Behavioral/Cognitive

Prelimbic Cortex Activity during a Distress Tolerance Task Predicts Cocaine-Seeking Behavior in Male, But Not Female Rats

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Distress tolerance (DT) is defined as the ability to persist in challenging goal-directed behavior in the face of stress, and individuals with low DT exhibit heightened drug-seeking behavior. However, no preclinical studies have examined the neurobiology underlying this phenomenon. To assess this, *in vivo* electrophysiology was used in Long Evans male and female rats during a DT task to record neural activity in the prelimbic cortex (PrL), a brain region implicated in drug-seeking. Rats were first assessed for DT, defined as the amount of time elapsed before rats quit seeking reward in an increasingly difficult operant task. Subsequently, rats underwent 2 weeks of self-administration for either water/saline or cocaine for 6 h/day. Animals then began a 1 month period of experimenter-imposed abstinence to induce heightened drug-seeking behavior. On day 28 of abstinence, DT and neural activity were reassessed; and on day 30, cocaine-seeking behavior was examined under extinction. Males had significantly higher DT than females and exhibited significantly more phasic PrL activity during the DT task. Furthermore, in male rats with a history of cocaine, PrL activity shifted to track DT; and this change in activity significantly correlated with the change in DT. Additionally, male (but not female) rats with low DT after 28 d of abstinence had significantly heightened drug-seeking behavior. Finally, PrL activity during the DT task predicted cocaine-seeking behavior. Collectively, these data demonstrate an important role for the PrL in DT in males, and link this neural activity and behavior to drug-seeking, particularly in males.

Key words: **addiction; behavior; cocaine; distress tolerance; prelimbic cortex; rats**

Significance Statement

Distress tolerance (DT) is defined as the ability to persist in challenging goal-directed behavior in the face of stress, and individuals with low DT exhibit heightened drug-seeking. Here, we investigated the role of the prelimbic cortex (PrL) in DT and its relationship to cocaine-seeking in male and female rats. We found that males had significantly higher DT than females and exhibited significantly more PrL activity during the DT task. Furthermore, male (but not female) rats with low DT after 28 d of abstinence had significantly heightened drug-seeking behavior. Finally, PrL activity during the DT task predicted cocaine-seeking. These data demonstrate an important role for the PrL in DT and link this neural activity and behavior to drug-seeking in males.

Introduction

The psychological distress associated with withdrawal and drug abstinence is a primary driver of drug craving and relapse (Baker et al., 2004; Koob, 2013). Of particular importance to this relationship is distress tolerance (DT), which is defined as the ability of an individual to persist in goal-directed behavior in the face of psychological distress (Magidson et al., 2013). For multiple drugs of abuse, studies in human subjects have found that low DT predicts higher substance use and relapse (Brown et al., 2002, 2009; Brandon et al., 2003; Daughters et al., 2005a,b; Strong et al., 2012).

Subsequent studies have investigated the neurologic substrates of DT and its relationship to substance use disorders. One area of prominence is the anterior cingulate cortex (ACC). High resting functional connectivity between the ACC and multiple regions, including the dorsolateral PFC and medial frontal gyrus,

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is associated with high DT (Daughters et al., 2017; Dezachyo et al., 2021). Furthermore, higher ACC activity predicts DT in cocaine users, but not controls (Daughters et al., 2017), and higher levels of cocaine use predict a stronger association of the ACC and the associated salience network with the default mode network (Reese et al., 2019).

However, such clinical studies are limited both in their ability to draw causal inferences about the impact of substances of abuse on DT and in their spatial and temporal ability to record neural activity during the specific events occurring in the DT task. To address this, we recently developed a DT task for rodents and examined its relationship to cocaine-taking and seeking (Moschak et al., 2018a). Here, we found that animals with a history of high cocaine intake had lower DT, and that animals with low DT had high drug-seeking behavior. Furthermore, DT only predicted drug-seeking following extended drug abstinence, which induces a period of heightened craving known as "incubation of craving" (Grimm et al., 2001; Pickens et al., 2011).

We sought to extend these findings by examining the role of the rodent prelimbic cortex (PrL) in DT and cocaine-seeking behavior. The PrL is homologous to Brodmann area 32 of the ACC in humans (Heilbronner et al., 2016) and is well known for its role in drug-seeking (Capriles et al., 2003; McFarland et al., 2003; Stefanik et al., 2013). Furthermore, the PrL dynamically changes during incubation of craving (Ma et al., 2014; West et al., 2014; Shin et al., 2018; Moschak and Carelli, 2021b). The latter finding is particularly relevant since we found that the effects of cocaine on DT and the ability of DT to predict subsequent cocaine-seeking and -taking only manifested following this period of extended (~30 d) abstinence from cocaine (Moschak et al., 2018a), suggesting that regions implicated in incubation of craving may be relevant to the interaction between DT and cocaine-seeking. Finally, the PrL has been implicated in behaviors that are associated with DT, such as negative affect (Stern et al., 2010; Sierra-Mercado et al., 2011) and cognitive effort (Hosking et al., 2016), further suggesting a role for this region in DT.

The relationship between DT and drug-seeking may also be influenced by sex. Studies have shown that DT may be a more important driver of relapse for women than for men (MacPherson et al., 2008; Ali et al., 2015), and in preclinical literature, females self-administer higher doses of cocaine (Becker and Koob, 2016; Sanchis-Segura and Becker, 2016) and exhibit heightened drug-seeking following abstinence (Kerstetter et al., 2008). These findings suggest that a history of cocaine self-administration may decrease DT more in females than in males, and that DT may be a stronger predictor of subsequent drug-seeking in females.

Here, we investigated PrL activity during a DT task in a drug-naive state and following extended abstinence from cocaine self-administration in male and female Long Evans rats. We then determined whether this neural activity changed following abstinence, and whether activity during the DT task could predict drug-seeking behavior.

