity during these behaviors could be given of the use of Long Evan rats instead of the Sprague Dawley rats that we utilized in our study. For anxiety (Hage et al. 2012) established that animals that spend less time in the open arm have higher C-Fos expression in the medial prefrontal cortex, which indicates neuronal activity, in their control group only. Discrepancies between that study and ours could rely on the different technique to study neuronal activity. C-Fos is a functional marker of neuronal activity after induction of stimuli and analyzed ex vivo. On the other hand, our utilization of endoscopic in vivo calcium imaging allows us to visualize the activity in real time when the behaviors are currently happening. A history of cocaine administration reduces locomotion and functional activity in the PrL (Calipari et at. 2013) which was not observed in our studies, but this could also be the result of lower locomotor activity at baseline as previously explained.

Similar to the behaviors, we sought to examine the relationship between neuronal activity during the behaviors of interest and self-administration and water- and drug-seeking behaviors. We noticed that a greater proportion of inhibited cells during naïve distress tolerance predicted lower drug self-administration. Meanwhile, we found that a high proportion of inhibited cells predicted low water self-administration and a high proportion of nonphasic cells during abstinence predicted increasing water self-administration. As described before, rats with higher distress tolerance typically have higher neuronal activity (Moschak et al. 2023) and tend to have less cocaine seeking (Ali et al. 2015, Moschak et al. 2017) but after prolonged exposure to cocaine, neuronal activity decreased, and cocaine intake decreased as well (Moschak et al. 2023). We replicated the study of Moschak et al. (2017) and Moschak et al. (2023) given that when the proportional inhibited cells from our rats increased, cocaine intake decreased.

Prelimbic activity during the incentive salience task also predicted reward-seeking. Specifically, rats with a high proportion of excited neurons during naïve incentive salience had high water self-administration. Conversely, during abstinence those with higher inhibited cells had higher water-seeking, those with more nonphasic neurons had less drug-seeking, and those with higher excited cells had increased drug-seeking. These findings therefore show that different aspects of prelimbic activity during the incentive salience task predict different aspects of both drug and natural reward-seeking. This may suggest how different population of neurons within the same brain region tap different behavioral responses that predict reward-seeking similar as it had been seen in prior investigations (Carelli et al. 1994) in where they observed different distinct patterns of phasic activity when animals were self-administering water or cocaine in the nucleus accumbens.

The only remaining significant relationship observed was in the elevated plus maze, where high relative neural activity while in the open arm predicted low water-seeking. According to prior literature (Dillen et al. 2012 and Hage et al. 2012) high anxiety was found to be positive correlated with cocaine self-administration as well as higher neuronal activity. Time in open arm is considered as a low anxiety behavior (Rodgers et al. 1997 and Pellow et al. 1985) therefore based on prior findings (Dillen et al. 2012) one could consider that neuronal activity in low anxious rats to have a low cocaine self-administration and possibly high water-seeking as we reported on our data.

7. Conclusion

Overall, we found sex differences in all behaviors with exception of impulsivity and incentive salience regardless of cocaine history. Cocaine did not significantly influence any behavior apart from distress tolerance, which decreased. There were significant interactions between behaviors and their neuronal activity with self-administration and reward-seeking. Specifically, sensation seeking behavior predicted water and cocaine seeking, prelimbic activity during DT predicted cocaine and water self-administration, and prelimbic activity during PCA predicted cocaine and water seeking.

Although cocaine did not have the impact on behavior and neuronal activity that we expected, we did observe that multiple behaviors and their associated prelimbic activity predicted self-administration and reward-seeking. Future work should expand the study to other brain regions that have been associated with drug addiction and associated behaviors of interest such as the nucleus accumbens (Fernandez-Teruel et al. 2021, Haight et al. 2014, Koob et al. 2016, Calipari et al. 2013, Badiani et al. 2013), the amygdala (Fernandez-Teruel et al. 2021, Koob et al. 2016, Calipari et al. 2013) or the paraventricular nucleus (Haight et al. 2014, Koob et al. 2016).

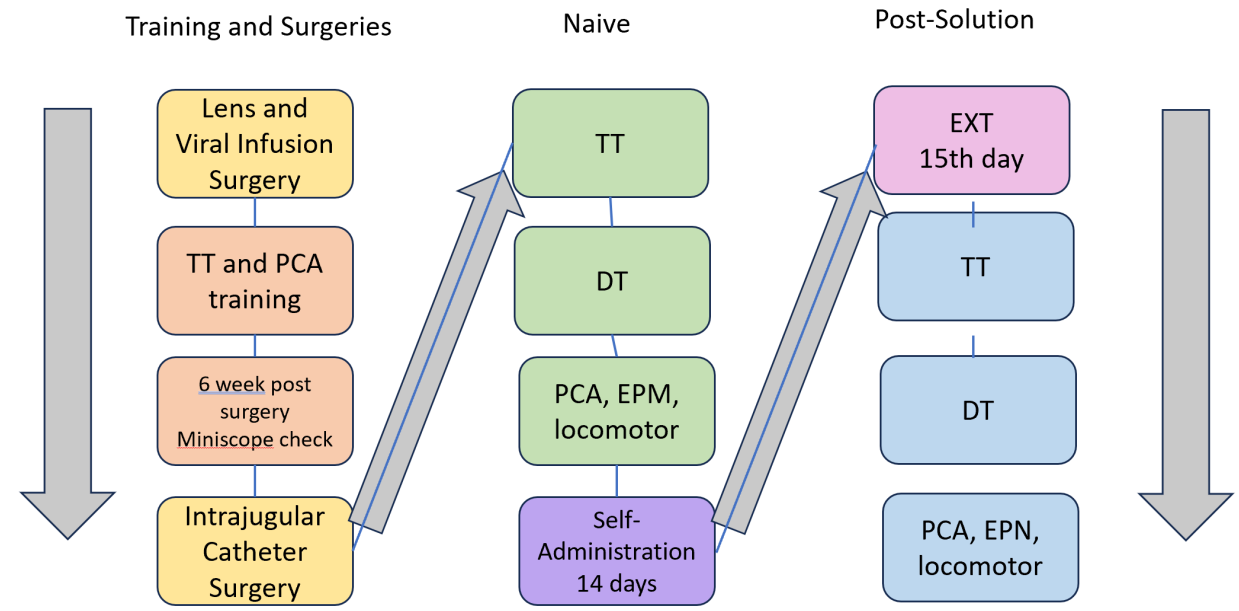

Figure 1. Experimental timeline.

