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Perirhinal cortex to the nucleus accumbens circuit in novelty salience following methamphetamine self-administration☆

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Abstract

Methamphetamine (meth) use disorder is part of an overarching use disorder that encompasses continued drug seeking and an increased risk of returning to drug use following periods of abstaining. Chronic meth use results in drug-induced cortical plasticity in the perirhinal cortex (PRC) that mediates responses to novelty. PRH projection targets are numerous and include the nucleus accumbens core (NAc). Whereas the PRH-prefrontal cortex is involved in object recognition; we propose that the PRH–NAc is involved in novelty salience. Rats underwent short-access (ShA, 1 hr) or long-access (LgA, 6 hr) meth self-administration (SA). We then used a dual viral strategy to inhibit or activate PRH–NAc during a novel cue test in which rats were presented with meth-associated and novel levers. Response patterns on these levers differ

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Declaration of competing interest

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Supplementary materials

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depending on the meth access protocol: ShA meth SA results in equal responding on both novel- and meth-associated levers, whereas LgA meth results in perseverative responding on the meth-associated lever. Inactivation of the PRH–NAc increased responding on the meth lever relative to the novel lever, resulting in a LgA behavioral phenotype. In contrast, activation in LgA rats was without a behavioral effect. We also report that male LgA sucrose SA animals perseverated on the novel lever rather than the meth-associated lever, which contrast their meth SA counter parts and female specific patterns of behavior. These data open a new line of interest in the role of the PRH–NAc circuit in novelty salience through identification of the behavioral relevance of this circuit.

Keywords

Entorhinal cortex; Novelty; Substance use disorder; Cognitive processes; Salience; Addiction

1. Introduction

Methamphetamine (meth) abuse in the United States has soared in recent years. For example, past year meth use in 2019 was 2.0 million people relative to 1.4 million reported in 2016 [1]. Of those, 92 % report daily or near daily meth use [1]. Further, in 2022 this increased to 2.7 million people with past year meth use [2]. People with meth use disorder (MUD) often score high on indices of sensation seeking [3,4] and a desire for novel and potentially risky experiences [5]. In humans, novelty seeking strategies are related to memory recall and drive exploratory behavior for intrinsically rewarding stimuli [6]. In a human laboratory experiment, abstinent (9–10 months) participants with MUD spent more time investigating novel objects in a novel environment relative to matched control participants, indicating novelty induced increases in exploration [7]. This test of novelty-induced exploratory behavior in humans demonstrates changes in cognitive processes after a history of MUD that involve responses to novelty, but this clinical report did not differentiate a preference between novel over familiar objects or determine if exploration was driven by novelty or by objects in general. A more recent study demonstrated that people with MUD do not differentiate between “neutral novel” (e.g., stapler, coffee mug, etc.) or “personal novel” (e.g., cell phone, wallet, etc.) objects [8].

Relevant to our studies described herein is if the presence of drug paraphernalia competes with the interest in novelty. Our novel cue responding task is a direct test of this question. Specifically, our lab previously developed a rodent novel cue responding test, to assess competition between novel and meth-associated stimuli following meth self-administration (SA) [9,10]. During this task, simultaneous access to novel and meth-associated stimuli compete to drive novelty interaction or relapse to meth seeking. Different meth SA protocols can emulate distinct patterns of drug taking in individuals who use meth. A short access protocol (ShA, 1 hr daily for 21 days) models individuals who use meth intermittently or in lower quantities. Clinically and preclinically, this pattern of meth use does not appreciably alter cognitive function [11]. In contrast, individuals who use meth chronically or in high amounts develop cognitive deficits, modeled by a long access meth SA protocol (LgA, 1 hr daily for 7 days then 6 hrs daily for 14 days) [12–14].

In the novel cue task, rats undergo ShA or LgA meth SA followed by an abstinence period. On test, rats are returned to the drug associated context in which a novel lever and cue light are present (see methods for specific details). We have previously reported that LgA meth SA results in perseverative responding on the meth-associated lever with little regard for novelty, whereas ShA meth SA leads to similar responding on both the novel and meth-associated levers [9]. Different manipulations can shift these patterns of responding. For example, chemogenetic activation of the perirhinal cortex (PRH) or positive allosteric modulation of the type 5 metabotropic glutamate receptor in the PRH of LgA meth SA rats ameliorates perseverative meth-associated responding, shifting to equal responding on both levers [9,10]. Conversely, a meth prime injection given to ShA meth SA rats shifts responding to the meth-associated lever [9].

The PRH, a cortical region in the temporal lobe of the brain, has numerous connections to other brain areas implicated in cognitive function, memory, reward, and relapse (e.g., the prefrontal cortex, hippocampus, amygdala, and nucleus accumbens (NAc) [15]. While the function of these brain areas has been studied for decades, far less is known about the PRH and its inputs to these areas. The PRH is essential for recognition memory, and it has been shown to support glutamatergic transmission [16–18]. Human fMRI studies show increased BOLD signals in the rhinal cortex in response to novel stimuli and in the NAc in response to reward-related stimuli [19]. There are anatomically defined projections from layers III and V of the PRH to the NAc core, however the functional significance of these projections is unknown [20–23]. Given the roles of the PRH and NAc in novelty processing, and our prior findings that LgA meth SA causes drug-induced plasticity within the PRH [16,24,17,18], we propose that LgA meth SA prevents effective recruitment of these PRH to NAc projections during novelty interaction, leading to altered responding during the novel cue test. Here, we propose that manipulations of this circuit will shift responding in response to novel and meth-associated cues. First, since prior iterations of the novel cue test used only male subjects, we determined if female rats had similar novel cue test performance as males. Subsequently, we use a dual viral chemogenetic approach to inactivate and activate the PRH–NAc circuit in ShA and LgA meth SA rats, respectively. We hypothesized that inhibition of the PRH–NAc circuit in ShA rats would impair novel cue test performance, shifting responding to the meth-associated lever, while activation of this projection in LgA rats would equalize meth-associated and novel lever responding.

2. Methods

2.1. Subjects

Subjects were age-matched adult male (250–275 g; $n = 60$) and female (225–250 g; $n = 30$) experimentally naïve Sprague Dawley rats (Envigo, Indianapolis, IN, USA). Rats were individually housed in a temperature and humidity-controlled vivarium and allowed to acclimate to a reverse 12:12 light cycle for a minimum of 72-hours before handling. Water was available ad libitum throughout the experiments and approximately 20 g of rat chow (standard rat chow, Harlan, Indianapolis, IN) was provided daily until meth SA training was complete, after which food was given ad libitum. All behavior took place during the dark phase of the light cycle. All procedures were approved by the MUSC Institutional

Animal Care and Use Committee (IACUC) and facilities are accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). All procedures were conducted in accordance with the guidelines established by the NIH, NRC, and the "Guide for the Care and Use of Laboratory Animals" [25].