Materials and Methods

Subjects and surgery

Female ($n = 14$) and male ($n = 19$) Long Evans rats were singly housed under a 12 h light/dark cycle. All experiments were conducted during the dark phase. Rats received standard lab chow (Prolab Isopro RMH3000, Purina) and were food- or water-restricted to no less than 85% free feed weight. All experiments were conducted in accordance with the National Institutes of Health's *Guide for the care and use of laboratory animals* and the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

During surgery, animals were anesthetized with a cocktail of 100 mg/kg ketamine and 10 mg/kg xylazine. A sterile polyurethane catheter (Access Technologies) was implanted into the right jugular vein and externalized at the back. Additionally, a 16 wire electrophysiological electrode array (NB Labs) was implanted into the PrL (AP: 2.8 mm, ML: ±0.6 mm, DV: −3.8 mm from bregma at skull surface), and a reference wire from the array was wrapped around a skull screw. Rats were given 1 week to recover with *ad libitum* food and water.

Apparatus

Experiments were conducted in operant chambers (43 × 43 × 53 cm, Med Associates) housed in a sound-attenuating cubicle lined with a mesh Faraday cage. Each chamber contained two retractable levers, a reward receptacle, two cue lights, a house light, and a white noise generator. Self-administration was conducted in distinct operant chambers (25 × 25 × 30 cm) housed in sound-attenuating chambers and equipped with an illuminated nosepoke, a houselight, a tone generator, and a fluid receptacle. Infusions of cocaine, saline, or water were delivered by a motorized pump. Behavioral events were controlled and recorded by Med-PC software (Med Associates).

Electrophysiology

Electrophysiological recordings were conducted as described previously (Moschak and Carelli, 2021a). Before the start of behavior, animals were connected to an Omniplex data acquisition system (Plexon) via 1× gain headstages and a flexible recording cable. Free movement of the rat was ensured using a commutator (Plexon) to connect the cables. Neural activity was recorded differentially between active and inactive (reference) wires. Wires with a lack of neural spike activity were chosen as reference wires (1 per array). Putative neurons were initially selected online via waveform analysis in PlexControl (Plexon), and behavioral events were sent to PlexControl via transistor-transistor logic from Med-PC (Med Associates). Additional cell sorting was conducted offline with principal component analysis in Offline Sorter (Plexon). Waveform principal components, interspike intervals, and auto-correlograms were used to ensure the putative neurons had biologically relevant characteristics. Finally, putative interneurons were defined as neurons with a peak-trough latency ≤200 μs (Mitchell et al., 2007) and were excluded from the analysis (6.9% of neurons). In female rats, vaginal swabs were collected shortly after each electrophysiological recording and the criteria outlined in Marcondes et al. (2002) were used to assess the stage of the estrous cycle with a brightfield microscope.

Histology

Following experiments, rats were deeply anesthetized with ketamine/xylazine. A 13.5 μA current was then passed through each recording wire for 5 s, after which the rat was decapitated and the brain was removed. Each brain was fixed in a solution of 20% sucrose and 3% potassium ferricyanide in 10% buffered formalin. Subsequently, brains were frozen and 40 μm sections were taken through the forebrain. Sections were mounted on slides, and electrode tip locations were visualized using a bright-field microscope. Placements were verified using a stereotaxic atlas (Paxinos and Watson, 2007).

Experimental design and statistical analysis

All rats were tested in multiple behavioral tasks. A timeline for these behaviors is found in Figure 1*A*, and detailed methodology is given in the following sections. Initially, drug-naive rats were trained to lever press for a sucrose pellet (45 mg, Purina TestDiet). After reaching criterion (50 pellets for 2 consecutive days), rats were then trained to lever press during a 5 s cue light presentation. Lever presses outside of the 5 s cue period resulted in an error and no pellet delivery. After 2 consecutive days of at least 50% accuracy, rats began the titration task (TT).

Titration Task

A diagram of the trial structure used in both the TT and DT task is illustrated in Figure 1*B*. The TT (100 trials) is composed of four distinct stages: a precue period, cue period, postcue period, and intertrial interval

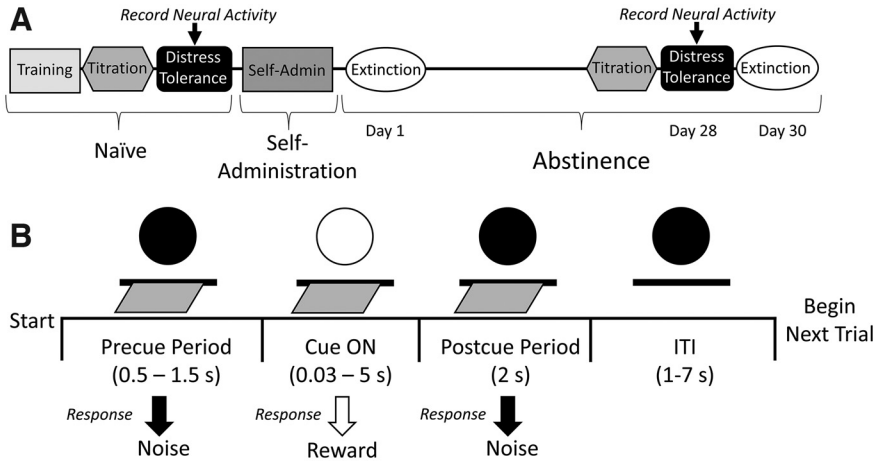

Figure 1. ***A***, Experimental timeline (for details of task design, see text). ***B***, Schematic of a single trial in the DT task and TT. Responses during the cue period (Cue ON) were rewarded with a single sucrose pellet. Responses during the periods before or after Cue ON were considered "errors" and were signaled by a noise and did not result in pellet delivery. The duration of the cue ON period titrated according to task performance in the TT, and progressively decreased in the DT task.