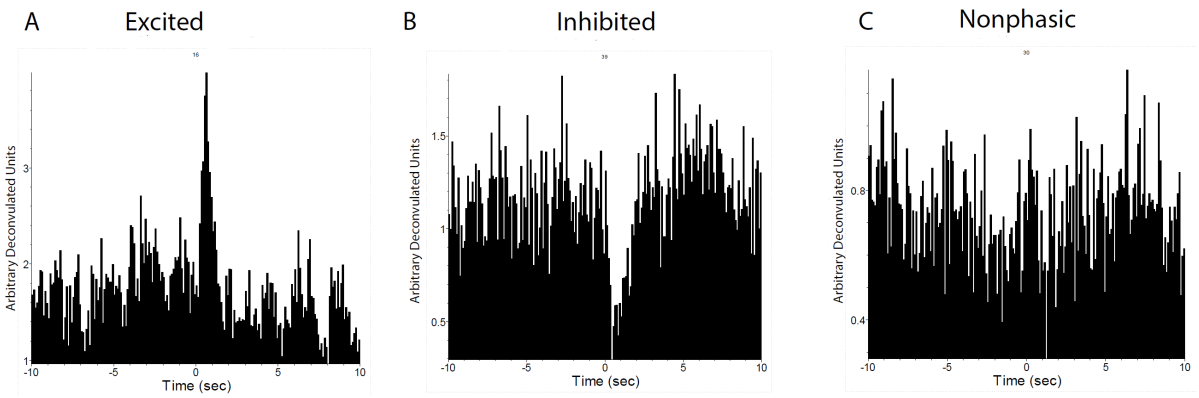

Figure 2. PeriEvent Histograms that shows neuronal activity of individual cells in the same behavior in a female rat. A) Excited cell when the lever comes out in the distress tolerance task. B) Inhibited cell when the lever comes out in the distress tolerance task. C) Nonphasic cells or cells without activity when the lever comes out in the distress tolerance task.

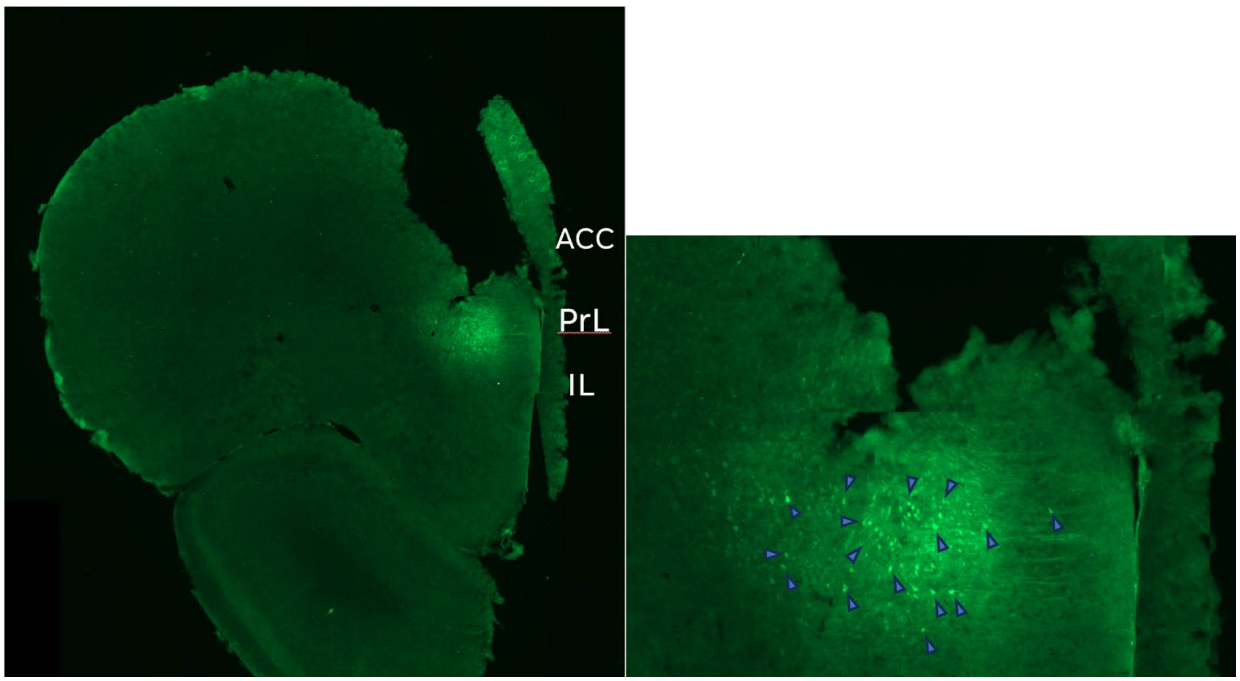

Figure 3. Immunofluorescence microscopy analysis of brain tissue. Lens placement in the prelimbic cortex (left). Magnified in order to visual neurons (right)

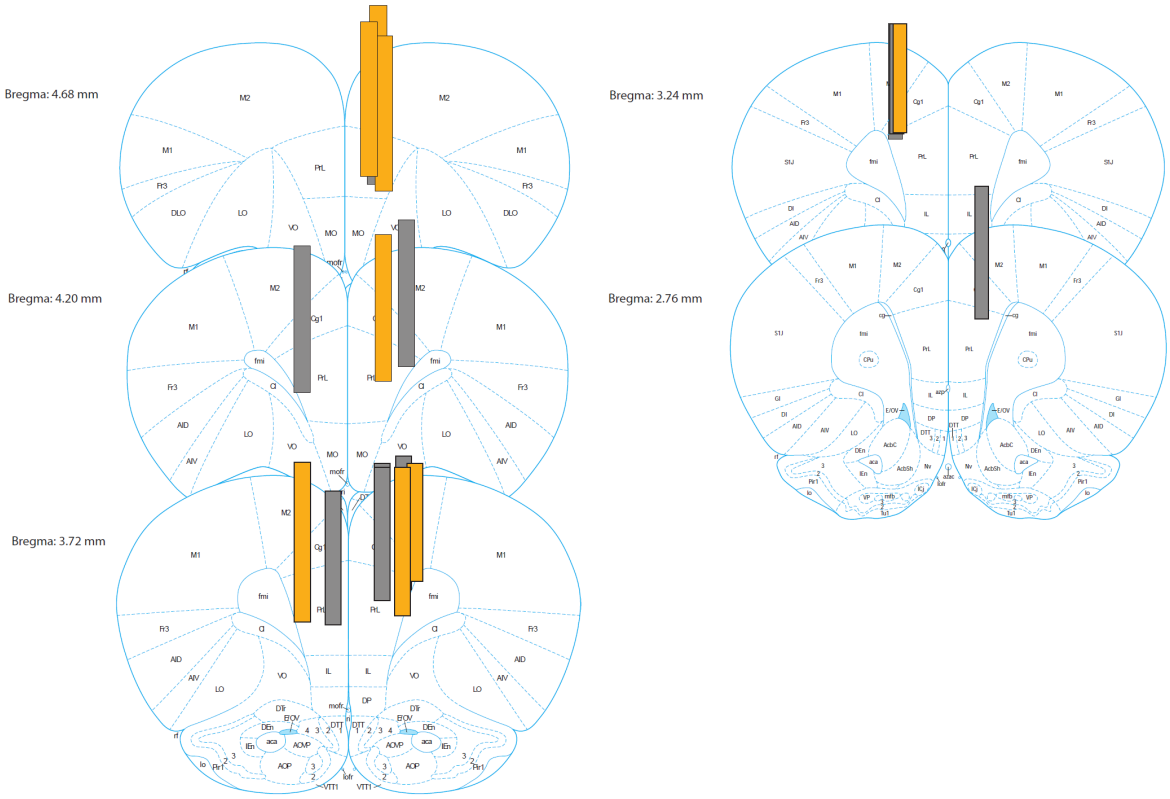

Figure 4. Lens placement verification in the prelimbic cortex for the water (grey) and cocaine rats (orange).