2.2. Drugs and sucrose

S(+)-methamphetamine hydrochloride (NIDA Drug Supply Program) was dissolved in sterile saline (0.9 % NaCl) to a concentration of 20 ug/50 uL. Sucrose pellets were used for sucrose SA training (45 mg Dustless Precision Pellets, unflavored sucrose, Bio-Serv, Flemington, NJ). Clozapine-N-Oxide (CNO; Hello Bio, Princeton, NJ; 10 mg kg^{-1}, i. p.), the DREADD ligand, was dissolved in sterile saline (Vehicle; 0.9 % NaCl).

2.3. Intravenous catheter surgery

Rats were anesthetized with vaporized isoflurane (4–5 % for induction, 1–3 % maintenance). Ketorolac (2.0 mg kg^{-1}, i.p.; Sigma Chemical, St. Louis, MO, USA) and cefazolin (0.2 g kg^{-1}, s.c.; Patterson Veterinary, Saint Paul, MN, USA) were given before surgery as an analgesic and antibiotic, respectively. Catheters (SAI Infusion Technologies, Lake Villa, IL, USA) were inserted 4 cm into the right jugular vein and secured with silk sutures. The opposite end of the tubing ran subcutaneously over the shoulder and exited through a small incision on the back below the shoulder blades where an external port (Single Channel Catheter Access Buttons with CannuLock technology, SAI Infusion Technologies) was exposed. Catheters were locked with 0.05 mL of TCS (Access Technologies, USA) after each use to maintain catheter patency.

2.4. Viral microinjections

Stereotaxic intracranial surgery was performed to bilaterally infuse viral constructs to isolate and manipulate the PRH–NAc circuit. Rats were anesthetized as described above. A retrograde AAV expressing EGFP-Cre (pENN.AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40; Addgene catalog number 105,540-AAVrg) was infused into the NAc "shore" (an area that encompasses both the core and shell: angled 15° medially, AP: +1.6 mm, ML: +1.6 mm, DV: −7.34 mm). Additionally, either an inhibitory hM4D-Gi (AAV2-hSyn-DIO-hM4D(Gi)-mCherry; Addgene catalog number 44,363-AAV2) or excitatory hM3D-Gq (AAV2-hSyn-DIO-hM3D(Gq)-mCherry; Addgene catalog number 44,361-AAV2) Cre-dependent DREADD was infused into the PRH (angled 10° laterally, AP: − 4.8 mm, ML: −5 mm, DV: −7.5 mm). Viral control animals received the same infusion into the NAc, but a Cre-dependent virus expressing only mCherry (no DREADD) was infused into the PRH (pAAV-hSyn-DIO-mCherry; Addgene, catalog number 50,459-AAV2). All coordinates were measured from bregma at the skull surface. All viruses were infused via Auto-Nanoliter Injector (Nanoject II or III, Drummond Scientific) at a volume of 50.6 nL/injection every 30 s at a rate of 23 nL/second (a total volume of 0.506 μL for the NAc and 0.607 μL for the PRH), followed by an additional 5-minute pause to allow the virus to diffuse before the glass micropipette was retracted. Holes were covered with bone wax (Sterile Natural Bone Wax; Medline) and the incision was sutured. Rats were allowed to recover for a minimum of 5 days following all surgical procedures prior to behavior.

2.5. Meth or sucrose self-administration

SA was conducted in standard operant chambers (30 × 20 × 20 cm, Med Associates, St Albans, VT). Operant chambers were housed in sound-attenuating cabinets and were equipped with a house light, a fan, two levers which could be extended, and a drug-delivery system consisting of an arm attached to a swivel and a spring tether which surrounded the tubing. Tygon tubing was connected to a 10 mL syringe fitted to an infusion pump located outside of the cabinet. At the start of each session, the house light turned on and both levers extended (active and inactive levers). Active lever responses resulted in the delivery of a 2-*sec*, 50 μL infusion of meth (0.4 mg/ml concentration; 0.02 mg/50ul infusion) followed by a 20-*sec* infusion timeout period during which responses were recorded but no drug was delivered. A white stimulus light above the active lever also illuminated for 5-*sec* with each meth infusion. Responses on the inactive lever were recorded but were without consequence. At the end of each training session, the house light turned off and the levers retracted. In both LgA and ShA conditions, days 1–7 of training consisted of 1 hr fixed ratio (FR) 1 sessions. Following this, rats either continued for an additional 14 days of 1 hr (ShA) FR1 sessions or increased to 6 hr (LgA) FR1 sessions. Rats were then returned to their home cage for 7 days of home cage abstinence prior to the novel cue test. Sucrose experiments followed the same training protocol, with the exception that they did not undergo any surgeries prior to training, and animals responded for sucrose pellets rather than drug infusions.

2.6. Novel cue test

Novel cue tests were conducted on the 8th day of abstinence. This timepoint was selected for consistency with our previous work [9,10]. Rats were returned to their original SA training chambers, now fitted with a novel lever and stimulus light on the opposite wall from the drug-paired lever. The novel cue stimulus lights were modified by the addition of two Velcro strips to create a narrow horizontal slit of light. This provided a different light shape, a novel tactile experience, and a reduction in light intensity compared to the meth cue light (See Supplemental Figure 3). CNO (10mg kg^{-1}, i.p.) was administered 30-min prior to novel cue testing. Control animals received saline (Vehicle, equivolume i.p.) instead of CNO. At the start of the novel cue test, both the novel cue light and active cue light turned on for 5-*sec*, serving as non-contingent cues to signal that levers were available. During the test, a response on either the active or novel lever resulted in the illumination of their respective cue light (5-*sec* duration, 20-*sec* time out) and responding on all 3 levers was recorded over the 60-min session. Rats were not tethered to receive meth and no extinction training was conducted prior to testing. Sucrose animals followed the same protocol for the novel cue test, but these animals did not receive CNO or Vehicle injections prior to test. All viral control animals received CNO injections prior to test. 30-min after test, rats were euthanized.