(variable time, 4 s). For each trial, the lever remained extended for the precue, cue, and postcue periods and was retracted until the start of the next trial only if the rat pressed or if the intertrial interval began without the rat responding. Correct presses (lever presses during the cue period) were rewarded with delivery of a sucrose pellet. If the rat lever pressed during either the precue or postcue period, an error was recorded, and no reward was delivered. Following an error response, there was a short burst of white noise. The precue period was variable time 1 s and the postcue period was 2 s. The cue period length was titrated based on the animal's past performance on the task: a 10% increase or decrease for error and correct responses, respectively. For omissions (trials where the rat did not lever press), the next cue period duration remained the same as the previous trial. For each TT session, the starting cue period duration was manually set to the previous session's final cue period duration. The TT continued until either the rat made 100 responses or 1 h had elapsed. For a rat to progress from the TT to the DT task, the individual must have had between 40% and 60% correct responses and met stability criteria (no significant changes in cue period duration or percent of correct responses over 3 consecutive days). Following acquisition of the TT, rats underwent surgery to have intrajugular catheters and electrophysiological electrode arrays implanted in the PrL. They were given 1 week to recover with *ad libitum* food and water, after which they were retrained on the TT. Once their behavior stabilized on the TT, electrophysiological activity was recorded in a single DT session (naive DT).

DT task

The DT task had similar structure to the TT; however, the cue period duration became progressively shorter until correct responses were practically impossible. The first cue period duration in the DT task was individually tailored based on the rat's TT performance: the average response latency on correct response trials multiplied by 2. In the early stage of the DT task, incorrect responses did not affect the subsequent cue period duration; however, correct responses rapidly and progressively decreased the cue period duration according to the formula: Cue Duration = Previous Cue Duration $\times$ 0.9^n (n = the number of correct responses in the DT task thus far). Only rats' first 20 responses were eligible to be rewarded (by which time the cue period duration is typically $<$250 ms), and they were allowed a maximum of 5 rewards. After either 20 responses or 5 correct responses, all subsequent responses decreased the next cue period duration by 10%. As in the TT, omissions did not affect cue period duration. The goal of the DT task was to determine individual rats' persistence in lever pressing when challenged with psychological distress (forced failure and negative feedback). When rats consecutively omitted 5 times directly preceded by 3 omissions out of 5 trials (i.e., 8 omissions out of 10 trials), they were considered to have quit the task. If the rat did not ever reach criterion, the DT task session time was capped at 1 h.

Self-administration

After the "naive DT" session, rats began the self-administration protocol. For 6 h per session for 14 d, rats had access to either cocaine ($n = 7$ females, $n = 10$ males) or water/saline ($n = 7$ females, $n = 9$ males). A nosepoke would deliver either an intravenous infusion of 0.33 mg of cocaine or an equivalent volume of intravenous saline and water (delivered to the water receptacle). All infusions were followed by a 30 s refractory period where nosepokes did not result in additional reinforcement. This refractory period was signaled by houselight illumination and a tone. For this period, water/saline rats were mildly water-restricted to 20 ml of water/day and cocaine rats received 35 ml of water/day (extra 15 ml to account for the extra water the water/saline rats self-administered). In addition, 10 of the 16 "water/saline" rats were not patent, and thus only received water. To account for this, we initially statistically examined nosepokes for "water" and "water/saline" rats separately to determine whether there were differences between the two. However, no significant differences were observed between the groups (F values < 0.81, p values > 0.387); thus, all reported analyses combined the two groups. Following 2 weeks of self-administration, rats entered a period of experimenter-imposed extended abstinence where they no longer received drug for the remainder of the experiment. Cocaine hydrochloride (National Institute on Drug Abuse) was dissolved in 0.9% saline.

Extinction task

On days 1 and 30 of abstinence, rats underwent a 2 h extinction task that was identical to the self-administration procedure described previously, but no drug infusions were delivered.

DT task (abstinence)

Following extended abstinence (day 28), a second DT session was conducted that was performed exactly as the naive DT task. Immediately before the abstinence DT test session, rats were retrained on the TT for at least 3 d. Electrophysiological recording sessions were conducted during each DT session ("Naive DT" before self-administration training and during the second DT session following abstinence).

Data analysis

Self-administration. To determine whether a significant escalation of intake took place for either group, a $2 \times 2 \times 14$ ANOVA (Drug $\times$ Sex $\times$ Session) was completed on the amount consumed (mg/kg cocaine or ml/kg water) obtained over the 14 d.

Extinction. To determine whether incubation of cocaine craving took place for either group, a $2 \times 2 \times 2$ ANOVA (Drug $\times$ Sex $\times$ Session) on the number of responses obtained on days 1 and 30 of extinction was used.

Distress tolerance. To determine sex differences in baseline DT, an unpaired t test comparing males and females was completed. To assess changes in DT following abstinence, a $2 \times 2 \times 2$ ANOVA (Drug $\times$ Sex $\times$ Session) on DT values was used. Pearson correlations were run to assess the effect of the amount of drug administered on the change in DT following abstinence. Finally, Pearson correlations were used to compare abstinent DT with the number of responses on day 30 extinction.

Electrophysiology

To classify neurons according to their firing pattern, perievent histograms were first constructed for each event as described previously (Moschak and Carelli, 2021a). Baseline for each perievent histogram was established as the average activity in the -2 to 0 s before each event.

Paired t tests were then used to compare baseline activity to event-related activity using the 200 ms bin following lever extension, cue illumination, and lever press as the event period. Neurons were classified as "Excited" if they significantly increased activity, "Inhibited" if they significantly decreased activity, or "Nonphasic" if there was no significant change in activity relative to baseline. χ^2 tests were used to assess differences in the proportions of neural subtypes across groups and conditions.

