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Circulating ovarian hormones interact with protein interacting with C kinase (PICK1) within the medial prefrontal cortex to influence cocaine seeking in female mice

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Abstract

Protein interacting with C kinase 1 (PICK1) is an AMPA receptor binding protein that works in conjunction with glutamate receptor interacting protein (GRIP) to balance the number of GluA2-containing AMPARs in the synapse. In male mice, disrupting PICK1 in the medial prefrontal cortex (mPFC) leads to a decrease in cue-induced cocaine seeking and disrupting GRIP in the mPFC has the opposing effect, consistent with other evidence that removal of GluA2-containing AMPARs potentiates reinstatement. However, PICK1 does not seem to play the same role in female mice, as knockdown of either PICK1 or GRIP in the mPFC leads to similar increases in cue-induced cocaine seeking. These previous findings indicate that the role of PICK1 in the prefrontal cortex is sex specific. The goal of the current study was to examine whether ovarian hormones contribute to the effect of prefrontal PICK1 knockdown on reinstatement of cocaine seeking. While we replicated the increased cue-induced cocaine seeking in prefrontal PICK1 knockdown sham mice, we did not see any difference between the GFP control mice and PICK1 knockdowns following ovariectomy. However, this effect was driven primarily by an increase in cocaine seeking in ovariectomized GFP control mice while there was no effect ovariectomy in PICK1 knockdown mice. Taken together, these findings suggest that circulating ovarian hormones interact with the effects of PICK1 on cue-induced reinstatement.

Keywords

PICK1; sex differences; cocaine; prefrontal cortex; reinstatement

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Ethics approval and consent to participate

All procedures using experimental animals were approved by Temple's Institutional Care & Use Committee.

Consent for publication

All authors read and approved the final manuscript for publication.

Competing interests

The authors declare no conflict of interest.

Introduction

Cocaine use is highly prevalent in the United States, with a reported 5.2 million American's having used cocaine in 2020 (U.S. Department of Health and Human Services, 2021). Further, cocaine use accounted for 45.52% of emergency department visits in adults between the ages of 26 to 44 in 2021 (U.S. Department of Health and Human Services, 2022). Despite this, effective treatments for cocaine use disorder (CUD) remain elusive. Gaining a better understanding of the mechanisms driving CUD, as well as whether these mechanisms differ across biological sex, could provide novel avenues for treatment.

While the primary pharmacological action of cocaine is to bind to the dopamine transporter and increase dopamine in the synapse, chronic cocaine also impacts glutamate neurotransmission (Pierce et al., 1996; Schmidt and Pierce, 2010). One way that cocaine alters glutamate transmission is through altering the trafficking of glutamate receptors (Boudreau et al., 2007; Boudreau and Wolf, 2005; Famous et al., 2008). Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors are made up of four subunits, GluA1–4. The distribution of these subunits can confer different receptor properties; GluA2-containing AMPA receptors are calcium impermeable while those lacking GluA2 subunits are calcium permeable. As calcium permeable AMPA receptors exhibit higher conductance, activity dependent alterations in the distribution of these receptors can lead to changes in excitability. For example, cocaine self-administration leads to GluA2 containing AMPAR internalization via protein kinase C (PKC)-mediated phosphorylation and subsequent dissociation between GluA2 and glutamate receptor interaction protein (GRIP) (Dong et al., 1997; Matsuda et al., 1999). GRIP anchors AMPARs to the synaptic membrane and when the association between GRIP and GluA2 is dissolved, GluA2 binds to protein interacting with C kinase 1 (PICK1) leading to receptor removal from the synapse, as PICK1 is responsible for regulating AMPAR trafficking and synaptic plasticity (Chung et al., 2000; Volk et al., 2010).

Alterations in glutamate neurotransmission due to chronic cocaine use are also influenced by baseline sex differences in AMPAR trafficking. In females, higher levels of glutamate have been observed during the proestrus stage of the estrous cycle – when levels of ovarian hormones are at their highest (Frankfurt et al., 1984). Additionally, under basal conditions, female rats have enhanced hippocampal AMPAR synaptic responses compared to their male counterparts, likely due to increased phosphorylation of the GluA2 subunit (Wickens et al., 2018). In males and females, the GluA2 subunit is internalized through dissociation from GRIP, allowing PICK1 to bind to the subunit in its place (Chung et al., 2000).

The prefrontal cortex plays a prominent role in cocaine relapse and cocaine can impact the function of the PFC to potentiate cocaine seeking (Bechard et al., 2021; Capriles et al., 2003; Guercio et al., 2020; Hofford et al., 2021; McFarland et al., 2003; Rubio et al., 2019; Warren et al., 2019). Previous work from our lab found that disruption of GRIP in the mPFC potentiated reinstatement of drug seeking behavior in male and female mice (Wickens et al., 2019). Because of this, we then hypothesized that prefrontal deletion of PICK1 would have an opposite effect due to a net decrease in AMPA transmission. In females, however,

this was not the case. Female mice with a PICK1 knockdown exhibited an increase in cue-induced cocaine responding compared to their male counterparts (Wickens et al., 2021). This suggests that PICK1 may play a different role in the prefrontal cortex of females compared to males (Wickens et al., 2021, p. 202).

These sex differences in glutamate signaling and AMPAR trafficking mechanisms may be driven by gonadal hormones, as glutamate levels fluctuate across the female menstrual cycle. Specifically, blood glutamate has an inverse relationship with plasma estrogen and progesterone, as females exhibit lower levels of blood glutamate during the beginning phases of menstruation (Zlotnik et al., 2011). In female rats, endogenous estrogen impacts excitatory and inhibitory synaptic transmission in the reward system (Freese et al., 2020). Specifically, estrogen acts through positive modulation of glutamate postsynaptic membranes and increases the number of NMDA receptors, leading to an increase in neuronal excitability (Freese et al., 2020).