2.7. Immunohistochemistry and microscopy

Following novel cue testing, rats were transcardially perfused with 10 % buffered formalin, and brains were removed for immunohistochemistry (IHC) on free-floating coronal (40 μm) sections. Sections were permeabilized and blocked in 0.1 M phosphate buffered saline (PBS) with 2 % Triton X-100 (PBST) with 2 % normal goat serum (Jackson ImmunoResearch

Laboratories Inc.; code 005–000–121) for 1-h at room temperature with agitation. Sections were then incubated overnight at 4°C with agitation in the appropriate primary antisera: rabbit α c-FOS: (1:1000, Synaptic Systems ref 226–008) or chicken α mCherry (1:2000, LS Bio ref LS-C204825) diluted in 2 % PBST with 2 % normal goat serum. Following this, the sections were washed 3 times for 5-min each in 2 % PBST, then incubated in the appropriate secondary antisera: goat α chicken 594 (1:1000, Invitrogen Alexa Fluor 594 ref A11042) or goat α rabbit 647 (1:1000, Invitrogen Alexa Fluor 647 ref A21245) diluted in PBST with 2 % normal goat serum for 2 hrs at room temperature with agitation. Sections were washed for a final 3 times for 5-min each in 2 % PBST, mounted on SuperFrost+ slides, and cover slipped with ProLong Gold Antifade Mountant. Slides were stored at 4°C and protected from light until imaging. Next, mounted sections were imaged to map viral expression, determine mCherry and cFOS expression and their colocalization. A Leica THUNDER Tissue Imager (Leica Microsystems) was used to determine the accuracy and spread of viral microinjections. Slides were then imaged using a Leica TCS SP8 laser-scanning confocal microscope (Leica Microsystems) and images were analyzed using Bitplane Imaris (Oxford Instruments). The “spots” tool was used to semi-manually label mCherry+ and cFOS+ cells. The “colocalize spots” extension (distance threshold = 5μm) was then used to determine the number of colocalized mCherry+/cFOS+ cells. Colocalization values were calculated from the results of semi-manually labeled “spots” placed over mCherry signal and c-fos signal. For each channel, a minimum spots diameter of 15um was utilized. Once Imaris was used to label all neurons (mCherry) and c-fos+ nuclei, an experimenter blind to treatment conditions reviewed each file to manually eliminate spots that were placed on the image by the algorithm that did not meet minimum standards for inclusion. Neurons or c-fos+ nuclei that were poorly labeled were consequentially eliminated before any colocalization analysis occurred. Subjects were eliminated from the final dataset if no viral expression was detected or if viral expression was off-target or spread to adjacent regions.

2.8. Statistical analyses

Data from each of assessment were analyzed and graphs were generated using GraphPad Prism 10 statistical software. 2- or 3-way analyses of variance (ANOVA) were used with Holm-Sidak’s post-hoc tests, as indicated. The between-subjects variable was sex and/or virus and the within-subjects were day and lever. Fos co-localization was analyzed with unpaired *t*-tests between CNO and vehicle treatment. On test days, planned comparisons were conducted between the active and novel lever regardless of the omnibus ANOVA, because of our *a priori* hypothesis is that responding on these levers shift according to the SA protocol. Statistical significance was set to $p < 0.05$.

3. Results

3.1. Experiment 1. male and female rats respond similarly on novel and meth-associated levers after short access meth self-administration, but differ after long access

Fig. 1A shows the experimental timeline used in Exp 1. Male ($n = 11$) and female ($n = 8$) rats that administered ShA meth did not display any differences in responding over the 21 days of meth SA (Fig 1B). There was, however, a Lever x Day interaction (3-way ANOVA, [$F(20,340)=4.9$, $p < 0.0001$]), a Day main effect [$F(20,340)=3.69$, $p < 0.0001$],

and Lever main effect [F(1,17)=43.11, $p < 0.0001$]. On the novel cue test (Fig 1C), ShA males and females both responded similarly on the novel and meth cue. There was a Lever main effect (2-way ANOVA, [F(2,34)=13.98, $p < 0.0001$]), but no other significant effects or interactions. Even though there was not a significant main effect of sex, planned comparisons of male and female rats were conducted separately to isolate patterns of responding between the sexes that may be obscured by the overall ANOVA. For males, responding on the meth cue lever was greater than the inactive lever ($p = 0.006$), but there were no differences relative to the novel lever. For females, the same pattern was observed, as meth-associated lever responding was greater than the inactive ($p = 0.007$), but not the novel, lever.

Male ($n = 12$) and female ($n = 7$) rats that administered LgA meth also did not differ during SA (Fig 1D). As with ShA, there was a Lever x Day interaction (3-way ANOVA, [F(20,336)=8.7, $p < 0.0001$]), a Day main effect [F(20,340)=9.49, $p < 0.0001$], and Lever main effect [F (1,17)=29.7, $p < 0.0001$]. On the novel cue test (Fig 1E), LgA males and females displayed different patterns of responding. The overall 2-way (Sex X Lever) interaction was significant [F(2,51)=4.58, $p = 0.015$] but there was no sex main effect. The Lever main effect was also significant [F(2,49)=22.49, $p < 0.0001$]. Post-hoc comparisons show that for males, responding on the meth-associated lever was greater than the inactive lever ($p < 0.0001$) and the novel lever ($p = 0.0022$). For females, responding on the meth lever was also increased relative to the inactive lever ($p = 0.006$), but not relative to the novel lever ($p = 0.38$). Together, these results replicate our previous work [9,10] showing that ShA males respond similarly on novel and meth associated lever, whereas LgA males preferentially respond on the meth lever. We also extend these findings to females during ShA, but females exhibit a different pattern of responding after LgA meth SA than males.

3.2. Experiment 2. inhibition of the PRH–NAc changes the pattern of lever responding to the meth cue in short access male and female rats

Previously [9], we demonstrated that a low dose meth injection prior to novel cue testing shifted responding in ShA rats to preferential responding on the meth-associated lever, akin to the LgA behavioral phenotype. In this experiment, we used a dual viral approach to inactivate PRH projecting neurons to the NAc, to determine if this circuit mediates novelty salience during the task. The experimental timeline and viral infusion schematic are shown in Figs. 2A and 2B, respectively. During ShA SA, rats were assigned to CNO ($n = 13$ total; $m = 10$; $f = 3$) or Vehicle ($n = 13$ total; $m = 10$; $f = 3$) condition based on meth intake, such that intake was approximately equal between groups. Lever responding between the groups (CNO and Vehicle) was equal throughout the SA period (Fig 2C, data is segregated by sex in Supplemental Fig 1B). The 3-way ANOVA showed a Lever x Day interaction [F (20,480)=7.7, $p < 0.0001$], a Day main effect [F(20,480)=6.50, $p < 0.0001$], and Lever main effect [F(1,24)=104.3, $p < 0.0001$].

