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Sex, stress, and prefrontal cortex: influence of biological sex on stress-promoted cocaine seeking

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Clinical reports suggest that females diagnosed with substance use disorder experience enhanced relapse vulnerability compared with males, particularly during stress. We previously demonstrated that a stressor (footshock) can potentiate cocaine seeking in male rats via glucocorticoid-dependent cannabinoid type-1 receptor (CB1R)-mediated actions in the prelimbic prefrontal cortex (PrL-PFC). Here, we investigated the influence of biological sex on stress-potentiated cocaine seeking. Despite comparable self-administration and extinction, females displayed a lower threshold for cocaine-primed reinstatement than males. Unlike males, footshock, tested across a range of intensities, failed to potentiate cocaine-primed reinstatement in females. However, restraint potentiated reinstatement in both sexes. While sex differences in stressor-induced plasma corticosterone (CORT) elevations and defensive behaviors were not observed, differences were evident in footshock-elicited ultrasonic vocalizations. CORT administration, at a dose which recapitulates stressor-induced plasma levels, reproduced stress-potentiated cocaine-primed reinstatement in both sexes. In females, CORT effects varied across the estrous cycle; CORT-potentiated reinstatement was only observed during diestrus and proestrus. As in males, CORT-potentiated cocaine seeking in females was localized to the PrL-PFC and both CORT- and restraint-potentiated cocaine seeking required PrL-PFC CB1R activation. In addition, *ex vivo* whole-cell electrophysiological recordings from female layer V PrL-PFC pyramidal neurons revealed CB1R-dependent CORT-induced suppression of inhibitory synaptic activity, as previously observed in males. These findings demonstrate that, while stress potentiates cocaine seeking via PrL-PFC CB1R in both sexes, sensitivity to cocaine priming injections is greater in females, CORT-potentiating effects vary with the estrous cycle, and whether reactivity to specific stressors may manifest as drug seeking depends on biological sex.

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INTRODUCTION

Clinical reports suggest that cocaine-dependent women may experience greater relapse vulnerability than men [1, 2]. Women reportedly experience greater difficulty abstaining [3] and relapse incidence [4], and resume use for longer periods of time following relapse [5]. This heightened relapse vulnerability is a crucial, yet currently untreated, women's health issue. However, the neurobiological basis for this sex difference is poorly characterized.

Stress is a problematic contributor to relapse, as it is unpredictable and unavoidable in everyday life. Females appear more vulnerable to stress-promoted relapse. Cocaine-dependent women display greater stressor reactivity [6] and female rats are more sensitive to yohimbine-promoted cocaine seeking [7]. Understanding the basis for sex differences that translate to heightened relapse susceptibility during stress has great relevance for treatment. Indeed, some medications that target the stress response appear to more effectively reduce cocaine craving in women [8].

Clinical reports point to a complex relationship between stress and drug seeking [9]. Rather than serving as a direct trigger for relapse, stress often interacts with other factors to promote drug

use [10, 11]. We have established a rodent model to study these potentiating effects of stress, wherein male rats undergo self-administration under conditions following which electric footshock is insufficient to reinstate extinguished cocaine seeking on its own but rather potentiates responding to an otherwise subthreshold priming dose of cocaine [12].

We have identified a role for glucocorticoid-dependent endocannabinoid mobilization in footshock-potentiated cocaine seeking in male rats. Corticosterone (CORT) potentiates cocaine-primed reinstatement via a mechanism that requires cannabinoid type-1 receptor (CB1R) activation [13]. These effects can be localized to the prelimbic prefrontal cortex (PrL-PFC), which sends a glutamatergic projection to the nucleus accumbens that has been implicated in the reinstatement of cocaine seeking in rodent models [14–18]. Stress-potentiated cocaine seeking is associated with increased excitability of pyramidal neurons attributable to a CB1R-dependent attenuation of inhibitory transmission resulting from reduced GABA release from PrL-PFC interneurons [13, 19].

Women display greater PFC activity during stress-induced craving than men [20]. Moreover, sex differences in PrL-PFC

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activation during cocaine seeking [21] and in response to stressors [22] have been identified. Sex differences in PrL-PFC endocannabinoid signaling have also been reported [23]. For example, while males and females express CB1Rs on the same PrL-PFC GABAergic interneuron terminals [19, 24], CB1R affinity is reduced in the female PrL-PFC [25].