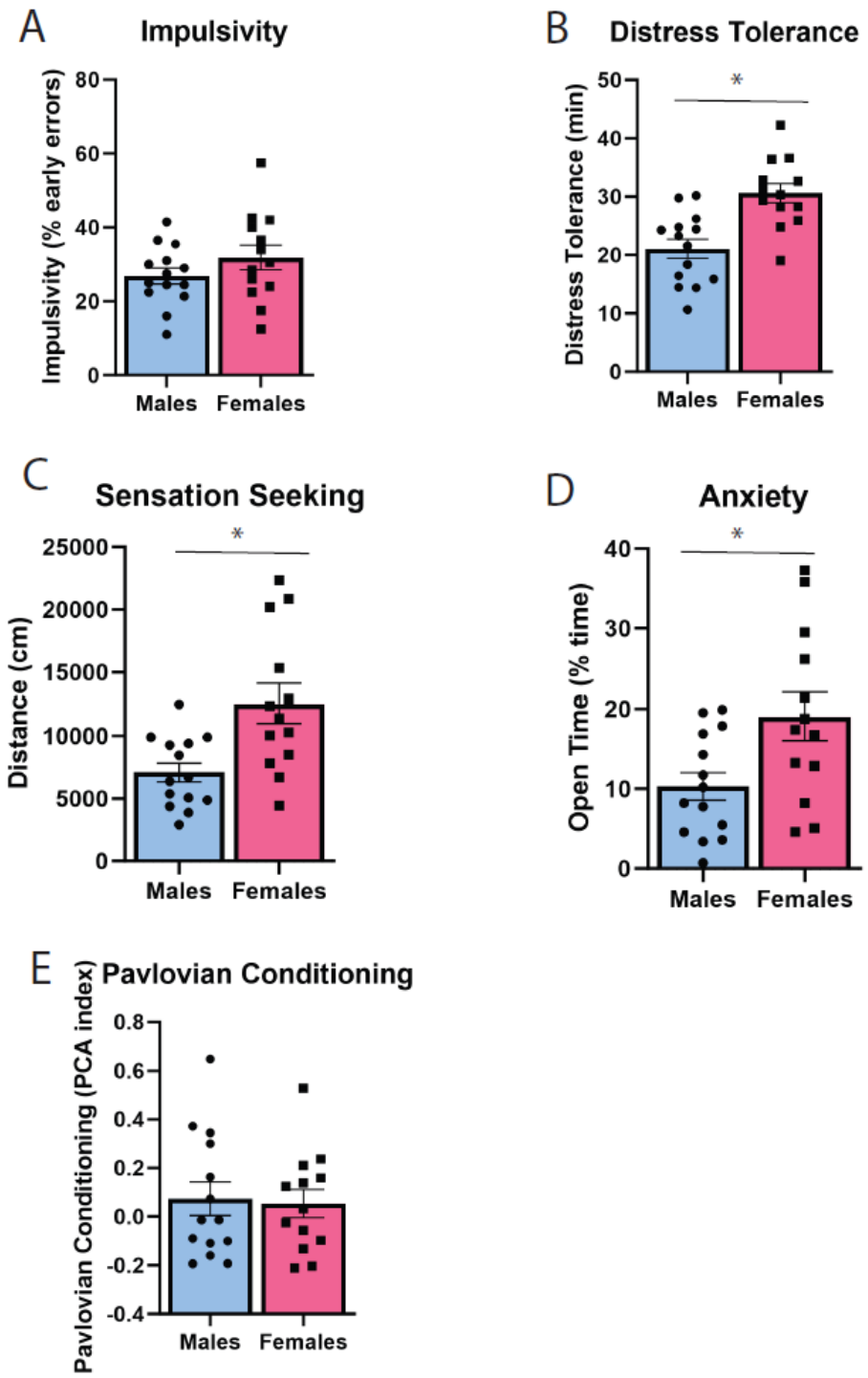

Figure 5. Sex differences in behaviors.