Gonadal hormones also play a role in cocaine taking and seeking. In female rats, administration of the reproductive hormone estradiol increases cocaine intake during self-administration (Zhao and Becker, 2010). Additionally, female rats treated with estrogen benzoate consistently choose cocaine over a food reward more than males (Kerstetter et al., 2012). In humans, females report more intense effects of cocaine when estrogen levels are highest (Evans et al., 2002). The same has been seen in rodents. When ovaries are removed in rats who are trained on cocaine self-administration, these effects are reversed (Kerstetter et al., 2012). In male mice, removal of gonadal hormones eliminates the effects of PICK1 knockdown on cue-induced reinstatement of cocaine seeking (Wickens et al., 2021). While prefrontal PICK1 knockdown on its own blunts cocaine seeking in a cue-induced reinstatement test, following gonadectomy, prefrontal PICK1 knockdown leads to an increase in cue-induced reinstatement responding, mimicking what is seen in female mice (Wickens et al., 2021). The current study buildings upon this work examining interaction between gonadal hormones and PICK1 to examine the effect of ovariectomy in female mice on cocaine self-administration following PICK1 knockdown in the mPFC.

Materials and methods

Subjects.

Mice homozygous for the Cre/lox-conditional allele of PICK1 (flox/flox) were bred on a C57bl/6J background. These mice were bred from founders provided by Dr. Richard Huganir at Johns Hopkins University as previously described(Lin and Huganir, 2007; Steinberg et al., 2006; Wickens et al., 2021). Adult female mice (2–6-months old, age matched across group) were group housed until three days before the first day of food training, at which time they were single housed and began food deprivation. Food deprivation (receiving 1 standard rodent chow pellet/day) lasted until the third day of cocaine self-administration. The single housing condition lasted throughout the duration of the experiment. All mice were housed in a temperature- and humidity-controlled animal care facility with a 12-h light/dark cycle (lights on at 0700 hours). All procedures were approved by the Temple University Animal Care and Use Committee. Cocaine was obtained from the

National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

Prefrontal Microinjections and Adeno-Associated Virus Constructs.

The adeno-associated virus (AAV) expressing Cre recombinase (AAV2/9.CMV.PI.CRE, titer 2.84 × 1013 vgc/μl) and the AAV expressing green fluorescent protein (eGFP) (AAV2/9.CMV.eGFP, titer 3.74 × 1013 vgc/μl) were generated by Addgene. PICK1 flox/flox mice (6–8 weeks) were anesthetized with isoflurane and 0.4μl of the viral construct (Cre or GFP) was injected bilaterally into the prefrontal cortex through a 30-gauge needle at a rate of 0.1 μl/min. Stereotaxic coordinates for the prefrontal cortex are (from Bregma) anterior-posterior 2.6, lateral +/− 0.3, dorso-ventral −2.3. Following recovery, mice remained in the home cage for 6 weeks prior to behavioral testing. The procedures involving the AAV viruses have all been approved by the Temple University Institutional Biosafety committee.

Ovariectomy.

Two weeks prior to food training, mice underwent ovariectomy or sham surgery. Mice were anesthetized with .80 mg/kg ketamine and 12 mg/kg xylazine via an IP injection. An incision was made into the midline and the ovaries were removed. The peritoneum and skin were then sutured, and a drop of betadine surgical scrub was applied to promote healing. Sham mice received incisions into the skin and peritoneum only. All mice were placed under a heat lamp until awake and mobile. For the week following ovariectomy or sham surgery, mice were monitored to ensure proper healing. Meloxicam (2.0 mg/kg, subcutaneous injection) was administered for the first three days following surgery, and triple antibiotic ointment applied to the surgical site until the skin had fully healed.

Operant Food Training.

Before catheterization, mice were trained to perform an operant response for sucrose pellets. The mice were placed in operant chambers (Med-Associates) and trained to spin a wheel manipulandum to receive a sucrose pellet, with one-quarter spin measured as a single active response. Mice performed 10 days of FR1 responding. A compound cue stimulus consisting of a cue light above the active wheel, a 2900-Hz tone, and house light off was concurrent with each pellet administration, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Mice were allowed to self-administer a maximum of 50 pellets per 60 min operant session. During the food training phase, mice were food restricted to >90% of their free-feeding weight. Mice returned to *ad libitum* food access 3 days following the start of the cocaine self-administration phase.

Jugular Catheterization Surgery.

Prior to surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (Instech Laboratories, Inc.) that secured the placement. Meloxicam (2.0 mg/kg, subcutaneous injection) was administered for the first three days following surgery

while animals were allowed to recover. Catheters were flushed daily with 0.1 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline.

Cocaine Self-Administration.

Following recovery from surgery, mice were tested for cocaine self-administration behavior in 2-hour sessions in the same chamber used for sucrose pellet self-administration. During testing, responding on the wheel now delivered an intravenous cocaine injection (0.6 mg/kg/infusion), paired with the same compound cue, under the same schedule as the food training. After the cocaine self-administration phase, mice began extinction training, in which cocaine-seeking behavior was extinguished by replacing the cocaine with 0.9% saline. During this time the light and tone cues paired with cocaine delivery were not present. Daily 2-h extinction sessions continued until mice met the extinction criterion of less than 25% of their self-administration responding (average of last 3 days). Twenty-four hours after meeting the extinction criterion, mice underwent a cue-induced reinstatement session. During the cue-induced reinstatement session, the light and tone cues were presented non-contingently for 20 seconds every 2 minutes during the first 10 minutes of the session. After this time period, the cues were presented contingent with operant responding, just as was done during the cocaine self-administration phase. During the reinstatement session, mice received saline infusions following responses on the active wheel. During self-administration, extinction, and reinstatement, experimenters were blind to the condition of the mice.