As part of the nature of the DT task, behavioral response rate decreases over the course of the session as animals "quit" in the task. Because of this, one goal of the study was to assess the change in neural activity across the course of the DT task. To do this, neural activity was averaged in the relevant event period 200 ms bin (e.g., the 200 ms bin following lever extension) across the first 20 trials, second 20 trials, and so forth to obtain 10 bins each with the average of 20 trials worth of data. Then the linear slope of the line across the 10 bins for each neuron was assessed, referred to as "neural slope." Inhibited and excited neurons showed no differences in neural slope, so these were grouped together by multiplying activity in inhibited neurons by −1. Then an unpaired t test was used to compare the neural slopes between male and female rats. To aid the comparison between neural activity and behavior, slopes for behavior (lever response rate) in the DT task were calculated. Female rats had very few phasic neurons following abstinence, so the effects of abstinence on neural slope were assessed in males only using a 2 × 2 ANOVA (Drug × Session). To determine how the neural slope related to the DT and cocaine- or water-seeking behaviors, Pearson correlations were completed. Calculations of neural slope resulted in a few extreme outliers as determined by Grubbs' outlier test (1.3% of data); these were removed from the analysis. All statistical analyses were completed with SPSS version 23. For all within-subjects analyses that did not pass Mauchly's test for sphericity, Huynh-Feldt adjusted degrees of freedom were used.

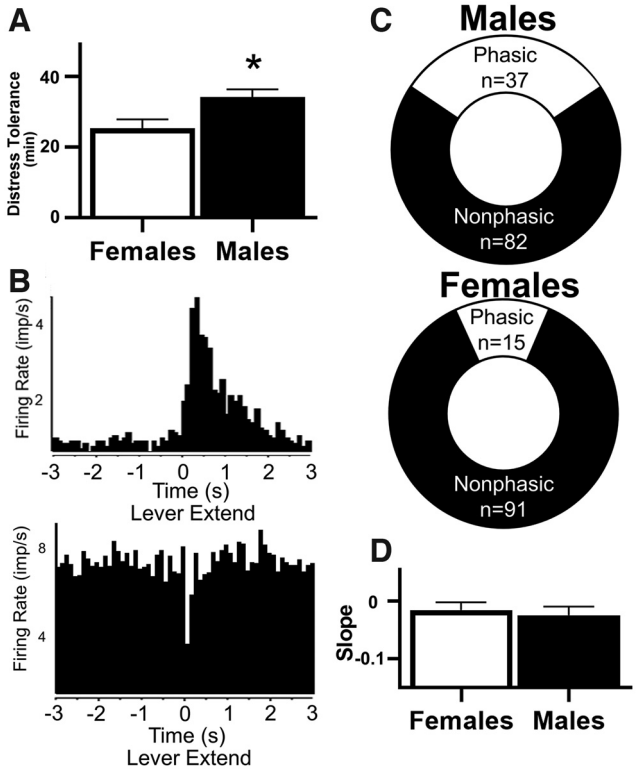

Figure 2. Drug-naive DT and neural activity. ***A***, Females exhibited significant lower DT than males. ***B***, Perievent histograms for representative PrL neurons showing either excitations (top) or inhibitions (bottom) following lever extension in the DT task. ***C***, Following lever extension, males had significantly more phasic neurons than did females. ***D***, The neural slope of lever extension-locked cells across the duration of the DT task was not significantly different from zero, indicating that neurons fired at the same rate for the duration of the task. Error bars: ± SEM; * $p < 0.05$.

Results

Female rats have lower DT and lower prelimbic activity during the DT task than male rats

Before any self-administration experience, females had significantly lower DT than males ($t_{(31)} = 2.72$, $p = 0.011$; Fig. 2*A*). Estrous cycle had no effect on DT ($t_{(11)} = 0.12$, $p = 0.906$); 225 neurons were recorded in the PrL during the drug-naive DT session. Examples of excited and inhibited neurons are shown in Figure 2*B*. During the task, males had significantly more phasic neurons in the PrL following the extension of the lever into the chamber than did females ($\chi^2 = 9.06$, $p = 0.003$; Fig. 2*C*). However, the relationship between percent phasic neurons and DT did not reach significance ($r = 0.33$, $p = 0.072$). Because behavioral response rate decreases over the course of the DT task, we also assessed the change in neural activity over the course of the task, or "neural slope." The neural slope of PrL phasic neurons did not change over the course of the DT task (one-sample t test vs zero: $t_{(23)} = 1.65$, $p = 0.112$), nor were there any differences between males and females in slope ($t_{(22)} = 0.223$, $p = 0.826$; Fig. 2*D*). This was in contrast to the slope in lever response rate, which was significantly lower than zero ($t_{(33)} = 18.19$, $p < 0.001$). Furthermore, the number of cue-responsive and error-responsive neurons did not significantly differ between males and females (Cue: $\chi^2 = 0.20$, $p = 0.658$; Error: $\chi^2 = 0.21$, $p = 0.642$), and these neurons also did not change slope during the DT task (Cue: $t_{(22)} = 0.37$, $p = 0.717$; Error: $t_{(22)} = -0.09$, $p = 0.930$). We did find that, in males, there were significantly more phasic neurons to lever extension than to cue ($\chi^2 = 5.84$, $p = 0.016$) or error ($\chi^2 = 7.57$, $p = 0.006$). We also found that there were no impacts of estrous cycle on phasic activity for lever extension, cue, or errors (Lever: $\chi^2 = 0.23$, $p = 0.628$; Error: $\chi^2 = 0.01$, $p = 0.927$; Cue: $\chi^2 = 1.28$, $p = 0.258$). Last, there were no sex differences in the ratio of excited to inhibited cells following lever extension, lever press, or cue (Lever: $\chi^2 = 0.01$, $p = 0.925$; Cue: $\chi^2 = 1.65$, $p = 0.200$; Error: $\chi^2 = 0.38$, $p = 0.536$). In total, this demonstrates that females have lower DT and lower PrL activity specifically to the lever extension during the DT task in the cocaine naive state.