Here we investigate potential sex differences in stressor-related cocaine seeking and the underlying neurobiological mechanisms. Stressor-potentiated cocaine-primed reinstatement following intravenous self-administration and corresponding behavioral and hormonal responses were systematically examined in male and female rats, as was the contribution of CORT-dependent PrL-PFC endocannabinoid signaling.

MATERIALS AND METHODS

Subjects

Female (90 days old/250 g minimum at study onset) and male (90 days old/ \leq 300 g at study onset) Sprague Dawley rats were single-housed under a reversed 12:12 h light–dark cycle (lights off at 0700 h; see Supplementary Methods). All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th ed.) and IACUC-approved.

Chemicals/drugs

Cocaine hydrochloride was obtained through the NIDA Drug Supply Program. CORT was obtained from Steraloids. 2-Hydroxypropyl- β -cyclodextrin- (HBC-) CORT and AM251 were purchased from Sigma-Aldrich. For electrophysiological recordings, TTX, CNQX, D-AP5, AM251, and CORT were obtained from Tocris Bioscience. All other chemical reagents were obtained from Sigma-Aldrich.

Intravenous catheterization and intracranial cannulation

Rats were implanted with intravenous catheters for cocaine self-administration and, in some cases, with PrL-PFC-targeted intracranial cannulae (A/P +2.8 mm, M/L +1.0 mm, D/V –3.5 mm) under isoflurane anesthesia as described [13, 26]. See Supplementary Methods for details.

Cocaine self-administration

Rats were trained to self-administer cocaine (0.2 mg/0.2 ml infusion) by pressing a lever under a fixed-ratio 4 schedule of reinforcement during daily 2 h sessions as previously described [13, 26], with minor adaptations (see Supplementary Methods). Once trained, rats self-administered for 10–14 daily sessions prior to undergoing extinction training, during which the cocaine solution was replaced with saline. Once extinction criteria were met ($<$ 15 lever presses within 2 of 3 consecutive sessions), rats underwent reinstatement testing using a counter-balanced design.

Footshock

Rats were exposed to pseudo-randomly delivered 0.2 s triplicate footshocks through the floor grids of the experimental chambers over a 15 min period, with 40 s (average) inter-trial intervals. Males were tested for shock-potentiated cocaine seeking at 0.6 mA, as previously described [13], while females were tested across a range of intensities (0.15, 0.30, 0.60, or 0.90 mA). Levers were retracted and stimulus lights were off during the shock period, after which rats received an intraperitoneal (ip) cocaine or saline injection prior to initiation of the 2 h reinstatement session.

Restraint

Rats underwent 15 min of immobilization restraint using Decapicone bags (BrainTree Scientific DC 200) outside of the operant conditioning chambers or remained in the home cage for 15 min prior to cocaine or saline administration and reinstatement testing.

Plasma CORT

Trunk blood was collected following rapid decapitation between 1030 and 1230 h after 15 min footshock, restraint, or context exposure, or 5, 15, 30, or 45 min following systemic CORT (2 mg/kg, ip) or vehicle administration. Plasma CORT levels were analyzed by ELISA (Enzo Life Sciences) per the manufacturer's protocol with the addition of a 5 min 98 °C incubation in steroid dissociation reagent to fully dissociate CORT from binding globulins. Absent this optimization, CORT levels erroneously appeared 2–3 \times lower in females likely due to greater binding globulin levels [27].

Defensive behaviors

Defensive behaviors elicited by footshock were assessed during the 15 min sessions. Freezing behavior was defined as the cessation of all movement except that required for respiration, and freezing duration of at least 2 s following footshock offset was used to define latency [28]. Defensive darts were defined as rapid, forward movement across the chamber that resembled an active escape-like behavior [29]. All behaviors were scored using FreezeScan 2.0 (CleverSys). See Supplementary Methods for details.

Ultrasonic vocalizations (USVs)

Sonorous activity was recorded using condenser microphones (CM16, Avisoft Bioacoustics) suspended inside chambers housed within sound-attenuating cubicles (Coulbourn Instruments). Recorded.wav files were manually scored for total number and average durations of 20–22 and 50–55 kHz USVs using Raven Pro 1.4 software (Cornell Lab of Ornithology) similar to previous reports [30, 31]. See Supplementary Methods for details.

Estrous phase tracking

Vaginal lavage samples were collected daily following behavioral testing. Estrous phases were identified using a Nikon Eclipse 55i microscope according to cell types visualized by Papanicolaou stain as previously described [26]. Predominant expression of large, round, nucleated epithelial cells defined proestrus; large, cornified anucleated epithelial cells defined estrus; leukocytes defined diestrus; and a combination of all three cell types defined metestrus.