On the novel cue test (Fig 2D), CNO and Vehicle rats exhibited different lever choices, evidenced by the Group x Lever interaction (2-way ANOVA, [F(2,35)=4.29, $p = 0.022$]; data segregated by sex in Supplemental Fig 1C). The Lever main effect was also significant [F (2,36)=35.23, $p < 0.0001$]. Post-hocs show that for Vehicle, responding on the meth-

associated lever was greater than the inactive lever ($p < 0.0001$), not the novel lever ($p = 0.36$). For CNO rats, responding on the meth lever was increased relative to the inactive lever ($p < 0.001$) and the novel lever ($p < 0.001$). Heatmaps of viral DREADD spread are shown in Fig. 2E. An unpaired *t*-test showed a significantly decreased mean number of c-Fos/mCherry colocalized cells in the PRH of CNO-treated animals as compared to Vehicle-treated animals [Fig 2F, t (10)=3.376, $p = 0.007$], with representative photomicrographs in Fig. 2G. CNO did not have an impact on lever choice in the absence of a DREADD, as evidenced by the viral control group (see Supplemental Figure 2).

3.3. Experiment 3. activation of the PRH–$_{NAc}$ does not change lever choice following long access meth

In our previous work [10], we demonstrated that CNO activation of an excitatory, Gq-DREADD in the PRH shifted focused lever responding from the meth cue lever to be equal on the novel and meth-associated levers. In this experiment, we sought to determine if PRH projecting neurons terminating in the NAc were impacted by LgA meth SA, such that increasing their activity can induce a ShA phenotype. This experiment is limited to male rats because LgA female rats did not exhibit the same perseveration on the meth lever. Fig. 3A shows the experimental timeline, with is a schematic of the viral infusions in Fig. 3B. During LgA SA, rats were assigned to CNO ($n = 8$ males) or Vehicle ($n = 6$ males) condition such that meth intake was approximately equal between groups. Lever responding between the groups were equal throughout the SA period (Fig 3C). There was, however, a Lever x Day interaction [F(20, 243)=4.8, $p < 0.0001$], a Day main effect [F(20,260)=4.7, $p < 0.0001$], and Lever main effect [F(1,13)=15.51, $p < 0.0017$].

On the novel cue test (Fig 3D), CNO and Vehicle rats exhibited the same pattern of responding. The overall two-way (Group x Lever) interaction was significant [F(2,37)=3.27, $p = 0.049$], as well as a Group main effect [F(1,37)=16.03 $p = 0.0003$], and Lever main effect [F (2,37)=22.12, $p < 0.0001$]. Post-hoc comparisons show that for Vehicle subjects, responding on the meth cue lever was greater than the inactive lever ($p < 0.0001$) and the novel lever ($p = 0.017$). For CNO, responding on the meth-associated lever was also increased relative to the inactive lever ($p < 0.005$) and the novel lever ($p < 0.0055$). Heatmaps of viral DREADD spread are shown in Fig. 3E. An unpaired *t*-test showed a significantly increased mean number of c-Fos/mCherry colocalized cells in the PRH of CNO-treated animals as compared to Vehicle-treated animals [Fig 3F, t(13)=6.35, $p = 0.0001$], with representative photomicrographs in Fig. 3G.

3.4. Experiment 4. overtraining does not explain perseveration on the meth cue in LgA rats

In a final experiment, we tested if perseverative responding on the meth lever in the LgA males was due to overtraining of the meth-associated cue during meth SA. To test this, we instead used a LgA sucrose SA protocol. During sucrose acquisition, males ($n = 12$) had higher lever pressing than female ($n = 12$) rats, but responses were similar over the 21 days of sucrose access (Fig 4A; Group x Lever x Day interaction, [F (20,440)=4.71, $p < 0.0001$]). On the novel cue test (Fig 4B), the overall two-way (Group x Lever) interaction approached significance [F(2,33)=3.09, $p = 0.059$]. The Sex main effect [F(1,33)=5.15 p

= 0.029] and Lever main effect [F(1,33)=63.75, $p < 0.0001$] were significant. For males, post-hoc comparisons show that responses on the sucrose cue lever were greater than the inactive lever ($p < 0.0001$) but reduced relative to the novel lever ($p = 0.0015$). For females, responding on the sucrose cue lever was increased relative to the inactive lever ($p < 0.0001$), but not the novel lever ($p < 0.59$).

4. Discussion

We have demonstrated that the PRH is important for novelty processing and salience of the novel stimuli; and that a history of LgA meth SA prevents the recruitment of this area when engaging with novelty [10]. Here, we hypothesize that PRH projections to the NAc comprise the circuit that mediates responses to novelty salience.

First, we found that female rats diverged from the established pattern of responding to novel and meth associated cues demonstrated by males [9,10]. Second, using a dual viral chemogenetic approach to inactivate and activate the PRH–NAc circuit in ShA and LgA meth SA rats, respectively; we found that inhibition of the PRH–NAc circuit shifts lever responding to the meth cue in ShA male and female rats, while activation of the PRH–NAc does not change lever choice following LgA meth. Finally, we assessed if the lack of a behavioral shift in the LgA animals was due to over-training by using a LgA sucrose condition. We found that LgA male sucrose rats perseverated on the novel cue during test, while females responded equally to the sucrose and novel levers. The discussion and implication of these results are expanded upon in the following sections.

4.1. Inhibition of PRH–NAc projecting neurons shifts lever responding in male and female ShA meth SA rats

Male and female rats that underwent ShA meth SA responded equally for novel and meth-associated cues during the novel cue relapse test (Fig 1C). Consistently, inactivation of the PRH–NAc shifts lever pressing to preferential responding on the meth-associated lever in both sexes (Fig 2D). As mentioned previously, this circuit has been identified anatomically but the behavioral relevance of this circuit previously undefined [20–23]. To date, we consider this to be the first reporting of behavioral implications for this circuit, specifically in novelty salience in rats.

ShA meth SA does not obstruct performance of object recognition memory in rats [26,27], which is in alignment with clinical data reporting typical cognitive responses in recreational meth users [11]. Inhibition of PRH projections to the prefrontal cortex, however, impaired object recognition memory in ShA meth SA rats [27]. Thus, our present data showing a shift in lever choice – novel vs. meth-paired – following inhibition of the PRH–NAc demonstrate the importance of the PRH in stimulus processing for both memory and novelty detection. Inhibition of this circuit resulted in a behavioral phenotype consistent with LgA meth SA rats. Importantly, CNO did not impact lever responding in rats that did not receive DREADD infusion.