Female rats exhibit heightened reward-taking and reward-seeking compared with male rats

Similar to previous findings (Ahmed and Koob, 1998), male and female cocaine rats both significantly escalated intake across self-administration ($F_{(5.80,98.58)} = 9.74$, $p < 0.001$; Fig. 3*A*). Females self-administered significantly more cocaine ($F_{(1,17)} = 8.59$, $p = 0.009$) and water/saline ($F_{(1,17)} = 15.70$, $p = 0.001$) than males, corroborating previous reports (Becker and Koob, 2016; Sanchis-Segura and Becker, 2016). Males and females decreased water/saline intake initially, but stabilized responding thereafter ($F_{(7.01,119.28)} = 4.55$, $p < 0.001$; Fig. 3*B*). Consistent with previous work demonstrating incubation of craving (Kerstetter et al., 2008), males and females increased reward-seeking following extended abstinence for both cocaine and water/saline ($F_{(1,29)} = 28.71$, $p < 0.001$; Fig. 3*C*,*D*). Females had significantly higher reward-seeking than males for both water/saline and cocaine ($F_{(1,29)} = 4.48$, $p = 0.043$).

A history of cocaine induces PrL activity to track DT in male rats

DT decreased following abstinence in males, but not females ($F_{(1,29)} = 9.47$, $p = 0.005$). However, there were no differences in this decrease between cocaine and water/saline rats ($F_{(1,29)} = 0.37$,

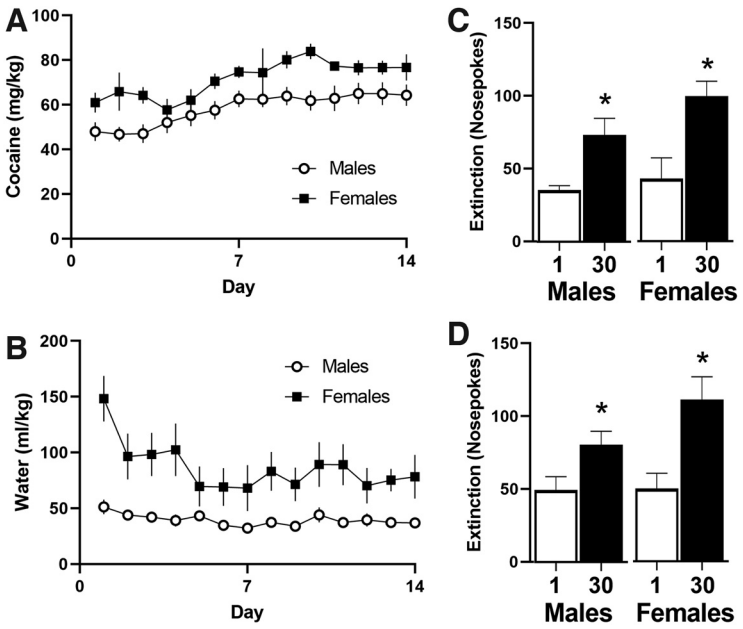

Figure 3. Self-administration. ***A***, Males and females significantly escalated cocaine intake, and females self-administered significantly more cocaine than did males. ***B***, Females self-administered significantly more water than did males. Males and females both showed incubation of craving for cocaine (***C***) and water/saline (***D***). Females had higher nosepokes across all extinction tests than did males. Error bars: ± SEM; * $p < 0.05$.

$p = 0.549$; see Fig. 4*A*). Furthermore, there was no relationship between the amount of cocaine self-administered and the change in DT in either male or female rats (males: $r = -0.54$, $p = 0.106$; females: $r = 0.63$, $p = 0.133$), 184 neurons were recorded in the PrL during the DT session following 28 d of abstinence. Neither males nor females changed the percent of PrL cells that were phasic to the lever extension, lever press, or cue duration following abstinence (Lever, male, cocaine: $\chi^2 = 0.68$, $p = 0.410$; Fig. 4*B*, top left; Lever, female, cocaine: $\chi^2 = 1.40$, $p = 0.236$; Fig. 4*B*, bottom left; Lever, male, water/saline: $\chi^2 = 0.92$, $p = 0.337$; Fig. 4*B*, top right; Lever, female, water/saline: $\chi^2 = 0.01$, $p = 0.904$; Fig. 4*B*, bottom right; Cue, male, cocaine: $\chi^2 = 0.01$, $p = 0.939$; Cue, female, cocaine: $\chi^2 = 0.01$, $p = 0.929$; Cue, male, water/saline: $\chi^2 = 0.40$, $p = 0.527$; Cue, female, water/saline: $\chi^2 = 0.05$, $p = 0.831$; Error, male, cocaine: $\chi^2 = 0.05$, 0.829; Error, female, cocaine: $\chi^2 = 0.00$, $p = 0.987$; Error, male, water/saline: $\chi^2 = 0.05$, $p = 0.829$; Error, female, water/saline: $\chi^2 = 0.08$, $p = 0.774$). Because behavioral response rate decreases over the course of the DT task, we also assessed the change in neural activity over the course of the task, or "neural slope." In males, cocaine, but not saline, rats had a significant decrease in the neural slope of PrL activity aligned to lever extension across the course of the DT task following 28 d abstinence (Day × Drug: $F_{(1,12)} = 6.01$, $p = 0.031$; Bonferroni *post hoc* tests found a significant difference between naive and abstinence slopes in the cocaine group; Fig. 4*C*). Furthermore, in cocaine male rats, the change in slope following abstinence significantly correlated with the change in DT ($r = -0.77$, $p = 0.024$; Fig. 4*D*). There was no relationship between the amount of cocaine self-administered and the change in neural slope ($r = 0.63$, $p = 0.095$). There were no changes in slope for cue or lever response aligned activity (Cue: $F_{(1,10)} = 0.10$, $p = 0.760$; Error: $F_{(1,8)} = 0.23$, $p = 0.659$), nor were there any changes in excited/inhibited ratio following lever extension, lever press, or cue after cocaine or water abstinence in males (Lever, cocaine: $\chi^2 = 0.07$, $p = 0.796$; Lever, water/saline: $\chi^2 = 3.30$, $p = 0.069$; Cue, cocaine: $\chi^2 = 0.01$, $p = 0.906$; Cue, water/saline: $\chi^2 = 3.54$, $p = 0.060$; Error, cocaine: $\chi^2 = 0.01$, 0.906; Error, water/saline: $\chi^2 = 0.18$; $p = 0.671$). These findings demonstrate that PrL activity shifted to track DT following extended abstinence from cocaine self-administration in male rats.