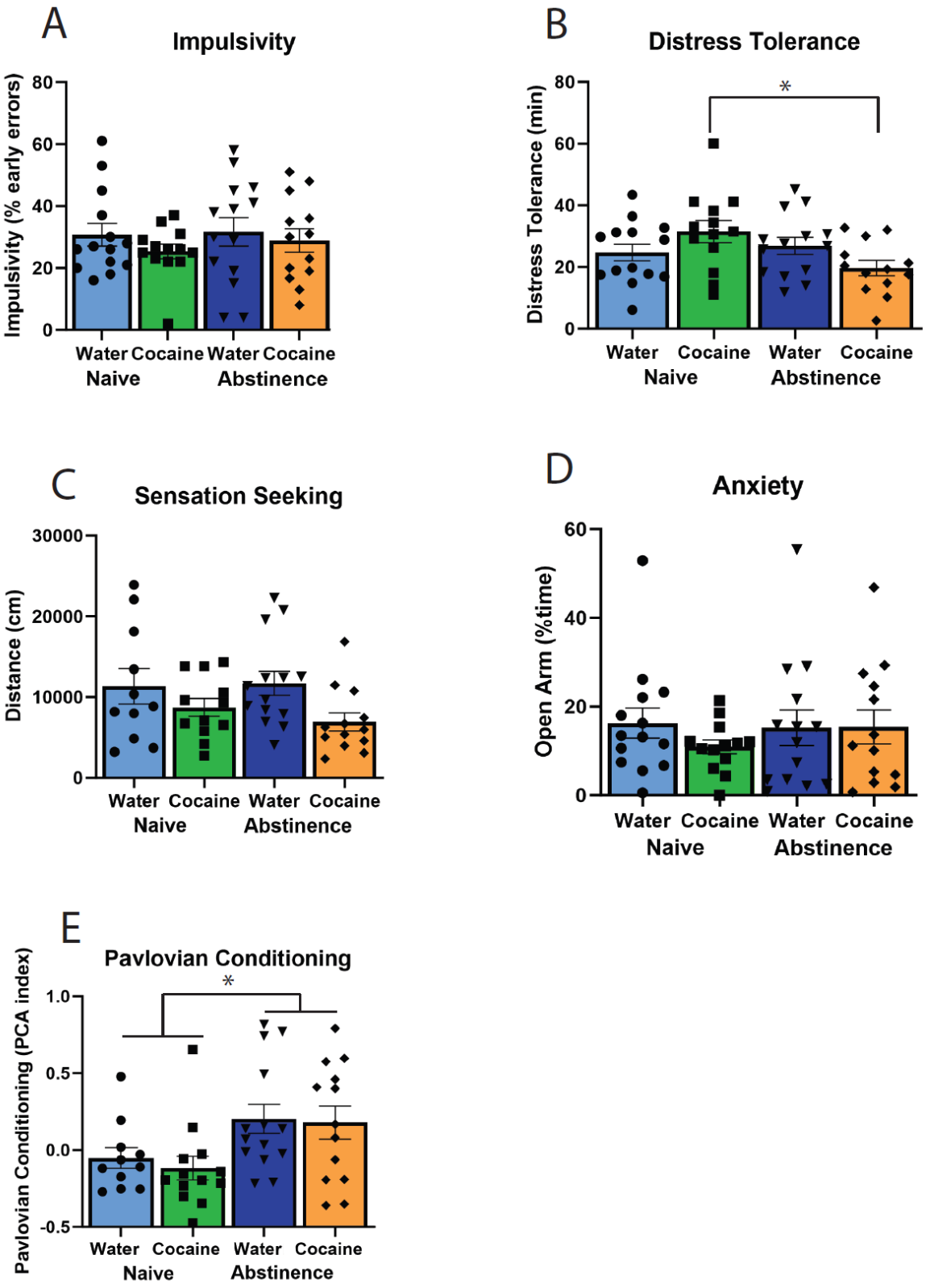

Figure 6. Cocaine influence on behaviors

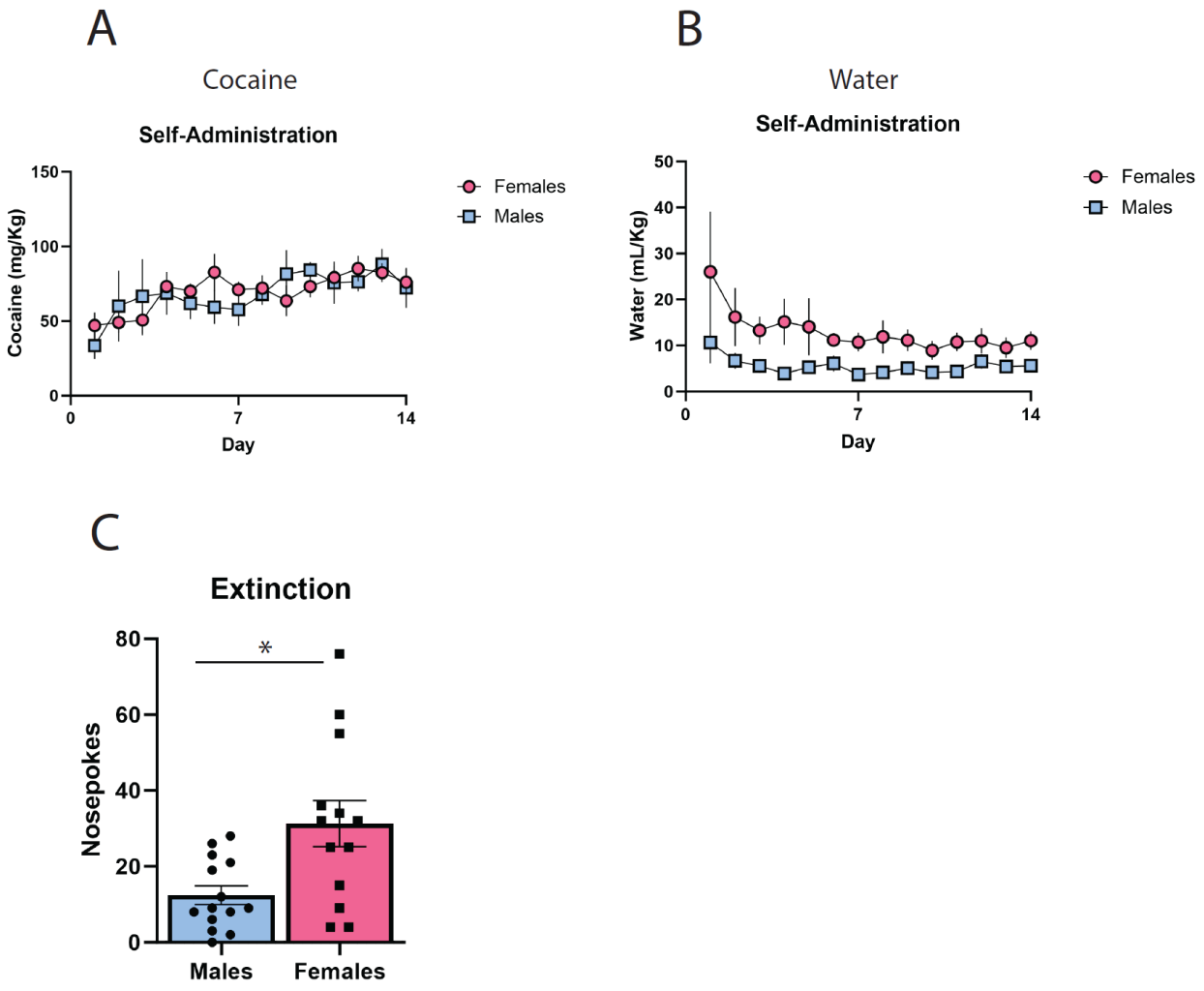

Figure 7. Reward-Seeking and Self-Administration

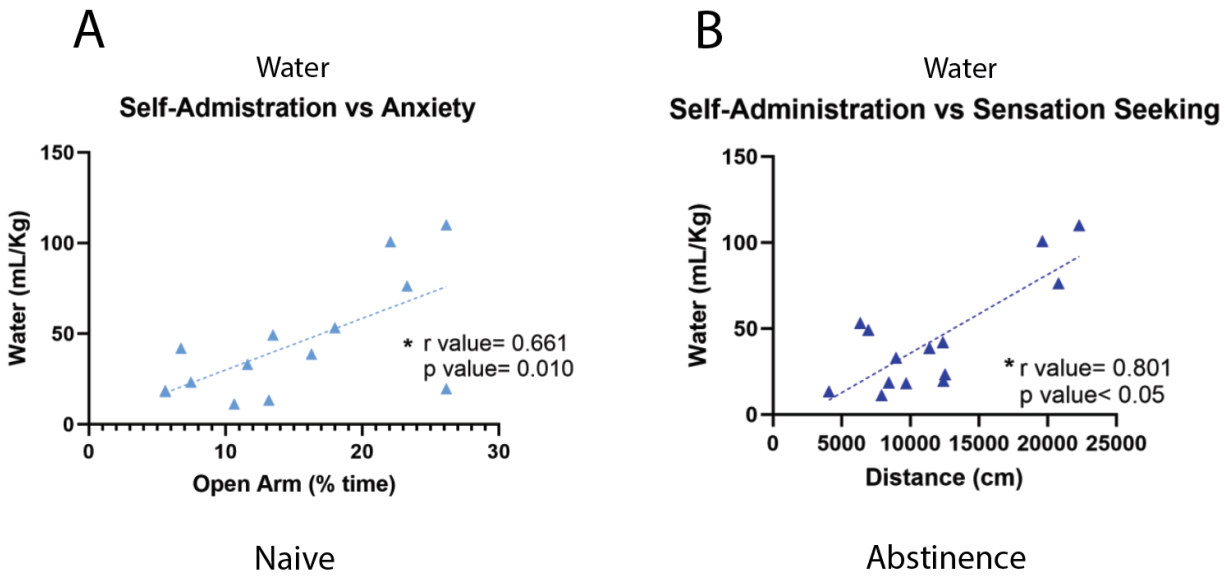

Figure 8. Behavioral correlations with self-administration.