We suggest that inhibiting PRH outputs projecting to the PFC and NAc emulates LgA meth SA induced changes in glutamatergic processing in the PRH [16–18]. A history of LgA

meth SA decreased surface expression of glutamate receptors (GluN2B [17,18] and mGluR5 [24] and led to a loss of long-term depression, impairing recognition memory and causing sustained focus on meth-associated stimuli [9,10,18,27]. Interestingly, pharmacological activation of these receptors via d-cycloserine (DCS) [18], 3-cyano-N-(1,3-diphenyl-IH-pyrazol-5-yl) benzamide (CDPPB) [16], and 1-(4-(2,4-difluorophenyl) piperazin-1-yl)–2-((4-fluorobenzyl)oxy)-ethanone (DPFE) [9] reversed memory deficits and equalized lever pressing in the both a novel object recognition (NOR) task and novel cue task, respectively.

Inhibition of the prefrontal cortex (both pre-limbic and infralimbic areas) decreased relapse to meth-associated cues [28]. But the NAc also receives and processes glutamatergic signals from multiple limbic and paralimbic brain areas [29] as well as other cortical areas [30]. We suggest, PRH glutamatergic projection neurons synapsing into the NAc mediate novelty salience. A history of meth SA reduces basal glutamate levels in the NAc and connected cortical areas [31]. Glutamate release is increased in response to meth cues and leads to increased meth seeking [31]; however, the source of this glutamate has not been determined. Although, we did not measure markers of glutamate release in the NAc, we suggest that inhibition of the PRH–NAc suppresses glutamatergic transmission like that seen in LgA meth rats thereby reducing the salience of the novel cue. Future cell-type circuit specific manipulations will shed light on importance PRH glutamate projections to the NAc.

4.2. Activation of PRH projecting neuron terminating in the NAc has no impact on lever responding in male LgA meth SA rats

Given that previous work from our lab found that novel object recognition memory and novelty salience (novel cue task) were restored in a LgA model of meth SA by an excitatory hM3D(Gq)-DREADD infused into the PRH [10], we isolated the PRH–NAc circuit to determine if activating this circuit alone (as opposed to activating all outgoing circuits from the PRH, as we have done previously) would also rescue novelty salience in the novel cue relapse test. Contrary to our hypothesis, there was no change in novel cue performance after activation of the PRH–NAc circuit. Subjects injected with CNO prior to test had the same pattern of responding as LgA animals injected with Vehicle, i.e. rats perseverated on the active/meth lever in both experimental conditions. Validation analyses revealed that the viral infusions were properly targeted to the PRH–NAc circuit and the brains of LgA animals treated with CNO had significantly more colocalization of mCherry-tagged DREADDs and eGFP-tagged neurons expressing cFOS as compared to LgA animals that received a Vehicle injection. These results imply that while the PRH–NAc circuit was activated by CNO as expected, activating this circuit alone was insufficient to shift responding to the novel lever. Given that global DREADD activation of the PRH rescued novelty salience previously, this suggests that other PRH circuits are required instead of, or in addition to, the PRH–NAc projections tested here [10].

In LgA male rats, it is possible that the PRH–NAc circuit may no longer be involved once the animals transition to an addictive phenotype due to changes in ventral vs. dorsal striatal control of behavior. Briefly, ventral striatal circuits are necessary for the initial formation of drug seeking but after repeated exposure the dorsal striatal circuits become engaged to support the formation of habitual responses to drug cues [for a review see [32]]. This implies

that while the PRH–NAc circuit may initially be engaged, over the course of training, alternative circuits may be recruited. Overtraining could contribute to this shift, but this is unlikely given that LgA sucrose male rats preferred the novel lever compared to the sucrose-associated lever (see Fig. 4B).

4.3. Sex differences in novel cue responding following LgA meth and sucrose

The literature suggests that female rats are more sensitive to meth [33], take more meth [26,34] and reinstate to meth-taking at a lower dose compared to males [26,35]. It is interesting, then, that in the current study LgA female rats displayed equal responding to both the active lever and novel lever during test, while males perseverated on the active lever. We have previously indicated that equal responding on both levers is an indication of intact novelty recognition/salience, as it is a pattern of behavior seen in ShA meth animals with an intact PRH–NAc circuit. We suggest sex differences in novel cue responding following LgA meth are due to changes in glutamatergic cortical transmission. Although the following examples are from the medial prefrontal cortex (mPFC), it is possible that the PRH may exert similar basal differences and resistance to plasticity after meth.

Female rats exhibit basal and evoked synaptic cortical differences when compared to males [36]. Specifically, drug-naïve female rats have decreased amplitude and kinetics of sEPSCs compared to male rats, but female rats with a history of meth SA exhibit higher amplitude eEPSCs. Additionally, the GluN2B blocker Ro256981 did not affect NMDA currents in meth SA females, but decreased NMDA currents in meth SA males. In regard to the PRH, LgA meth SA impairs the induction of long-term depression (LTD) in the PRH of male rats. Importantly, PRH LTD requires activation of GluN2B [18,37] and blockade of GluN2B-containing NMDA receptors with Ro256981 inhibits induction of PRH LTD [38]. Importantly, LTD in the PRH is required for the recognition of novelty [39]. In previous experiments, we restored PRH LTD after meth exposure with administration of d-cycloserine (DCS), and subsequently blocked DCS restoration through blockade of GluN2B with Ro256981. It is unknown if the same baseline sex differences and meth-induced sex differences found in the PFC [36], are present in the PRH and influence novel cue responding dependent on the PRH–NAc circuit. Alternatively, LgA meth SA females may be protected from adverse cognitive changes due to circulating ovarian hormones [40,41]. Estrogen has neuroprotective effects against meth exposure [42,43] mediated by various factors, including antioxidant effects [44], alterations of dopamine transporter function [45,46] or potentiation of excitatory neurotransmission [47,48].