Statistics

For behavioral and stressor assessments, statistical analyses were conducted using SPSS software and consisted of ANOVAs followed by Bonferroni-corrected *t*-tests. Electrophysiological data were analyzed using Minianalysis (Synaptosoft) by two-way ANOVA followed by Tukey's-corrected post-hoc and Kolmogorov-Smirnov *t*-tests. All results are expressed as mean \pm SEM. Statistical significance was defined as $P < 0.05$.

Experiment 1: Sex comparison of cocaine-primed reinstatement

Male ($n = 19$) and female ($n = 16$) rats underwent cocaine self-administration and extinction and were tested for reinstatement in response to cocaine priming doses (1.25, 2.5, or 5.0 mg/kg, ip) or saline in counter-balanced sequence, administered immediately prior to the 2 h reinstatement session. Rats were re-extinguished to criterion between tests.

Experiment 2: Sex comparison of stressor-potentiated cocaine seeking and reactivity

Rats were tested for the ability of footshock (male $n = 29$; female $n = 6$) or restraint (male $n = 10$; female $n = 11$) to potentiate reinstatement when combined with subthreshold cocaine doses identified in Experiment 1 (1.25 mg/kg, ip in females and 2.5 mg/kg, ip in males). Generally, rats were tested under the following conditions in counter-balanced sequences: no stressor/saline, stressor/saline, no stressor/subthreshold cocaine, stressor/subthreshold cocaine. However, males tested for the effects of footshock on reinstatement were only tested once in one of the

previously listed conditions and were consequently analyzed using a between-subjects two-way (treatment condition \times day) ANOVA. Plasma CORT levels, defensive behaviors, and USVs were assessed (male $n = 16$; female $n = 16$).

Experiment 3: Sex comparison of CORT-potentiated reinstatement
Sex differences in stress-level CORT-potentiated reinstatement (male $n = 12$; female $n = 19$) were tested under the following conditions: CORT (2 mg/kg, ip in 10% ethanol in saline; 40 min pretreatment)/saline, CORT/subthreshold cocaine, vehicle/saline, vehicle/subthreshold cocaine. CORT effects on reinstatement responding to the identified subthreshold cocaine priming dose for the opposite sex were also assessed (male $n = 12$; female $n = 8$). Females were tested for CORT-potentiated reinstatement across the estrous cycle ($n = 24$; testing between 0730 and 1200 h) determined using daily vaginal lavage samples. Plasma CORT levels were measured in both sexes following CORT administration (male $n = 32$; female $n = 32$). The influence of the estrous cycle on cocaine self-administration and extinction was not assessed.

Experiment 4: Sex comparison of PrL-PFC CB1R-dependent restraint-potentiated reinstatement
Males ($n = 7$) and females ($n = 7$) were implanted with bilateral intracranial cannulae targeting the PrL-PFC and tested for reinstatement testing under the following conditions: intra-PrL vehicle/restraint (15 min)/saline; intra-PrL vehicle/no restraint/saline; intra-PrL vehicle/no restraint/cocaine (1.25 or 2.5 mg/kg, ip), intra-PrL vehicle/restraint/cocaine, intra-PrL AM251 (300 ng/0.3 μ L/site, 15 min pretreatment)/restraint/cocaine. Cannula placements were confirmed. No misses were found. AM251 effects on cocaine seeking under no restraint/saline, no restraint/cocaine, and restraint/saline conditions were not tested.

Experiment 5: Assessment of PrL-PFC CORT- and CB1R-dependent reinstatement and electrophysiological activity in females
Female rats ($n = 13$) were implanted with bilateral cannulae targeting the PrL-PFC and tested for reinstatement under the following conditions: intra-PrL HBC-CORT (50 ng/0.3 μ L/site, 15 min pretreatment)/saline, intra-PrL HBC/saline, intra-PrL HBC-CORT/cocaine (1.25 mg/kg, ip), intra-PrL HBC/cocaine. Cannula placements were confirmed, and misses were excluded ($n = 1$).

Female rats ($n = 7$) were tested for CB1R regulation of reinstatement under the following conditions: PrL vehicle/CORT (2 mg/kg, ip; 1 h pretreatment)/saline; PrL vehicle/vehicle/saline; PrL vehicle/vehicle/cocaine (1.25 mg/kg, ip), PrL vehicle/CORT/cocaine, PrL AM251 (300 ng/0.3 μ L/site, 15 min pretreatment)/CORT/cocaine. Anatomical misses were excluded ($n = 1$). AM251 effects on cocaine seeking under vehicle/saline, vehicle/cocaine, and CORT/saline conditions were not tested.