Statistical Analysis.

Self-administration results were analyzed with two-way repeated measures ANOVAs in which viral vector injection and day were the independent variables and pellets/infusions/responses as the dependent variable. Groups were separated into ‘sham control’ or ‘ovariectomy’. An additional two-way ANOVA was performed on the reinstatement data with surgery condition and viral vector injection as the independent variables and responses as the dependent variable. Sidak’s post hoc comparisons were made when main effects or interactions were detected ($p < 0.05$).

Results

3.1 Prefrontal knockdown of PICK1 and ovariectomy have no effect on sucrose self-administration.

Six weeks following viral injections into the mPFC, PICK1 knockdown mice and GFP controls were trained for 10 days on an FR1 schedule of reinforcement to acquire sucrose self-administration. Both ovariectomized and sham treated mice from both groups acquired sucrose self-administration, evidenced by a significant increase in number of pellets earned across the 10 sessions [sham: effect of session, $F(2.131, 68.19)=13.4$, $p< 0.0001$; OVX: effect of session, $F(1.846, 59.08)=12.3$, $p< 0.0001$; Figure 1A, B] and increase in responses [sham: effect of session, $F(1.627, 52.07)=11.2$, $p=0.0002$; OVX: effect of session, $F(1.600, 49.59)=13.01$, $p<0.0001$; Figure 1C, D]. There was no effect of virus on number of pellets earned [sham: effect of viral infusion, $F(1, 32)=0.0057$, $p=0.947$; OVX: effect of viral infusion, $F(1, 32)=0.18$, $p=0.67$; Figure 1A, B] or active responses [sham: effect of viral

infusion, $F(1, 32)=0.47$, $p=0.50$, OVX: effect of viral infusion $F(1, 31)=0.77$, $p=0.39$; Figure 1C, D].

3.2 Prefrontal knockdown of PICK1 and ovariectomy have no effect on acquisition of cocaine self-administration.

Following 10 days of operant food training, mice were implanted with a jugular catheter and trained on cocaine self-administration. During the 10-day period, sham mice exhibited a slight increase in cocaine intake, while mice in the OVX conditions did not [sham: main effect of session, $F(1.883, 52.71)=3.8$, $p=0.03$; OVX: effect of session, $F(2.618, 68.06)=2.30$, $p=0.09$; Figure 2A, B]. There was no effect of PICK1 knockdown on responding for cocaine in either the sham of OVX conditions [sham: $F(1, 28)=1.55$, $p=0.22$; OVX: $F(1, 26)=0.57$, $p=0.46$; Figure 2A, B]. Additionally, there were no differences in responding either over the 10 days [sham: $F(2.749, 76.97)=0.34$, $p=0.78$; OVX: $F(1.876, 50.65)=1.25$, $p=0.29$] or between the different viral infusion groups [sham: $F(1, 28)=0.03$, $p=0.87$; OVX: $F(1, 27)=1.58$, $p=0.22$; Figure 2C, D].

3.3 Ovariectomy eliminates effects of prefrontal PICK1 knockdown on cue-induced reinstatement.

After completion of cocaine self-administration, mice began extinction training followed by a single cue-induced reinstatement test. There were no significant differences between groups in responding on day 1 of extinction [effect of surgery condition, $F(1,21)=2.34$, $p=.14$; effect of viral injection, $F(1,21)=1.85$; $p=.19$; Table 1]. or in the number of days needed to meet extinction criterion [effect of surgery condition, $F(1,21)=0.33$, $p=.57$; effect of viral injection, $F(1,21)=0.26$; $p=.62$; Table 1]. Sham treated PICK1 knockdown mice exhibited a significantly higher number of active responses during their cue-induced reinstatement test compared to GFP controls [effect of virus, $F(1, 9)=10.97$, $p=0.009$, effect of session, $F(1,9)=24.6$, $p=.0008$, interaction, $F(1,9)=10.2$, $p=.01$; Sidak post-hoc, GFP control vs. Cre PICK KD, $p=0.0005$; Figure 3A]. In contrast, following OVX, there are no differences between the GFP and PICK1 knockdown mice in cue-induced reinstatement responding [effect of virus, $F(1, 12)=0.23$, $p=0.64$, effect of session, $F(1,12)=34.7$, $p<.0001$, interaction, $F(1,12)=0.59$, $p=.46$; Figure 3B]. A two-way, independent measures ANOVA comparing reinstatement responding across both surgical conditions reveals as significant interaction between PICK1 knockdown and ovariectomy [effect of virus, $F(1, 21)=10.23$, $p=0.004$, effect of OVX, $F(1,21)=0.87$, $p=.36$, interaction, $F(1,21)=4.85$, $p=.039$].