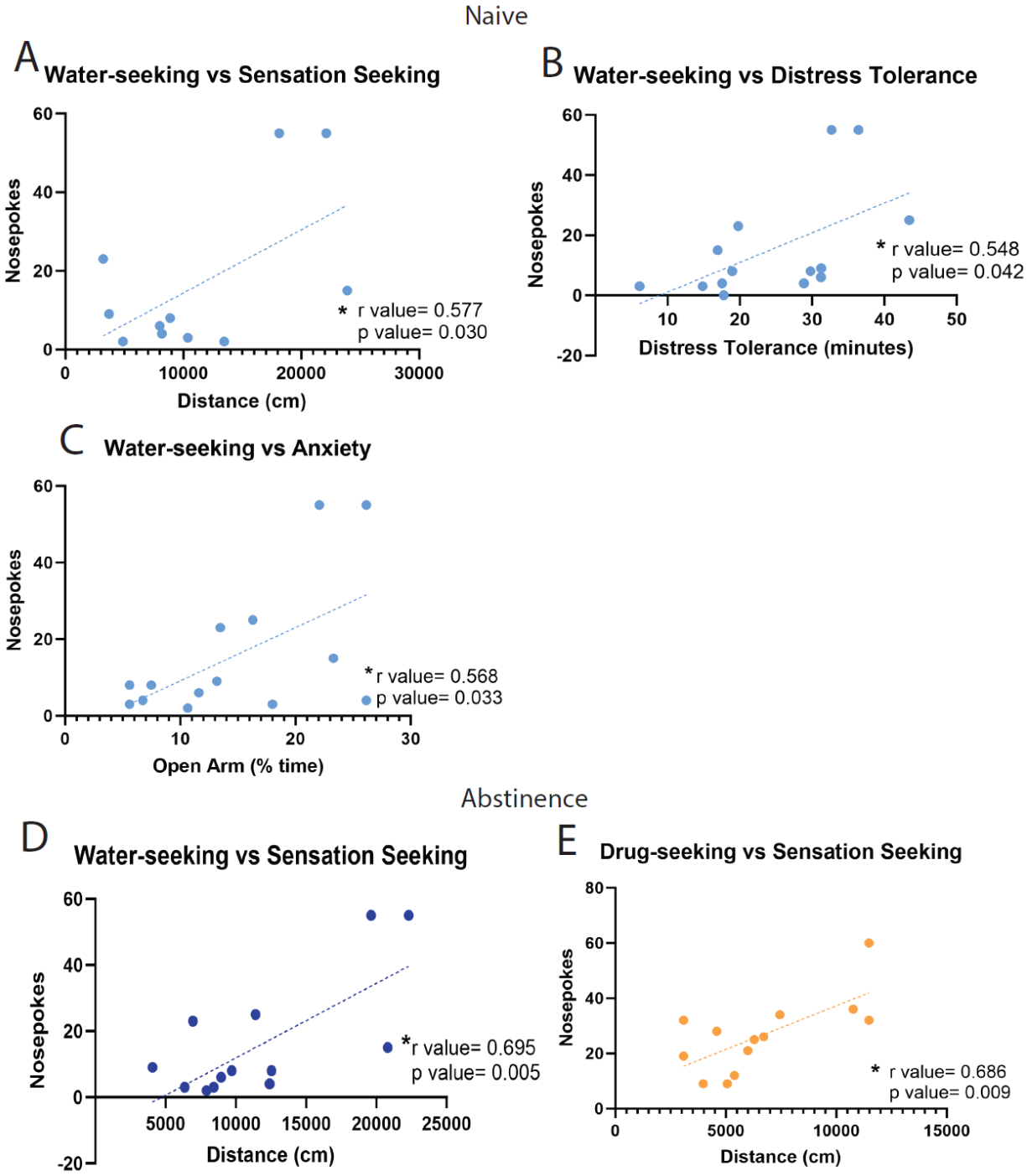

Figure 9. Relationship between the behaviors with water- and drug-seeking.