4.4. Concluding remarks

In the present study, rats were trained to SA meth before the PRH–NAc circuit was manipulated using a dual-viral chemogenetic approach during novel cue testing. Our results supported our hypothesis, that inhibiting the PRH–NAc circuit in ShA rats would lead to perseverative responding on the meth-associated lever, similar to the behavior of LgA rats who have meth-induced cognitive deficits. However, our other hypothesis was not supported, as activating the PRH–NAc circuit in LgA rats did not restore equal responding on both levers. Finally, we found that male and female rats respond differently following LgA sucrose, an indication that overtraining does not explain the pattern of responding after LgA

meth SA in males. Further studies are needed to understand this lack of behavioral change in LgA meth rats by exploring other related circuits, specific cell-types, behavioral tasks, and with additional female subjects. By understanding the neurobiology of meth-induced changes to the brain and behavior, we aim to identify ways to reverse these changes and reduce relapse propensity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

NIDA Drug Supply for providing the methamphetamine. Images were created with Biorender.

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Data availability

Data will be made available on request.

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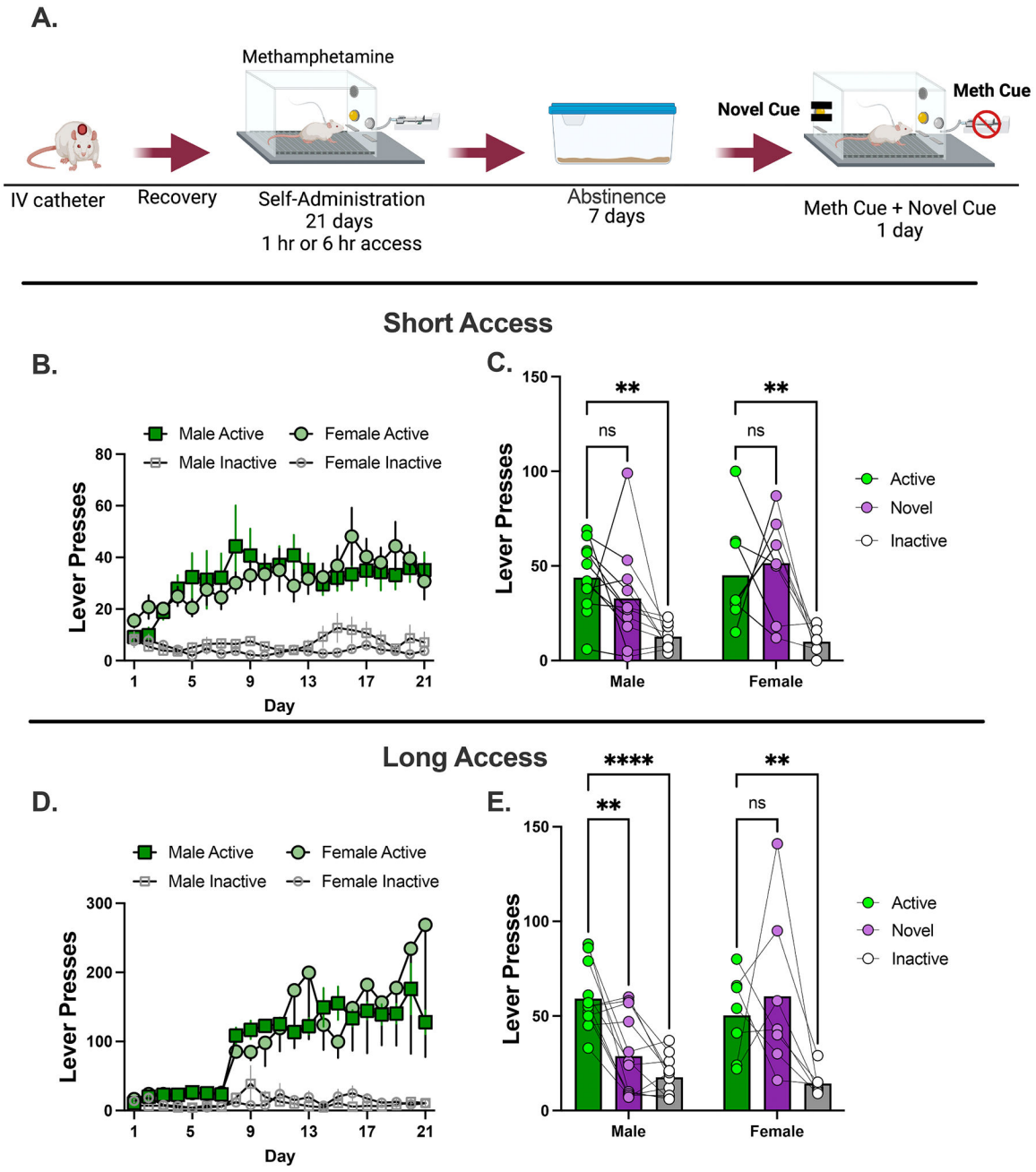

Fig. 1.

Male and female rats went through short or long access meth self-administration. A) Depicts the experimental timeline. Rats first underwent surgery to receive indwelling jugular vein catheters. Following a recovery period of at least 3 days, animals were trained to self-administer (SA) meth for 21 days in either a short-access (1 hour per day) or long-access (1 hour per day for 7 days followed by 6 h per day for 14 days) condition. All subjects then had a 7 day abstinence period in their homecages. On abstinence day 8, subjects were placed back in their training boxes for the novel cue test where they could respond on the meth-associated lever, inactive lever or a novel lever for 1 hour. B) Male (n = 11) and female

(n = 8) rats that administered ShA meth (1 hour a day) did not display any differences in responding over the 21 days of meth SA. C) On the novel cue test ShA males and females both responded similarly on the novel and meth cue. D) Male (n = 12) and female (n = 7) rats that administered LgA meth (1 hour a day for 7 days, then 6 h a day for 14 days) did not differ during SA training. E) On the novel cue test, LgA males and females displayed different patterns of responding on the novel cue test. Males responded on the meth cue lever significantly more than the inactive lever and the novel lever. There was no significant difference in responses on the active and novel levers in LgA females.