Discussion

Overall, we found that ovariectomy eliminated the differences in cue-induced reinstatement driven by mPFC PICK1 knockdown in female mice. Similar to our previously published findings in gonadally-intact mice (Wickens et al., 2021), sham surgerized mice exhibit an increase in cue-induced cocaine seeking during a reinstatement test following mPFC knockdown of PICK1. However, following ovariectomy, there was no difference between GFP control mice and mPFC PICK1 knockdown mice. We did not see any effects of either mPFC PICK1 knockdown or ovariectomy on sucrose self-administration or cocaine taking during the drug self-administration phase. This suggests that the effects of mPFC PICK1

knockdown in females may be mediated in part by ovarian hormones. A closer look at the data reveals that this lack of effect of PICK1 knockdown may be driven, in part, by an increase in cue-induced cocaine seeking seen in control mice following ovariectomy. Of note, the current study did not track estrous cycle in our sham mice, so it is possible there are differences in cycle stage across groups that are driving some of these differences in the control groups.

4.1 Prefrontal PICK1 knockdown enhances cue-induced reinstatement in sham females while not influencing reinstatement following ovariectomy.

Knockdown of PICK1 in the mPFC resulted in a significant increase in cue-induced reinstatement responding in sham treated mice when compared to their GFP counterparts. However, following OVX, there were no differences between the GFP and PICK1 knockdown mice, with both exhibiting greater reinstatement than the sham treated GFP controls. The lack of effect seen between the OVX GFP control mice and the OVX PFC PICK1 knockdown mice is driven by an increase in cue-induced reinstatement in the control mice following OVX rather than a decrease in cue-induced cocaine seeking in PICK1 KO mice following OVX. This data may seem to conflict with other work suggesting that estrogen promotes drug seeking. Acute and chronic estrogen treatment following ovariectomy enhances escalation of cocaine intake and cocaine-primed reinstatement (Doncheck et al., 2018; Larson et al., 2007, 2005). However, the influence of estrous cycle on cue-induced cocaine seeking are less clear. In rats, female mice in estrus exhibit similar rates of cue-primed reinstatement following extinction training compared to females in non-estrus at many doses (Bechard et al., 2018; Fuchs et al., 2005). Similarly, no differences were seen between estrus and non-estrus female rats in cue-induced cocaine seeking in the absence of extinction (Kerstetter et al., 2008). However, other studies utilizing similar incubation of craving models, have seen higher levels of cue-induced cocaine seeking in estrus females compared to non-estrus females following longer withdrawal time periods (Corbett et al., 2021; Nicolas et al., 2019). These differences could be due, in part, to differences in the self-administration paradigms, including differences in the length of the sessions, presence of prior food training, or differences in dose, as lower doses often uncover subtle sex differences as well as effects of estrous cycle stage (Fuchs et al., 2005).

As OVX disrupts the cyclicity of multiple hormones beyond simply estrogen, the current findings may be caused by the disruption of progesterone or allopregnanolone. In line with this hypothesis, progesterone treatment in sham animals dampens escalation of cocaine intake in an extended access self-administration paradigm (Larson et al., 2007) and decreases reinstatement of cocaine seeking (Anker et al., 2007). Further, higher progesterone levels can be associated with lowered levels of cocaine craving in humans (Sinha et al., 2007). Similarly, allopregnanolone administration also dampens reinstatement behavior in female rats (Anker et al., 2009).

While many studies have investigated the difference between groups treated with high levels of estrogen and OVX groups, these levels are not physiologically accurate. Because of this, research has been done to examine these differences in free-cycling rodents. In free-cycling rodents tested during estrus and non-estrus, results found that there was a

significant effect of withdrawal day – with females in the estrus stage of their cycle exhibiting significantly increased responding on a cue-induced reinstatement test following cocaine self-administration (Corbett et al., 2021). Levels of estradiol and progesterone fluctuate greatly across the estrous cycle, with estradiol being higher than progesterone during proestrus, and levels of progesterone levels being higher during the subsequent stage of metestrus and diestrus (Becker et al., 2005). Further research needs to be conducted on the relationship between PICK1 and the estrous cycle, noting that OVX reverses the effects of PICK1 knockdown on cue-induced reinstatement as described here.

4.2 Ovariectomy and PICK1 knockdown have no effects on self-administration of sucrose or cocaine.

Neither PICK1 knockdown nor OVX influenced either sucrose or cocaine taking over the course of self-administration. This is consistent with previous findings showing that PICK1 has no effects on natural reward reinforcement (Turner et al., 2020). Our lab has previously demonstrated that knockdown of PICK1 has no effects on natural reward in males or females who had not experienced OVX or sham surgeries (Wickens et al., 2021). These data indicate that the effects of OVX and PICK1 knockdown on cue-induced reinstatement are specific to the rewarding effects of cocaine, and not a result of operant learning. Additionally, these data show that OVX and PICK1 knockdown did not cause any deficits in operant learning. Previous work in rats has shown that there are no differences in motivation to self-administer sucrose between males and females, regardless of estrous cycle stage (Datta et al., 2017). Furthermore, compulsive reward seeking behavior in female rats was not predictive of cocaine-seeking behavior during self-administration, indicating that increased cocaine responding is specific to the rewarding effects of the drug (Datta et al., 2018). This finding is consistent throughout the literature, indicating that the rewarding effects of cocaine are distinct from natural reward.