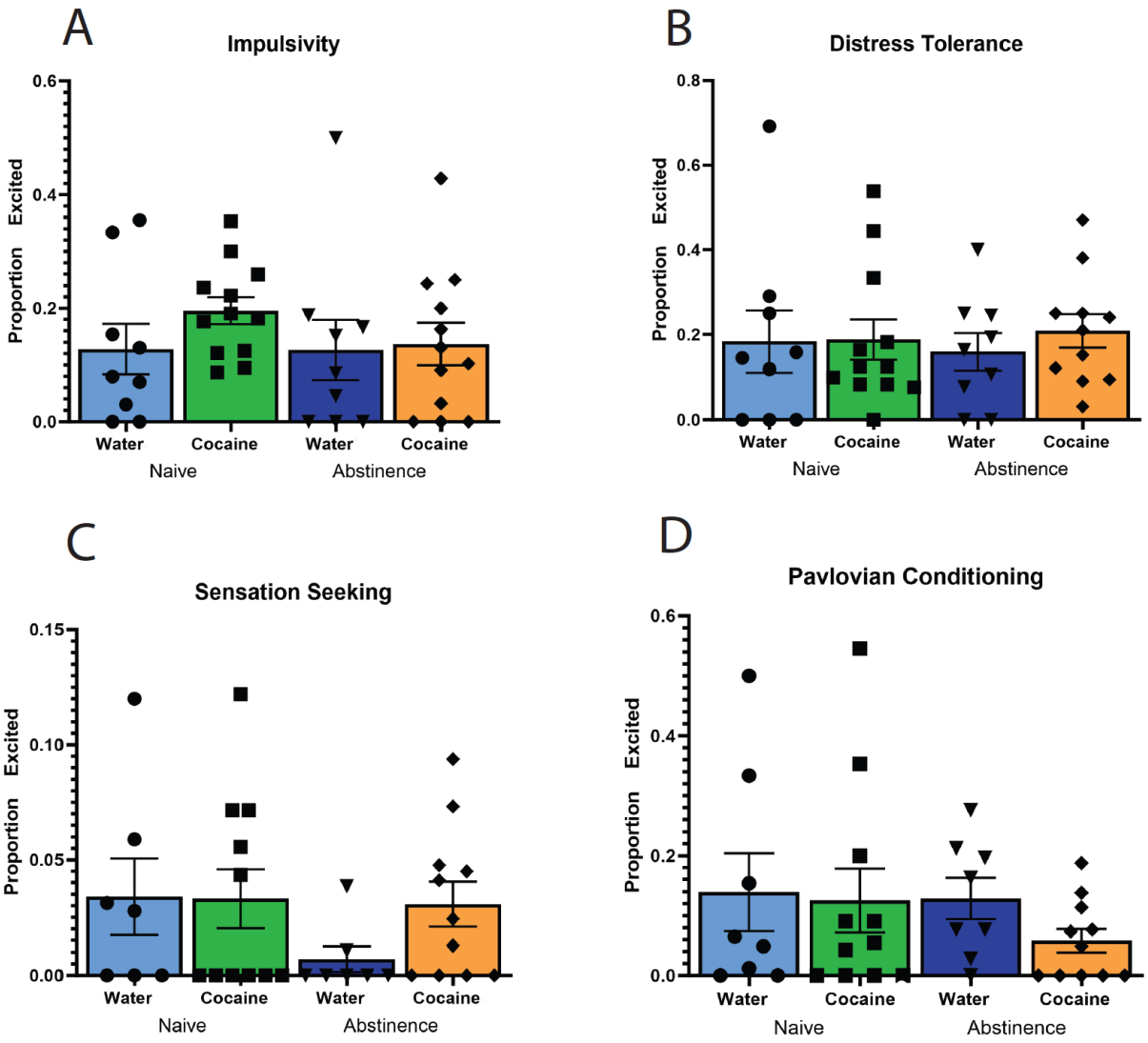

Figure 10. Behavioral interactions and effects of cocaine and water in excited cells.

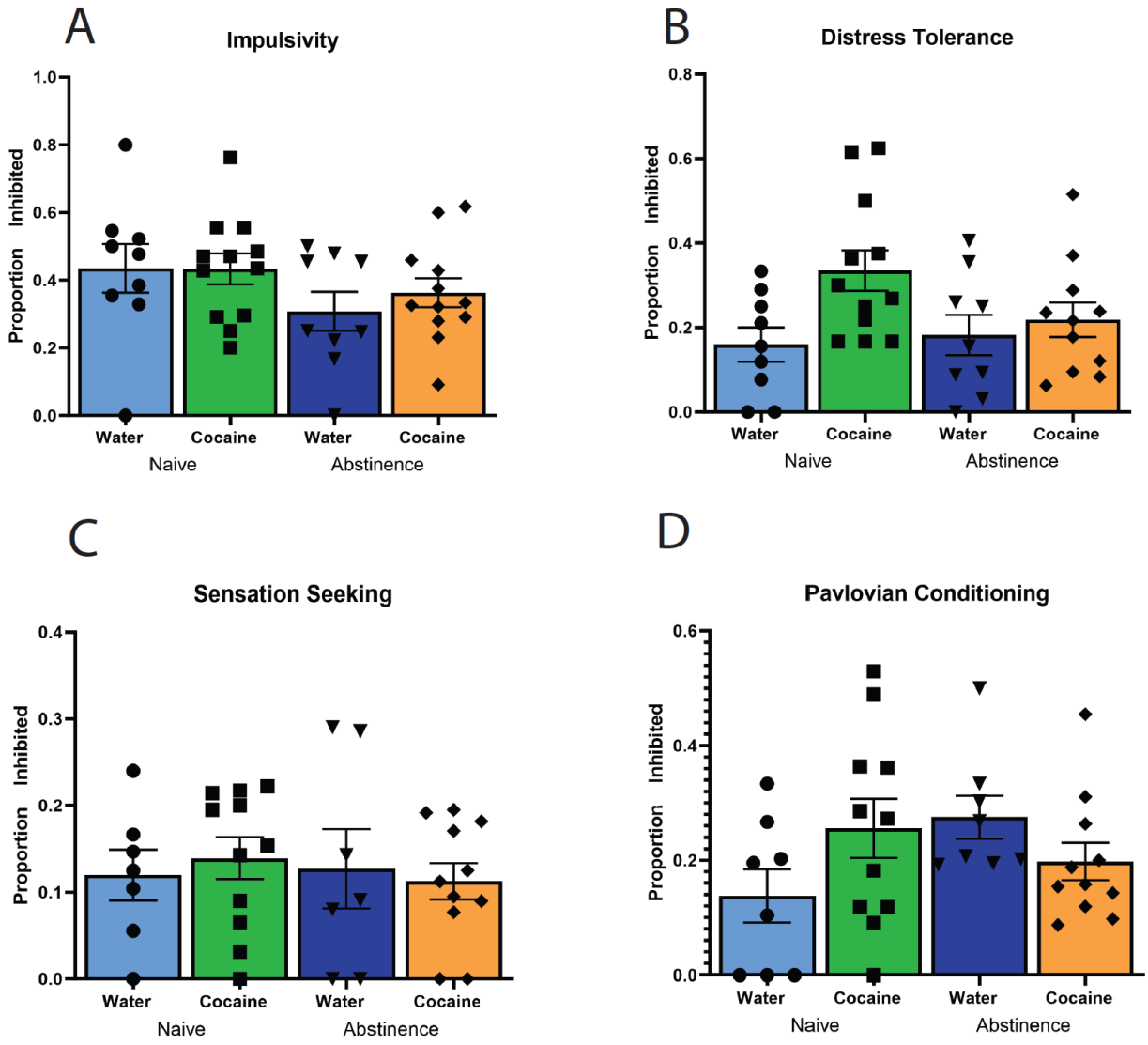

Figure 11. Behavioral interactions and effects of cocaine and water in inhibited cells.