DT and PrL activity predict drug-seeking in male rats

We found that male rats with low DT after abstinence had significantly higher drug-seeking ($r = -0.64$, $p = 0.048$; Fig. 5*A*, top left). However, there was no relationship between DT and cocaine-seeking in females ($r = 0.22$, $p = 0.624$; Fig. 5*A*, bottom left), nor were there any relationships between DT and water-seeking in either sex (Water, males: $r = 0.01$, $p = 0.977$; Water, females: $r = 0.39$, $p = 0.500$; Fig. 5*A*, right). Because behavioral response rate decreases over the course of the DT task, we also assessed the change in neural activity over the course of the task, or "neural slope." This is especially relevant given the cocaine-induced change in neural slope following lever extension reported above. A multiple regression assessing DT and neural slope as independent predictors of drug-seeking was not significant (adjusted $r^2 = 0.46$, $p = 0.091$). Nonetheless, male rats with the strongest negative neural slope (i.e., greatest decrease in phasic activity across the course of the DT task) had the lowest levels of cocaine-seeking ($r = 0.73$, $p = 0.041$; Fig. 5*B*, left). There were no relationships between PrL activity and water-seeking in males ($r = 0.05$, $p = 0.935$; Fig. 5*B*, right). Thus, both DT and PrL activity during DT predicted cocaine-seeking behavior following extended abstinence in male rats.

Histology

Data were only included for cells located in the PrL. The location of these cells is depicted in Figure 6.

Discussion

The relationship between DT and substance use disorders is well established (Brown et al., 2002, 2009; Brandon et al., 2003; Daughters et al., 2005a,b; Strong et al., 2012), but the underlying neurocircuitry is poorly understood. Here, we demonstrate that the PrL tracks drug-induced changes in DT and predicts cocaine-seeking behavior in a sex-dependent manner. Importantly, the PrL did not track DT in the drug-naive state, although there were significant sex differences in both DT and in PrL activity during the DT task. Following extended abstinence from cocaine, the shift in PrL activity predicted the change in DT in male rats. Furthermore, PrL activity during the DT task predicted cocaine-seeking following extended abstinence in males as well. These findings are summarized in Table 1.

Females exhibit lower DT and lower PrL activity during the DT task

We found that drug-naive female rats had significantly lower DT than drug-naive male rats. This replicates clinical findings demonstrating lower DT in women than men (Falavarjani and Yeh, 2019), although other studies found no differences between men and women (Kerstetter et al., 2008; Ali et al., 2015). While females

had lower DT than males, there was no relationship between DT and phase of the estrus cycle, suggesting that this decrease was not driven by fluctuations in circulating hormones.

Neurons in the PrL tracked multiple events in the DT task, including lever extension, cue illumination, and lever press. In males, we found that the PrL encoded the lever extension to a significantly greater extent than the cue or lever press. This fits well with data from our laboratory and others using similar tasks that have seen the PrL encode more activity before the cue light and have found that this "precue" PrL activity is more predictive of behavior (Narayanan and Laubach, 2006; Moschak and Carelli, 2021a). We also found that PrL activity following lever extension was significantly lower in females than in males, suggesting that the PrL may play a significantly larger role in males than in females in DT. Females have been shown to exhibit lower PrL activity than males for several behaviors, including impulsivity (Moschak and Carelli, 2021a), contextual renewal for appetitive cues (Anderson and Petrovich, 2017), and both controllable and uncontrollable stress (Bland et al., 2005; Baratta et al., 2018). Furthermore, females exhibited lower activity in the ACC than men in response to stress-related cues (Seo et al., 2011). Thus, the PrL's dampened activity in females during the DT task may be a result of its reduced role in stress for females.

PrL activity tracks DT following a history of cocaine

In the drug-naive state, we found that PrL activity did not track DT. Contrary to the sharp decrease in behavioral responses over the course of the DT task, PrL neurons continued to fire at the same rate at the beginning of each trial for the duration of the DT task. This was somewhat unexpected, as the PrL plays a role in processing goal-directed behavior (Tran-Tu-Yen et al., 2009; West et al., 2021). However, it should be noted that BOLD activity in the ACC did not change during a DT task in control subjects (Daughters et al., 2017). Furthermore, prior findings demonstrate that inactivation of the PrL does not disrupt break point in a progressive ratio task (Swanson et al., 2019), which shares some similarities with the DT task.

Following abstinence from self-administration, PrL neurons in Water/Saline controls continued to fire at the