Conclusion

The data in this study replicate our previous finding that knockdown of PICK1 in the mPFC in female mice leads to an increase in responding on a cue-induced reinstatement test following cocaine self-administration. However, there is no effect of PICK1 knockdown in female mice treated with ovariectomy to stop the circulation of ovarian hormones. Of note, the lack of effect of PICK1 knockdown in OVX mice was driven in part by increased cue-induced reinstatement in GFP control mice following OVX. These data indicate that ovariectomy can occlude the effects of PICK1 knockdown on cue-induced reinstatement, emphasizing the importance of examining the effects of estrous cycling on addiction circuitry. Further study is needed to examine the specific connections between prefrontal PICK1, and the hormones involved in the estrous cycle.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

References

Anker JJ, Holtz NA, Zlebnik N, Carroll ME, 2009. Effects of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats. Psychopharmacology (Berl) 203, 63–72. 10.1007/s00213-008-1371-9 [PubMed: 18998113]

Anker JJ, Larson EB, Gliddon LA, Carroll ME, 2007. Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. Experimental and Clinical Psychopharmacology, Effects of Progesterone on Cocaine Abuse 15, 472–480. 10.1037/1064-1297.15.5.472

Bechard AR, Hamor PU, Schwendt M, Knackstedt LA, 2018. The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: estrous cycle effects on behavior and protein expression in the nucleus accumbens. Psychopharmacology (Berl) 235, 837–848. 10.1007/s00213-017-4802-7 [PubMed: 29197981]

Bechard AR, Logan CN, Mesa J, Padovan-Hernandez Y, Blount H, Hodges VL, Knackstedt LA, 2021. Role of prefrontal cortex projections to the nucleus accumbens core in mediating the effects of ceftriaxone on cue-induced cocaine seeking. Addict Biol 26, e12928. 10.1111/adb.12928 [PubMed: 32558119]

Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E, 2005. Strategies and methods for research on sex differences in brain and behavior. Endocrinology 146, 1650–1673. 10.1210/en.2004-1142 [PubMed: 15618360]

Boudreau AC, Reimers JM, Milovanovic M, Wolf ME, 2007. Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. J Neurosci 27, 10621–35. 10.1523/JNEUROSCI.2163-07.2007 [PubMed: 17898233]

Boudreau AC, Wolf ME, 2005. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25, 9144–51. 10.1523/JNEUROSCI.2252-05.2005 [PubMed: 16207873]

Capriles N, Rodaros D, Sorge RE, Stewart J, 2003. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology 168, 66–74. 10.1007/s00213-002-1283-z [PubMed: 12442201]

Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL, 2000. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J Neurosci 20, 7258–67. [PubMed: 11007883]

Corbett CM, Dunn E, Loweth JA, 2021. Effects of Sex and Estrous Cycle on the Time Course of Incubation of Cue-Induced Craving following Extended-Access Cocaine Self-Administration. eNeuro 8, ENEURO.0054–21.2021. 10.1523/ENEURO.0054-21.2021

Datta U, Martini M, Fan M, Sun W, 2018. Compulsive sucrose- and cocaine-seeking behaviors in male and female Wistar rats. Psychopharmacology (Berl) 235, 2395–2405. 10.1007/s00213-018-4937-1 [PubMed: 29947917]

Datta U, Martini M, Sun WL, 2017. Sex Differences in the Motivational Contrast between Sucrose and Cocaine in Rats. J Drug Des Res 4, 1042. [PubMed: 34622250]

Doncheck EM, Urbanik LA, DeBaker MC, Barron LM, Liddiard GT, Tuscher JJ, Frick KM, Hillard CJ, Mantsch JR, 2018. 17beta-Estradiol Potentiates the Reinstatement of Cocaine Seeking in Female Rats: Role of the Prelimbic Prefrontal Cortex and Cannabinoid Type-1 Receptors. Neuropsychopharmacology 43, 781–790. 10.1038/npp.2017.170 [PubMed: 28825421]

Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL, 1997. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature 386, 279–84. 10.1038/386279a0 [PubMed: 9069286]

Evans SM, Haney M, Foltin RW, 2002. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology (Berl) 159, 397–406. 10.1007/s00213-001-0944-7 [PubMed: 11823892]

Famous KR, Kumaresan V, Sadri-Vakili G, Schmidt HD, Mierke DF, Cha JH, Pierce RC, 2008. Phosphorylation-dependent trafficking of GluR2-containing AMPA receptors in the nucleus accumbens plays a critical role in the reinstatement of cocaine seeking. J Neurosci 28, 11061–70. 10.1523/JNEUROSCI.1221-08.2008 [PubMed: 18945913]

Frankfurt M, Fuchs E, Wuttke W, 1984. Sex differences in gamma-aminobutyric acid and glutamate concentrations in discrete rat brain nuclei. Neurosci Lett 50, 245–50. 10.1016/0304-3940(84)90493-2 [PubMed: 6149503]

Freese L, Fraga de Souza M, Schuler Nin M, Calleti G, Flores Peres V, Gomez R, Maria Tannhauser Barros H, 2020. Elevated GABA levels in the medial prefrontal cortex and lower estrogen levels abolish cocaine sensitization behavior in ovariectomized female rats. Brain Res 1749, 147144. 10.1016/j.brainres.2020.147144 [PubMed: 33038296]

Fuchs RA, Evans KA, Mehta RH, Case JM, See RE, 2005. Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats. Psychopharmacology (Berl) 179, 662–672. 10.1007/s00213-004-2080-7 [PubMed: 15682307]

Guercio LA, Wimmer ME, Schmidt HD, Swinford-Jackson SE, Pierce RC, Vassoler FM, 2020. Deep brain stimulation of the infralimbic cortex attenuates cocaine priming-induced reinstatement of drug seeking. Brain Res 1746, 147011. 10.1016/j.brainres.2020.147011 [PubMed: 32652146]

Hofford RS, Euston TJ, Wilson RS, Meckel KR, Peck EG, Godino A, Landry JA, Calipari ES, Lam TT, Kiraly DD, 2021. Granulocyte-Colony Stimulating Factor Reduces Cocaine-Seeking and Downregulates Glutamatergic Synaptic Proteins in Medial Prefrontal Cortex. J Neurosci 41, 1553–1565. 10.1523/JNEUROSCI.1452-20.2020 [PubMed: 33361463]

Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE, 2008. Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology (Berl) 198, 63–75. 10.1007/s00213-008-1089-8 [PubMed: 18265959]

Kerstetter KA, Ballis MA, Duffin-Lutgen S, Carr AE, Behrens AM, Kippin TE, 2012. Sex differences in selecting between food and cocaine reinforcement are mediated by estrogen. Neuropsychopharmacology 37, 2605–14. 10.1038/npp.2012.99 [PubMed: 22871910]

Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME, 2007. Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. Exp Clin Psychopharmacol 15, 461–71. 10.1037/1064-1297.15.5.461 [PubMed: 17924780]

Larson EB, Roth ME, Anker JJ, Carroll ME, 2005. Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. Pharmacol Biochem Behav 82, 98–108. 10.1016/j.pbb.2005.07.015 [PubMed: 16111740]

Lin DT, Huganir RL, 2007. PICK1 and phosphorylation of the glutamate receptor 2 (GluR2) AMPA receptor subunit regulates GluR2 recycling after NMDA receptor-induced internalization. J Neurosci 27, 13903–8. 10.1523/JNEUROSCI.1750-07.2007 [PubMed: 18077702]

Matsuda S, Mikawa S, Hirai H, 1999. Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein. J Neurochem 73, 1765–8. [PubMed: 10501226]

McFarland K, Lapish CC, Kalivas PW, 2003. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23, 3531–7. [PubMed: 12716962]

Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You Z-B, McCarthy MM, Shaham Y, Ikemoto S, 2019. Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. Biological Psychiatry, Mechanisms of Addiction 85, 915–924. 10.1016/j.biopsych.2019.01.015

Pierce RC, Bell K, Duffy P, Kalivas PW, 1996. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 16, 1550–60. [PubMed: 8778304]

Rubio FJ, Quintana-Feliciano R, Warren BL, Li X, Witonsky KFR, Valle FSD, Selvam PV, Caprioli D, Venniro M, Bossert JM, Shaham Y, Hope BT, 2019. Prelimbic cortex is a common brain

area activated during cue-induced reinstatement of cocaine and heroin seeking in a polydrug self-administration rat model. Eur J Neurosci 49, 165–178. 10.1111/ejn.14203 [PubMed: 30307667]

Schmidt HD, Pierce RC, 2010. Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. Ann N Y Acad Sci 1187, 35–75. 10.1111/j.1749-6632.2009.05144.x [PubMed: 20201846]

Sinha R, Fox H, Hong K-I, Sofuoglu M, Morgan PT, Bergquist KT, 2007. Sex steroid hormones, stress response, and drug craving in cocaine-dependent women: implications for relapse susceptibility. Exp Clin Psychopharmacol 15, 445–452. 10.1037/1064-1297.15.5.445 [PubMed: 17924778]

Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S, Jin W, Thomas GM, Linden DJ, Huganir RL, 2006. Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. Neuron 49, 845–60. 10.1016/j.neuron.2006.02.025 [PubMed: 16543133]

Turner C, De Luca M, Wolfheimer J, Hernandez N, Madsen KL, Schmidt HD, 2020. Administration of a novel high affinity PICK1 PDZ domain inhibitor attenuates cocaine seeking in rats. Neuropharmacology 164, 107901. 10.1016/j.neuropharm.2019.107901 [PubMed: 31805281]

U.S. Department of Health and Human Services, S.A. and M.H.S.A., Center for Behavioral Health Statistics and Quality., 2022. Preliminary Findings from Drug-Related Emergency Department Visits, 2021. https://www.samhsa.gov/data/report/dawn-2021-preliminary-findings-report.

U.S. Department of Health and Human Services, S.A. and M.H.S.A., Center for Behavioral Health Statistics and Quality., 2021. National Survey on Drug Use and Health 2020. https://www.samhsa.gov/data/release/2020-national-survey-drug-use-and-health-nsduh-releases.

Volk L, Kim C-H, Takamiya K, Yu Y, Huganir RL, 2010. Developmental regulation of protein interacting with C kinase 1 (PICK1) function in hippocampal synaptic plasticity and learning. Proc Natl Acad Sci U S A 107, 21784–21789. 10.1073/pnas.1016103107 [PubMed: 21106762]

Warren BL, Kane L, Venniro M, Selvam P, Quintana-Feliciano R, Mendoza MP, Madangopal R, Komer L, Whitaker LR, Rubio FJ, Bossert JM, Caprioli D, Shaham Y, Hope BT, 2019. Separate vmPFC Ensembles Control Cocaine Self-Administration Versus Extinction in Rats. J Neurosci 39, 7394–7407. 10.1523/JNEUROSCI.0918-19.2019 [PubMed: 31331999]

Wickens MM, Bangasser DA, Briand LA, 2018. Sex Differences in Psychiatric Disease: A Focus on the Glutamate System. Front Mol Neurosci 11, 197. 10.3389/fnmol.2018.00197 [PubMed: 29922129]

Wickens MM, Deutschmann AU, McGrath AG, Parikh V, Briand LA, 2019. Glutamate receptor interacting protein acts within the prefrontal cortex to blunt cocaine seeking. Neuropharmacology 157, 107672. 10.1016/j.neuropharm.2019.107672 [PubMed: 31233823]

Wickens MM, Kirkland JM, Knouse MC, McGrath AG, Briand LA, 2021. Sex-specific role for prefrontal cortical protein interacting with C kinase 1 in cue-induced cocaine seeking. Addict Biol 26, e13051. 10.1111/adb.13051 [PubMed: 34110073]