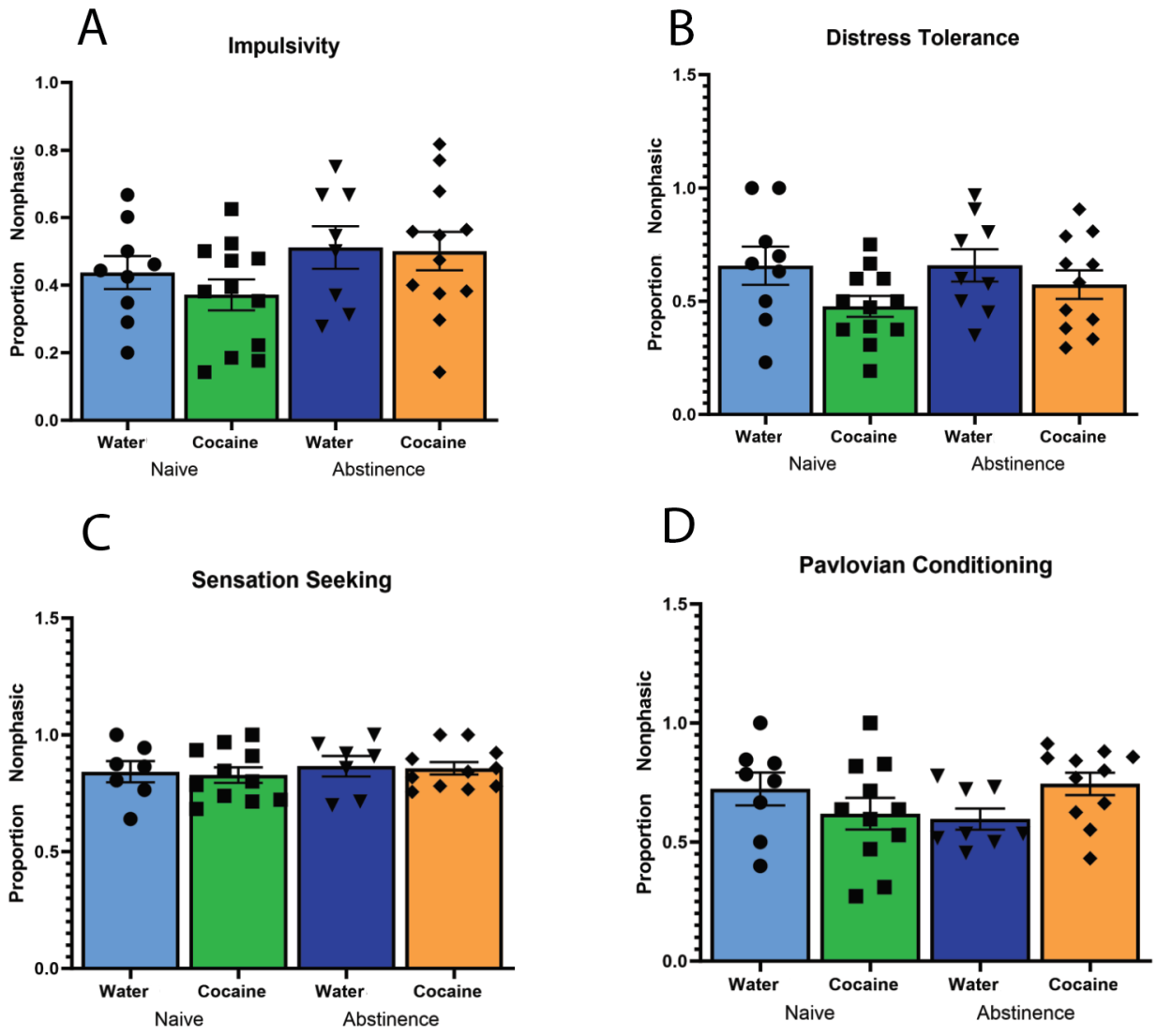

Figure 12. Behavioral interactions and effects of cocaine and water in nonphasic cells.

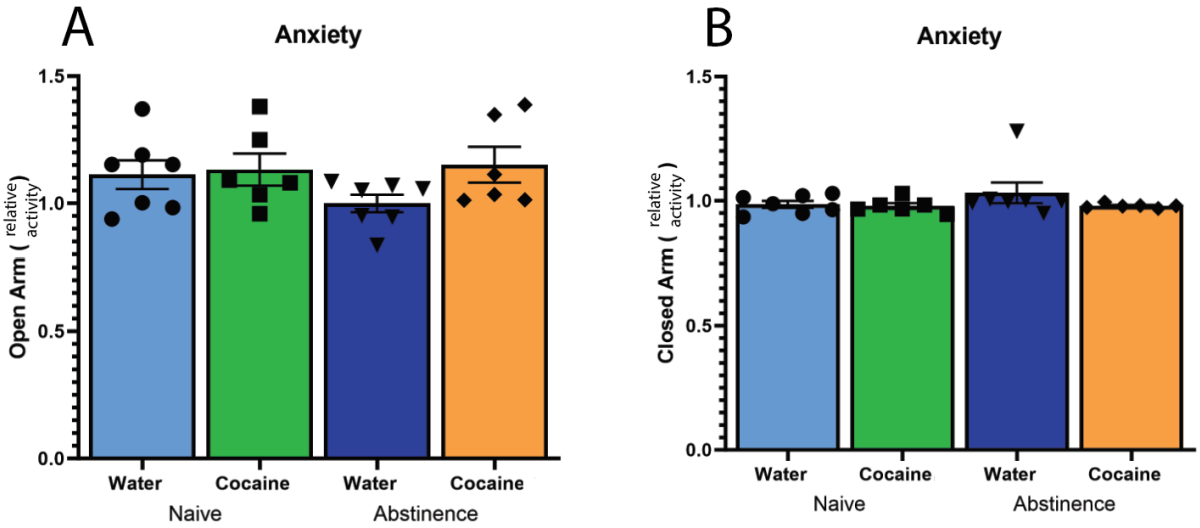

Figure 13. Anxiety's neuronal activity during the elevated plus maze.