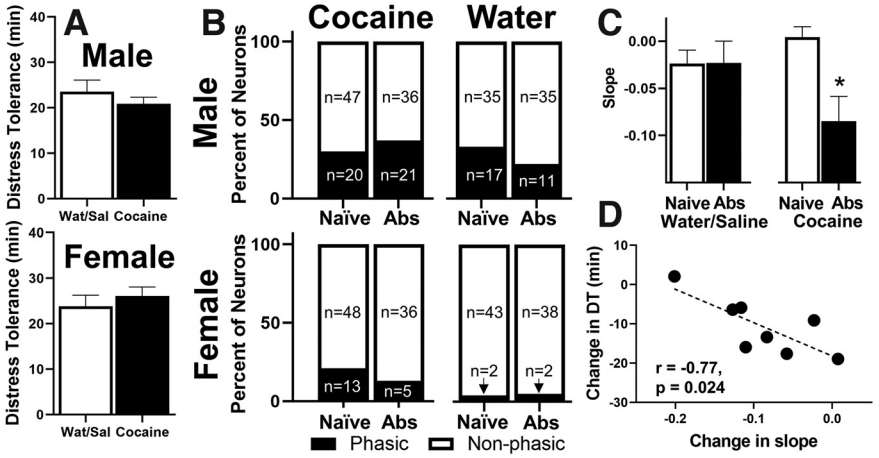

Figure 4. Drug-abstinent DT and neural activity. ***A***, There was no impact of a history of cocaine on DT for males (top) or females (bottom). ***B***, There were no differences in the percent of phasic neurons to lever extension following abstinence from cocaine or water/saline in males and females. ***C***, In males following abstinence from cocaine, the neural slope across the duration of the DT task significantly decreased for neurons aligned to the lever extension. This indicates that these neurons decreased their phasic activity across the duration of the DT task. ***D***, The change in neural slope significantly predicted the change in DT in male cocaine rats. Error bars: ± SEM; $^{*}p < 0.05$.

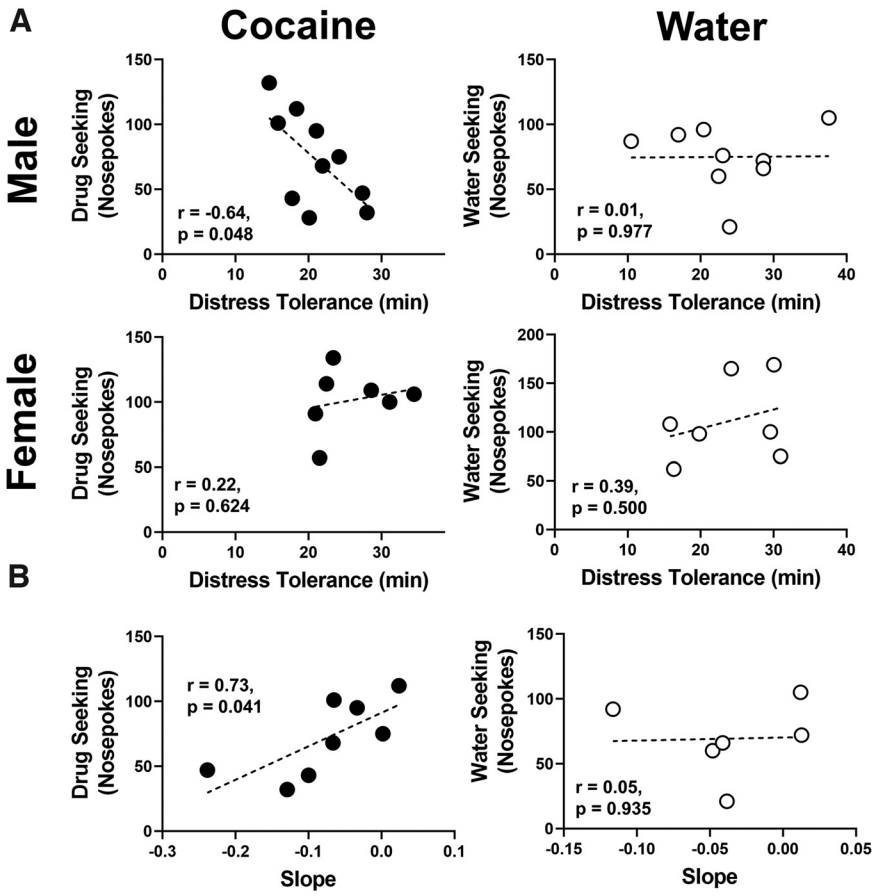

Figure 5. Behavioral and neural predictors of cocaine-seeking behavior. ***A***, Relationship between DT and cocaine-seeking behavior. In males, low DT significantly predicted high cocaine-seeking behavior (top left), which was not seen in females (bottom left). There was no relationship between DT and water-seeking behavior in either sex (right). ***B***, In males, animals with the steepest neural slope in neurons aligned to the lever extension had the lowest cocaine-seeking behavior (left). There was no relationship between neural slope and water-seeking behavior (right).