Zhao W, Becker JB, 2010. Sensitization enhances acquisition of cocaine self-administration in female rats: estradiol further enhances cocaine intake after acquisition. Horm Behav 58, 8–12. 10.1016/j.yhbeh.2009.09.005 [PubMed: 19769978]

Zlotnik A, Gruenbaum BF, Mohar B, Kuts R, Gruenbaum SE, Ohayon S, Boyko M, Klin Y, Sheiner E, Shaked G, Shapira Y, Teichberg VI, 2011. The effects of estrogen and progesterone on blood glutamate levels: evidence from changes of blood glutamate levels during the menstrual cycle in women. Biol Reprod 84, 581–6. 10.1095/biolreprod.110.088120 [PubMed: 20980684]

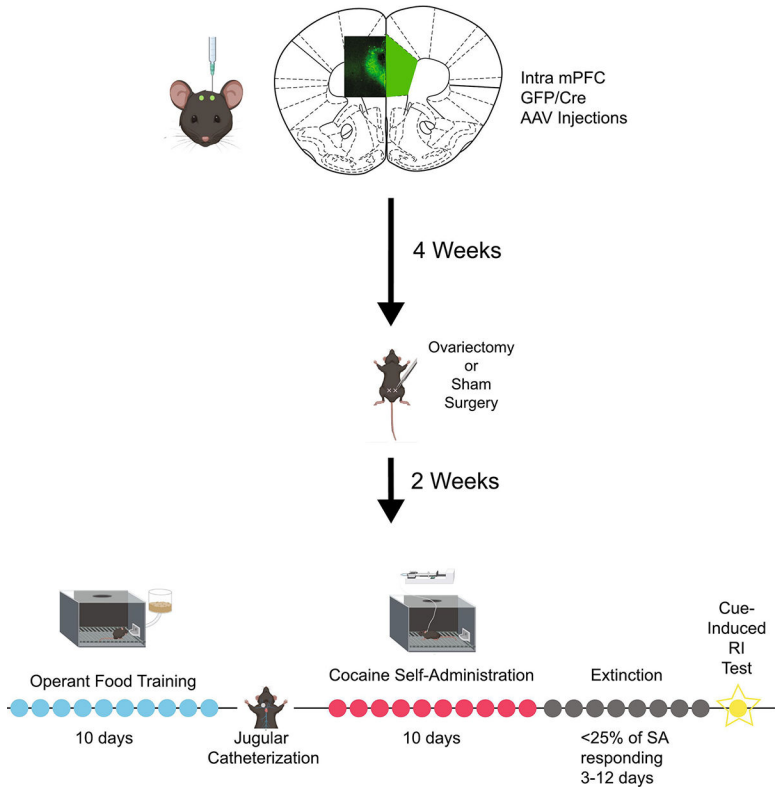

Figure 1. Experimental Design.

Male and female mice were injected bilaterally with 0.4μl of either AAV-Cre recombinase or AAV-GFP into the medial prefrontal cortex. Four weeks following viral injection, mice underwent either ovariectomy or sham surgery. Two weeks later, mice began behavioral training and testing. Mice were run on operant food training for 10 days prior to jugular catheterization surgery. After 4 days of recovery, mice ran for 10 days on cocaine self-administration, followed by extinction and cue-induced reinstatement.

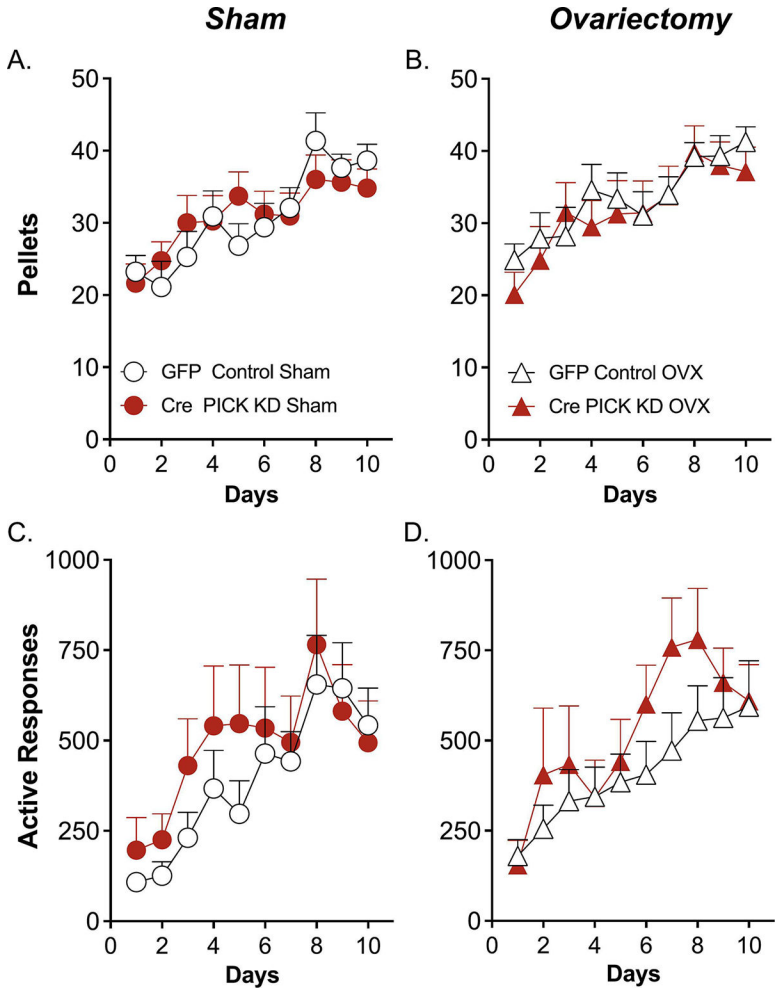

Figure 2. PICK1 knockdown in the mPFC does not alter operant learning during sucrose self-administration in female mice.