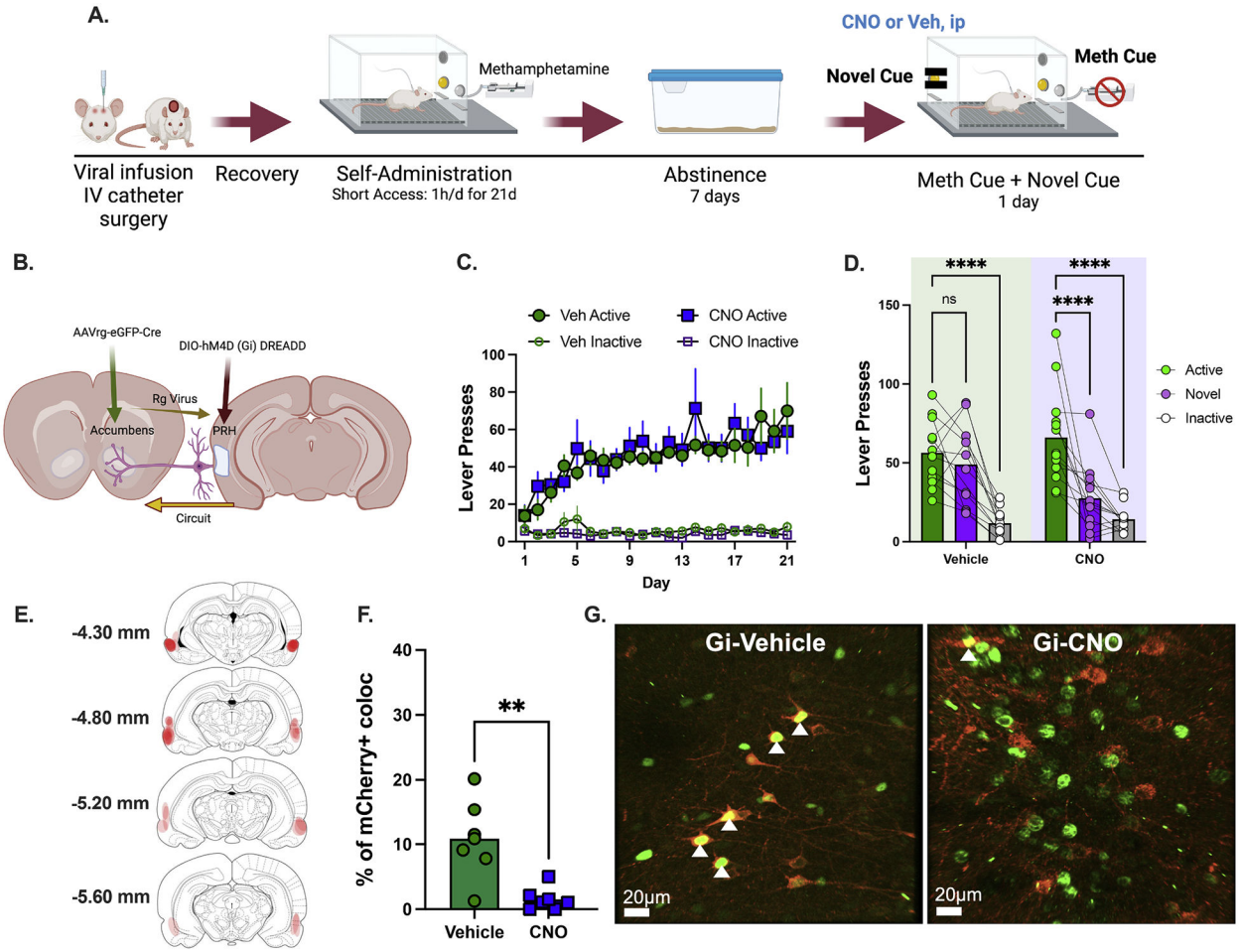

Fig. 2.

Inhibition of the PRH–NAc circuit. A) Illustration of the experimental timeline. Rats had surgery for both indwelling jugular vein catheters and intracranial microinfusions of virus in the PRH and NAc. Following this, the timeline is the same as in Experiment 1 with the addition of either CNO or Vehicle i.p. injections 30 min before test. Animals were sacrificed 90 min after the start of the novel cue test, perfused with 10 % buffered formalin and then their brains were collected for immunohistochemistry and analysis. B) Schematic of the PRH to NAc circuit and the placements of an AAVrg-eGFP-Cre virus into the NAc and DIO-hM4D-Gi DREADD into the PRH. C) During ShA SA rats were assigned to CNO (n = 13; males n = 10; females n = 3) or Vehicle (n = 13; males n = 10; females n = 3) conditions. Lever responding between the groups were equal throughout the SA period. D) On the novel cue test, CNO rats responded significantly more on the active lever compared to the inactive and novel levers whereas Vehicle rats responded significantly more on the active lever compared to the inactive lever, but not when compared to the novel lever. E) Heat map depiction of viral spread in the PRH. F) CNO-treated animals showed significantly decreased colocalization of mCherry and cFOS positive neurons in the PRH as compared to Vehicle-treated animals. G) Representative images of DREADDs tagged with mCherry (red) and cFOS (green). Arrows indicate overlap of cFOS and DREADD (yellow).