Patch-clamp electrophysiological recordings were taken from female coronal slices containing the PrL-PFC following bath application of CORT (1 μ M) or vehicle with AM251 (1 μ M) or vehicle. Recording details are included in Supplementary Methods. For analysis, mIPSC amplitude was normalized to baseline, and the depression (%) of mIPSCs by CORT was calculated as follows: $100 \times [\text{mean amplitude of IPSCs during final 5 min of treatment}/\text{mean amplitude of baseline mIPSCs}]$. Analysis of mIPSCs was performed with cumulative probability plots [32].

RESULTS

Experiment 1: Cocaine-primed reinstatement dose–response testing

No sex differences in acquisition, maintenance, or extinction of cocaine self-administration were observed (Table S1). Males and females exhibited equivalent latencies to acquire (Fig. 1a), responding during maintenance (Fig. 1b), and extinction behavior (Fig. 1c). Despite similarities in self-administration and extinction, females

reinstated in response to lower cocaine priming doses (Fig. 1d, e). A three-way repeated-measures ANOVA revealed a significant sex (male vs. female) \times day (extinction vs. reinstatement) \times dose (0, 1.25, 2.5, or 5 mg/kg cocaine) interaction ($F_{(3,170)} = 8.736$, $p < 0.0001$). In males, a two-way day \times dose ANOVA revealed main effects of day ($F_{(1,98)} = 34.59$, $p < 0.0001$) and dose ($F_{(3,98)} = 5.345$, $p < 0.01$), and a significant interaction ($F_{(3,98)} = 5.747$, $p < 0.01$). Post-hoc testing showed that significant reinstatement was only observed at 5.0 mg/kg cocaine ($p < 0.001$ vs. extinction). In females, a two-way day \times dose ANOVA also revealed main effects of day ($F_{(1,72)} = 39.47$, $p < 0.0001$) and dose ($F_{(3,72)} = 17.45$, $p < 0.0001$), and a significant interaction ($F_{(3,72)} = 15.19$, $p < 0.0001$). Significant reinstatement (vs. extinction) was observed in response to 2.5 mg/kg ($p < 0.05$) and 5.0 mg/kg ($p < 0.001$) cocaine. Based on these results, 2.5 mg/kg cocaine was selected as the subthreshold priming dose to be used for testing for potentiated cocaine seeking in males and 1.25 mg/kg as the subthreshold dose in females. Post-hoc analysis revealed that reinstatement following administration of the 5 mg/kg cocaine dose, but not the other doses tested was significantly increased in females relative to males ($p < 0.05$).

Experiment 2: Sex comparison of stressor-potentiated cocaine seeking and stressor reactivity

Footshock stress. To determine if stressors can similarly potentiate cocaine-induced reinstatement in males and females, rats were initially tested for the effects of footshock (Fig. 2a). A three-way repeated-measures ANOVA revealed a significant sex (male vs. female) \times day (extinction vs. reinstatement) \times treatment (no shock/saline, no shock/cocaine, shock/cocaine) interaction ($F_{(2,66)} = 6.499$, $p < 0.01$). Footshock-potentiated cocaine-induced reinstatement in males (Fig. 2b). A two-way ANOVA revealed a significant reinstatement \times shock/cocaine condition interaction ($F_{(3,38)} = 6.823$, $p < 0.001$). Post-hoc analysis showed that neither 2.5 mg/kg cocaine nor footshock alone reinstated cocaine seeking. However, when combined, the two stimuli produced significant reinstatement ($p < 0.001$ vs. extinction and the no shock/saline, no shock/cocaine, and shock/saline conditions). By contrast, females did not exhibit footshock-potentiated reinstatement at any intensity tested (0.15, 0.3, 0.6, 0.9 mA) in combination with the 1.25 mg/kg cocaine priming dose (Fig. 2c).