There were no significant effects of prefrontal PICK1 knockdown among OVX or SHAM females during sucrose self-administration, as measured by the number of sucrose pellets received (A,B) or the number of responses made (C,D) during a 1-hour testing session (n=14–20/group).

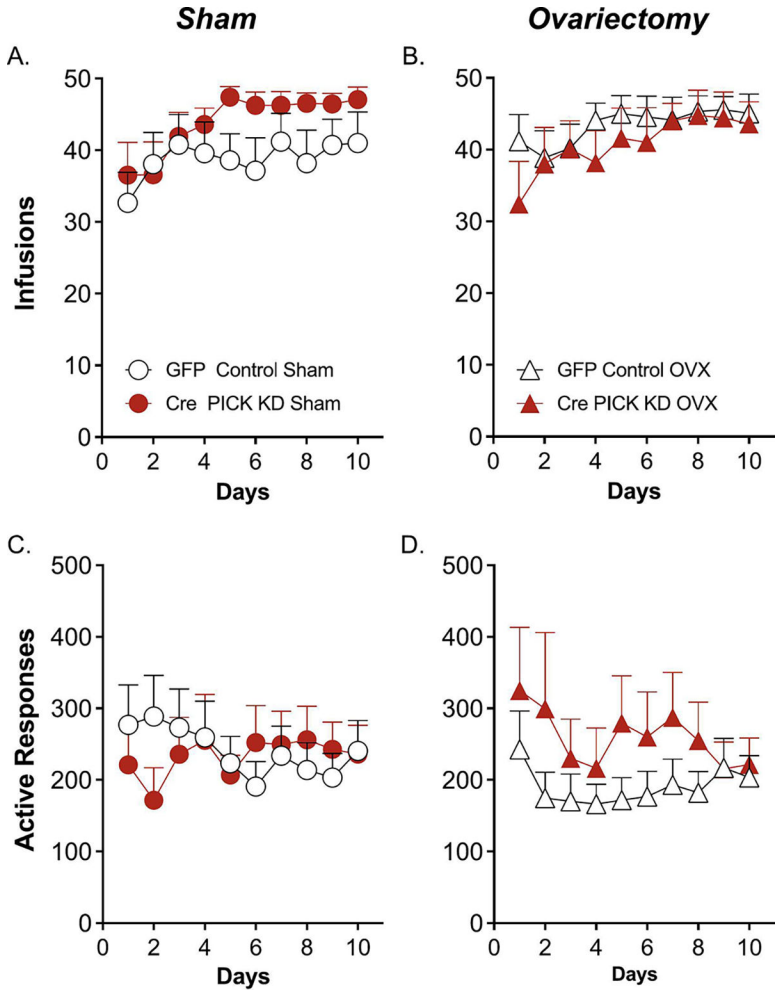

Figure 3. PICK1 knockdown in the mPFC does not alter acquisition of cocaine self-administration in female mice.

There were no significant effects of prefrontal PICK1 knockdown among OVX or SHAM females during cocaine self-administration, as measured by the number of infusions received (A-B) or the number of responses made (C,D) during a 2-hour testing session (n=12–15/group).

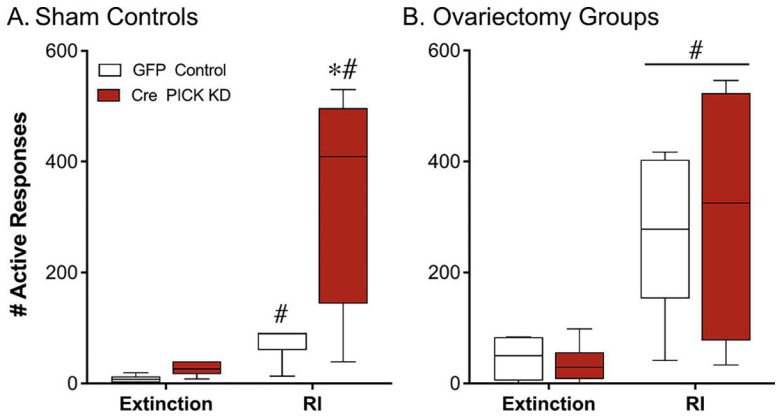

Figure 4. Ovariectomy interfered with the ability of prefrontal knockdown of PICK1 to enhance reinstatement of cocaine seeking.

In sham surgerized animals, prefrontal knockdown (KD) of PICK1 led to an increase in responding during a cue-indued reinstatement session compared to GFP injected controls (A, #p<.05 main effect of test session; *p<.05 PICK KD RI vs. GFP Control RI). However, in OVX treated mice, the only significant effect was a main effect of test session (#p<.05) and no effect of PICK1 knockdown (n=5–7/group).

Table 1

Neither PFC PICK knockdown nor ovariectomy influenced extinction of cocaine seeking.

Group	Extinction Day 1 Responding (Mean +/− SEM)	Days to Extinction Criterion (Mean +/− SEM)
Sham GFP Control	122 +/− 53	10.0 +/− 4.3
Sham Cre PICK Knockdown	215 +/− 43	18.0 +/− 5.5
OVX GFP Control	105 +/− 20	13.4 +/− 3.9
OVX Cre PICK Knockdown	116 +/− 34	9.7 +/− 3.4