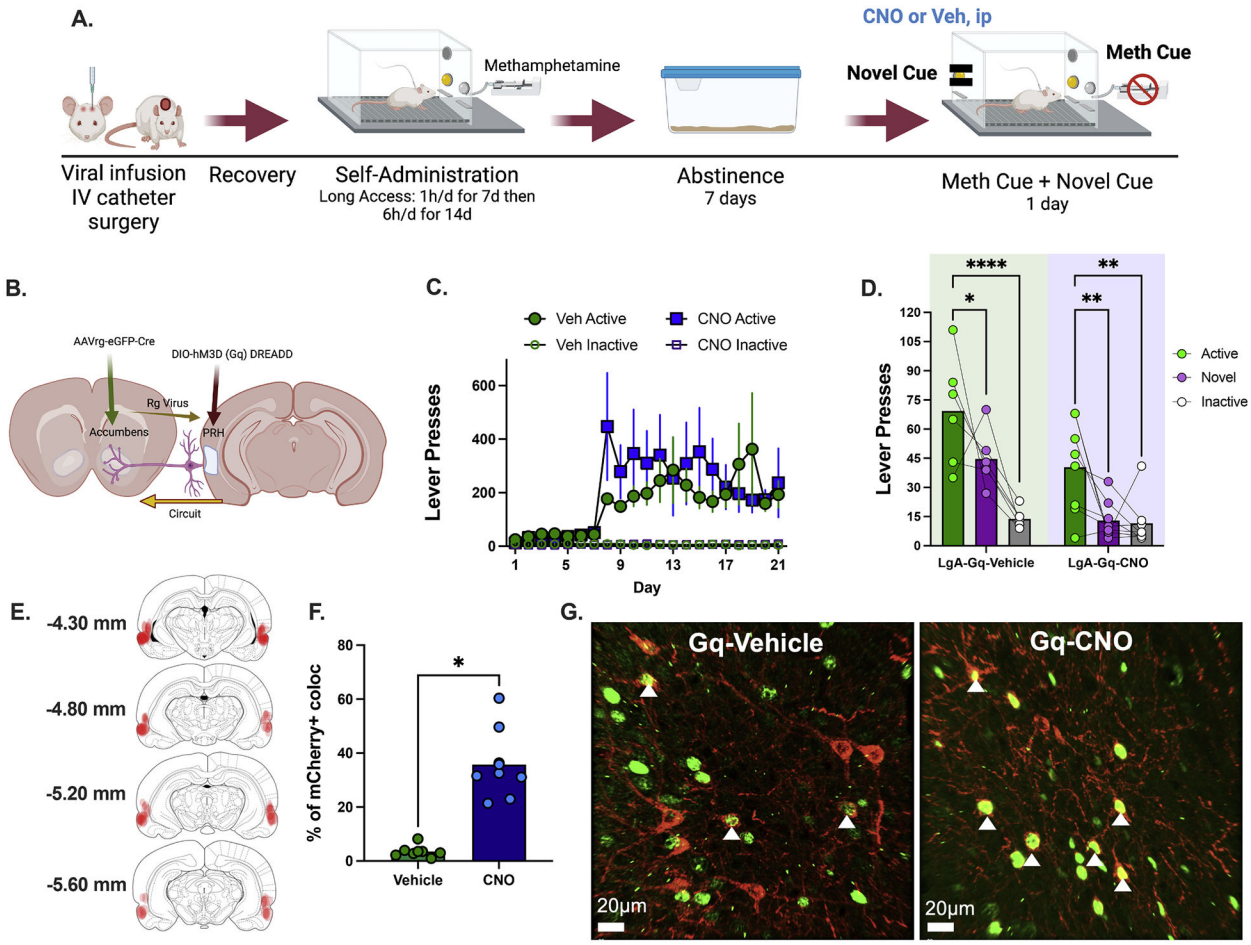

Fig. 3.

Activation of the PRH–NAc circuit. A) Illustration of the experimental timeline. Procedures were the same as in Experiment 2, with the exception of an excitatory DREADD being infused into the PRH instead of an inhibitory DREADD. B) Schematic of the PRH to NAc circuit and the placements of an AAVrg-eGFP-Cre virus into the NAc and DIO-hM3D-Gq DREADD into the PRH. C) During LgA SA rats were assigned to CNO (n = 8 males) or Vehicle (n = 7 males) conditions. Lever responding between the groups were equal throughout the SA period. D) On the novel cue test, CNO and Vehicle rats exhibited the same pattern of responding where animals interacted significantly more on the active lever compared to the novel or inactive levers. E) Heat map depiction of viral spread in the PRH. F) CNO-treated animals showed significantly increased colocalization of mCherry and cFOS positive neurons in the PRH as compared to Vehicle-treated animals. G) Representative images of DREADDs tagged with mCherry (red) and cFOS (green). Arrows indicate overlap of cFOS and DREADD (yellow).

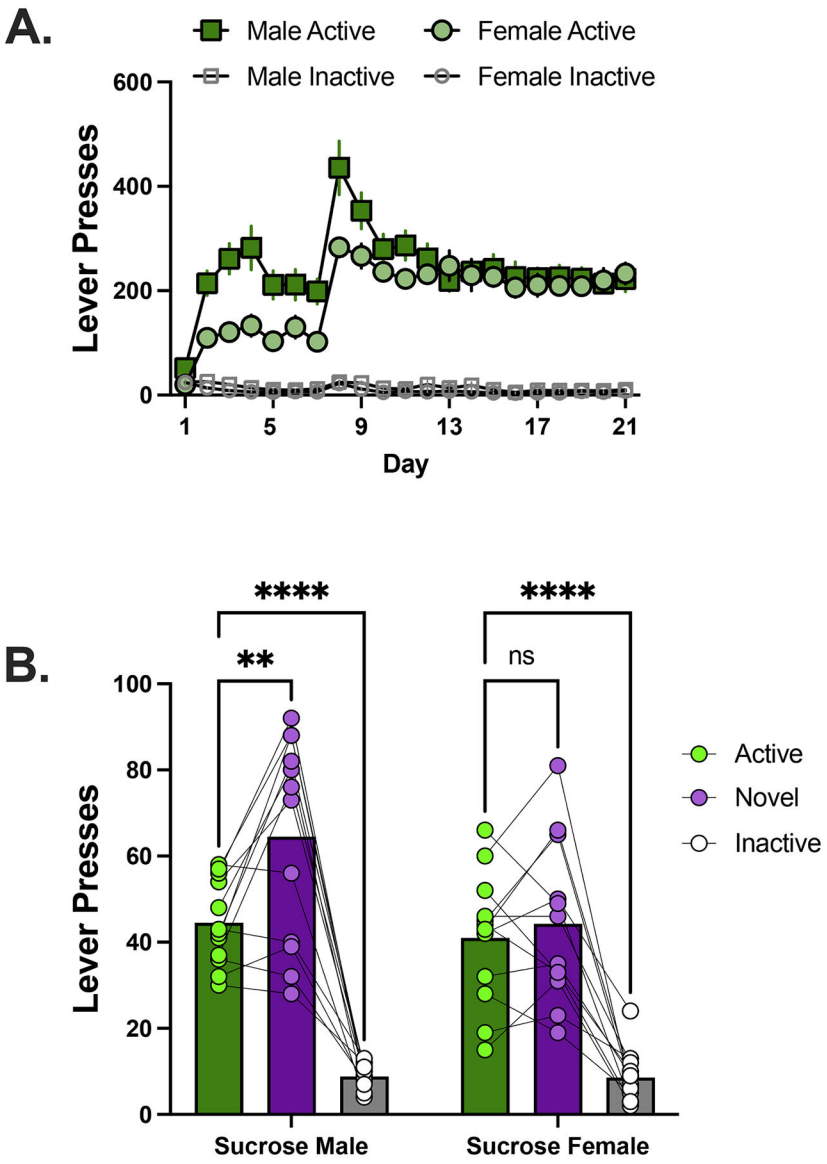

Fig. 4.

Male and female rats went through long access sucrose self-administration. A) During sucrose acquisition males (n = 12) had higher lever pressing than female (n = 12) rats, but the pattern of responses were similar over training. B) During the novel cue test males interacted significantly more on the novel lever compared to the active lever, but significantly more on the active lever compared to the inactive lever. Females pressed both the novel and active levers equally, but pressed the active lever significantly more than the inactive lever.