Restraint stress. Restraint potentiated cocaine-primed reinstatement in both males (Fig. 2d) and females (Fig. 2e). A three-way between-subjects ANOVA did not reveal a significant interaction between sex (male vs. female), day (reinstatement vs. extinction), and treatment (no restraint/saline, restraint/saline, no restraint/cocaine, restraint/cocaine; ($F_{(3,136)} = 0.310$), $p = 0.818$). Two-way ANOVAs revealed significant day \times treatment condition interactions in both males and females. Post-hoc analysis revealed that while neither restraint nor subthreshold cocaine (2.5 mg/kg in males; 1.25 mg/kg in females) alone reinstated cocaine seeking, restraint in combination with cocaine produced significant reinstatement in both sexes (males: $F_{(3,28)} = 3.159$, $p < 0.05$, post-hoc $p < 0.01$ vs. extinction, no restraint/saline, and restraint/saline conditions; females $F_{(3,40)} = 3.021$, $p < 0.05$, post-hoc $p < 0.05$ vs. extinction, no restraint/saline, and restraint/saline conditions).

Plasma CORT. To determine if differences in stressor-potentiated reinstatement were associated with differences in the CORT response, basal and stressor-induced plasma CORT immediately following 15 min of shock or restraint was compared between sexes (Fig. 2f). A sex \times stress/condition ANOVA found that, while there were significant main effects of sex ($F_{(1,18)} = 26.39$, $p < 0.01$) and stressor ($F_{(2,18)} = 37.38$, $p < 0.0001$) on plasma CORT, there was no sex \times stressor interaction. Overall females had higher CORT levels. A planned comparison revealed that this was likely attributable to increased basal CORT levels (unpaired t test, $t_{(6)} = 3.332$, $p < 0.05$). Overall, shock and restraint elevated CORT

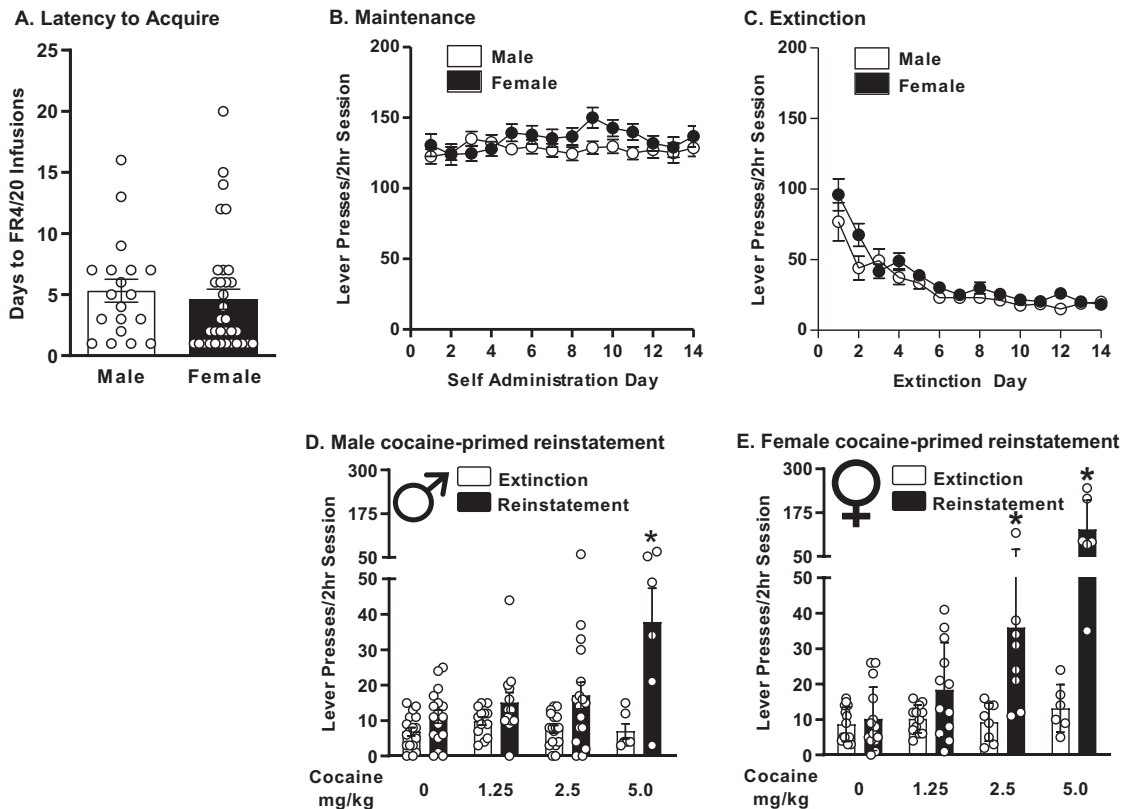


Fig. 1 Sex comparison of acquisition, maintenance, and extinction of cocaine self-administration, and cocaine-primed reinstatement (male $n = 49$, female $n = 34$). **a** Latency to acquire cocaine self-administration under a fixed-ratio 4 schedule of reinforcement with a minimum requirement of ≥ 20 0.2 mg/0.2 mL cocaine infusions. **b** Maintenance of cocaine self-administration. **c** Extinction of cocaine self-administration. **d** Male cocaine-primed reinstatement dose–response curve (0, 1.25, 2.5, and 5.0 mg/kg, ip; *** $p < 0.001$ vs. prior extinction). **e** Female cocaine-primed reinstatement dose–response curve (0, 1.25, 2.5, and 5.0 mg/kg, ip) (* $p < 0.05$ or *** $p < 0.001$ vs. prior extinction). Data are represented as mean \pm SEM.