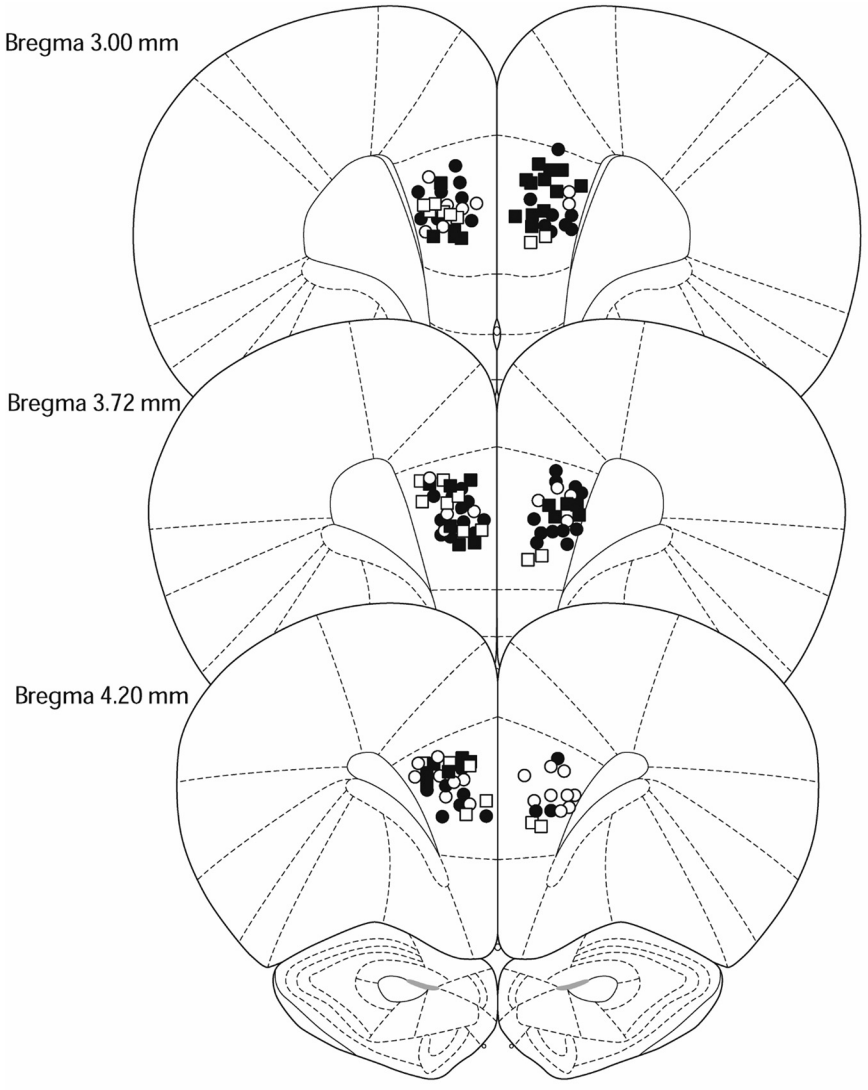

Figure 6. Location of electrodes in the PrL. ○, Male water/saline; ●, male cocaine; □, female water/saline; ■, female cocaine.

Table 1. Summary of results

Sex differences in DT and prelimbic activity before self-administration
• Females had lower DT than males
• Females had lower phasic prelimbic activity than males during DT
Effects of extended cocaine abstinence on prelimbic activity during DT
• In males, a history of extended cocaine abstinence induced prelimbic activity to track behavior in the DT task
• The change in prelimbic activity correlated with the change in DT following extended abstinence from cocaine
Relationship between DT and prelimbic activity to cocaine-seeking
• DT following extended cocaine abstinence predicted drug-seeking in males, but not females
• Prelimbic activity during the DT task following extended cocaine abstinence predicted drug-seeking in males

same rate throughout the course of the DT task. However, PrL neurons in Cocaine rats shifted to track DT, and were no longer persistently active throughout the course of the DT task. These findings fit with clinical literature demonstrating that ACC activity tracks DT in cocaine users, but not healthy controls (Daughters et al., 2017). However, rats with the sharpest decreases in "neural slope" were unexpectedly the rats with the least amount of change in DT following a history of cocaine. Thus, the change in neural slope may not cause the decrease in DT but instead act as an adaptive response to counter the decrease in DT. We recently reported a similar adaptive neural response in the PrL with respect to cocaine's effects on impulsivity (Moschak and Carelli, 2021a); we and others have also found such counteracting neural responses in other brain regions following a history of cocaine (Goldstein et al., 2009; Moschak et al., 2018b). Together, these findings suggest an important role for adaptive homeostatic processes in addition to the dysregulated homeostasis that has been well reported in substance use disorders (Koob, 2008).

DT and PrL activity predict cocaine-seeking behavior

Consistent with the clinical literature (Daughters et al., 2005a,b) and replicating our previous findings (Moschak et al., 2018a), we found that DT following extended abstinence from cocaine predicted cocaine-seeking behavior in male rats. Interestingly, we found no such relationship in female rats. This is in contrast to previous clinical studies which found no sex differences in DT's ability to predict treatment outcome in cocaine-dependent patients (Daughters et al., 2005a,b). However, other studies have found sex differences in DT's relationship with substance use. In particular, several studies found that DT was only predictive of high substance use problems (including cocaine) in women with high depressive symptoms (Ali et al., 2015; Pedrelli et al., 2018). Given this interaction, future studies should investigate the role of affective state and sex on DT and its relationship to drug-seeking.

In addition to these behavioral results, we also found that, in male rats, PrL activity during the DT task predicted cocaine-seeking behavior following extended abstinence from cocaine self-administration. On its face, this fits with our hypothesis that PrL activity during the DT task would predict cocaine-seeking behavior, and corroborates existing literature demonstrating a role for both DT and PrL activity in drug-seeking (Capriles et al., 2003; McFarland et al., 2003; Daughters et al., 2005b; Stefanik et al., 2013; West et al., 2014; Moschak et al., 2018a). However, in line with our earlier counterintuitive findings, rats with the steepest neural slope during the DT task unexpectedly had the lowest levels of cocaine-seeking behavior. Coupled with our aforementioned results demonstrating that animals with the largest change in PrL neural slope had the least amount of change in DT, this suggests that, in some animals, the PrL engages an adaptive process in response to cocaine abstinence that promotes high DT and low drug-seeking following incubation of craving. Notably, the PrL undergoes synaptic plasticity that drives incubation of

craving (Ma et al., 2014; Shin et al., 2018; Moschak and Carelli, 2021b) and increases PrL activity in response to drug cues (Sun and Rebec, 2006; Zavala et al., 2008; Hearing et al., 2013; West et al., 2014). However, our data suggest that a counteracting effect occurs in response to nondrug cues (e.g., lever extension, the cue signaling the trial start in the DT task) in a subset of animals. Future work should investigate individual differences in PrL activity across multiple behavioral contexts (drug, nondrug) following incubation of craving.

In conclusion, the current study demonstrates that neural activity in the PrL during the DT task is altered (steeper "neural slope") following a history of cocaine and predicts subsequent cocaine-seeking behavior. This corroborates existing research demonstrating a role for both DT and the PrL in drug-seeking (Capriles et al., 2003; McFarland et al., 2003; Daughters et al., 2005a; Stefanik et al., 2013; West et al., 2014; Moschak et al., 2018a) and demonstrates a strong link between the two of them as drivers of drug-seeking behavior. Future research is needed to investigate the role of PrL afferents and efferents in DT and to examine the relationship between DT and other drugs of abuse.

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