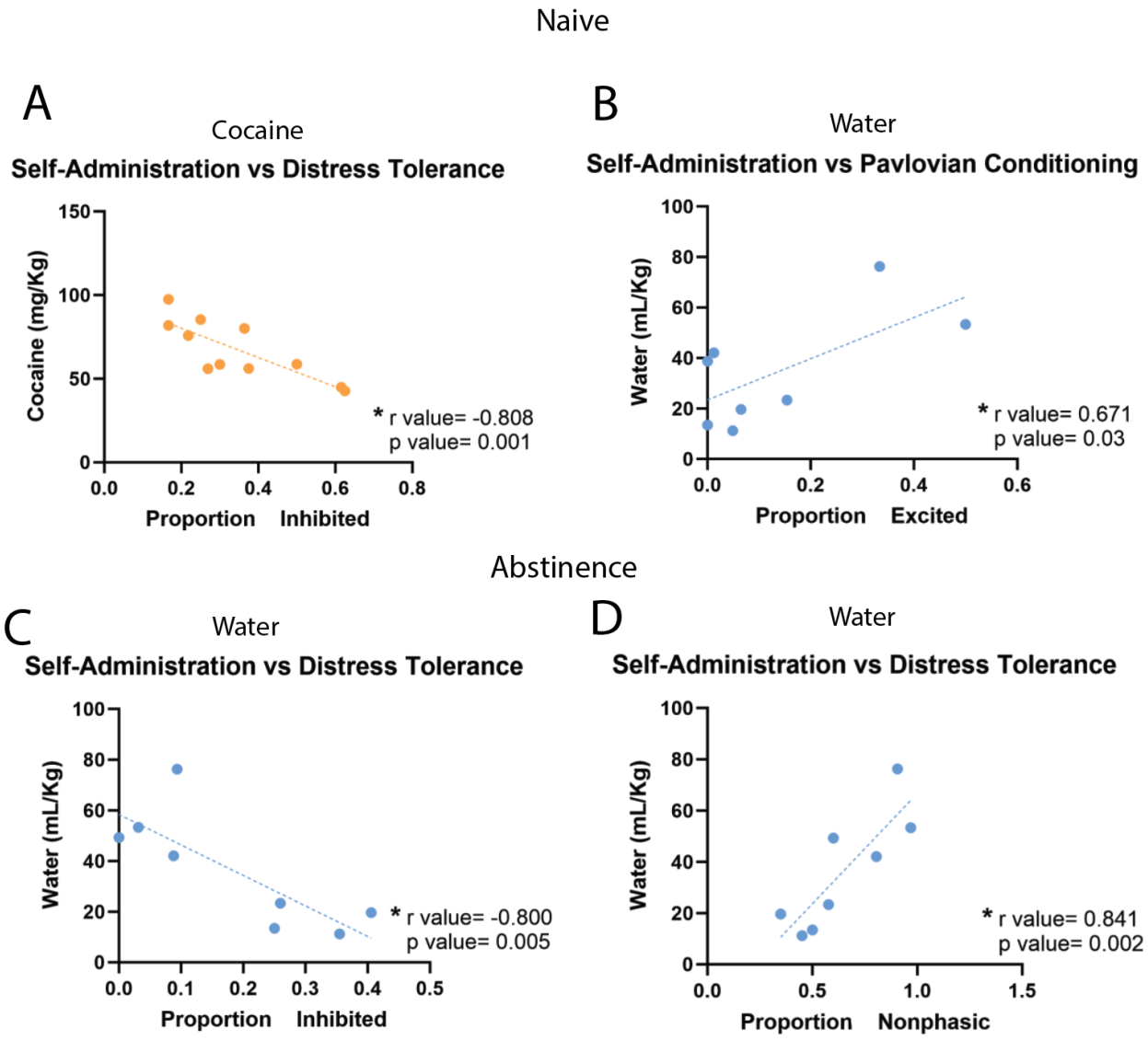

Figure 14. Relationship between neuronal activity in the behavior with water or cocaine self-administration.

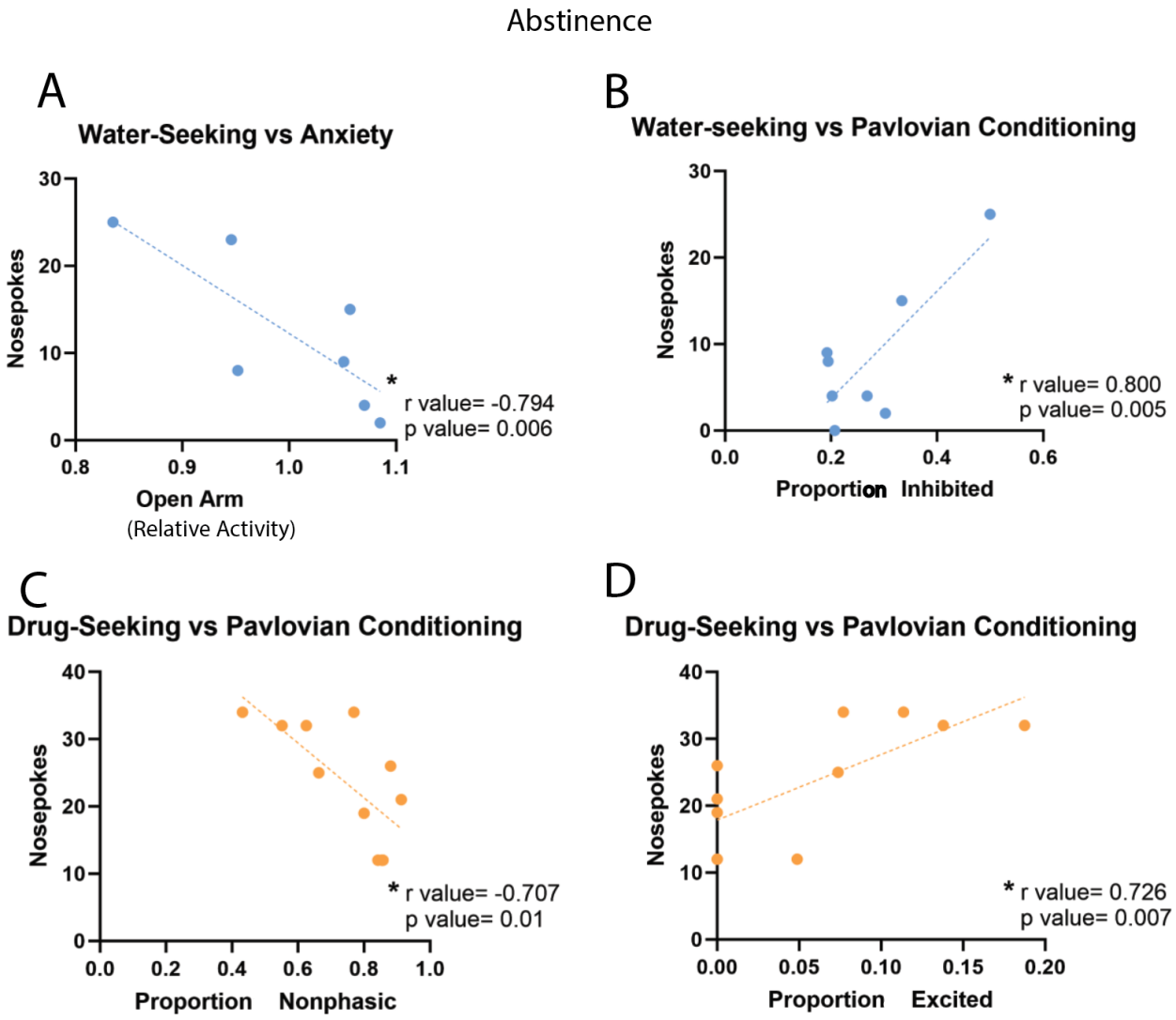

Figure 15. Relationship between neuronal activity in the behavior with water- and drug-seeking

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Vita

Investigation of neuronal activity population across predictive drug-seeking behaviors in the prelimbic area. Given that all behaviors relay back to the brain, understanding the neuronal activity in set behaviors particularly the unique neuronal activity exclusive to a behavior or the shared neuronal activity across multiple behaviors can assist in the development of better therapeutics to treat drugs of abuse disorder rather than simply abstinence. Skills I acquired includes mastery of in vivo calcium imaging, stereotaxic surgery, microscopy, undergraduate mentorship, and attendance of local and professional conferences. Management of paperwork for biohazard waste pick up with the University's Environmental and Safety Department. Further, coordination and administration of package receive and return of laboratory equipment to national and international research companies. Conducted and evaluated statistical analysis for significant data across multiple projects including the usage of programs such as Anaconda, IBM SPSS, Excel, and MATLAB. Graduating with an M.S in Biology with a GPA of 3.71.