relative to baseline ($p < 0.001$ for each stressor) and the magnitude (i.e., increase from baseline) of the stressor-induced CORT response did not differ across stressors or between sexes. A sex \times stressor (shock vs. restraint) ANOVA failed to reveal significant effects of sex, stressor type or a sex \times stressor interaction (Fig. 2g).

Defensive behaviors. To determine if the lack of footshock-potentiated cocaine seeking in females was attributable to a general increase or decrease in shock reactivity relative to males, we also examined defensive behaviors, including freezing and defensive darting. No sex differences in average percent freezing behavior over the 15 min session were observed (Fig. 2h). A two-way repeated-measures ANOVA revealed a main effect of footshock ($F_{(1,14)} = 262.1$, $p < 0.0001$), but no main effect of sex or interaction. We further assessed freezing in response to the first and last three shocks in the 15 min session (Fig. S1A) and the post-shock freezing latency across the session (Fig. S1B) and found no sex differences. We also assessed darting behavior, previously characterized as activity bursts primarily observed in females following shocks [33] and found a trend toward an increase in females (Fig. S1C; $p = 0.07$).

Ultrasonic vocalizations (USVs). To examine potential sex differences in emotional/affective processing, USVs were recorded over a 15 min baseline period and during exposure to footshock or restraint. Total numbers of 20–22 kHz USVs and 50–55 kHz USVs during each 15 min period are shown in Fig. 2i, j, respectively. In contrast to defensive behaviors, sex differences in stressor-evoked USVs were observed, but only during footshock. For each frequency range, a two-way sex \times stressor condition (baseline, restraint, footshock)

ANOVA examining effects on the total number of calls revealed significant main effects of stressor condition ($F_{(2,16)} = 14.92$, $p < 0.001$) and sex ($F_{(1,8)} = 12.17$, $p < 0.01$) for 20–22 kHz calls, and sex \times stressor condition interactions for 20–22 kHz ($F_{(2,16)} = 11.39$, $p < 0.001$) and for 50–55 kHz calls ($F_{(2,12)} = 3.84$, $p < 0.05$). Overall, the number of 20–22 kHz USVs increased while the number of 50–55 kHz USVs generally decreased during stressor exposure. While there were no sex differences in USVs at baseline, males emitted significantly more 20–22 kHz USVs than females ($p < 0.001$). While there were no differences from respective baselines for either sex, males also emitted significantly fewer 50–55 kHz USVs than females ($p < 0.05$) during the footshock session. When deconstructed by stressor, a main effect of restraint (baseline vs. restraint) was observed in both 20–22 kHz ($F_{(1,8)} = 9.93$, $p < 0.05$) and 50–55 kHz ($F_{(1,6)} = 11.39$, $p < 0.05$) total calls, regardless of sex, with an increase in 20–22 kHz calls and a reduction in 50–55 kHz calls. For 20–22 kHz calls during baseline vs. footshock stress, there was a main effect of shock condition ($F_{(1,8)} = 17.77$, $p < 0.01$), sex ($F_{(1,8)} = 12.15$, $p < 0.01$), and a significant stress \times sex interaction ($F_{(1,8)} = 11.72$, $p < 0.01$). Sex differences in the distribution of USVs during stressor sessions were not observed, nor were differences in the durations of USVs when analyzed according to frequency subtype (i.e., >250 ms, versus <100 ms 22 kHz calls; Fig. S2B–F, H–I). However, overall, females emitted more short-call 22 kHz USVs, whereas males emitted more long-call 22 kHz USVs.

Experiment 3: Sex comparison in CORT-potentiated reinstatement of cocaine seeking

CORT-potentiated reinstatement. CORT (2 mg/kg, ip; 40 min pretreatment; Fig. 3a) potentiated cocaine-induced reinstatement in both sexes (males Fig. 3b; females Fig. 3c). A three-way

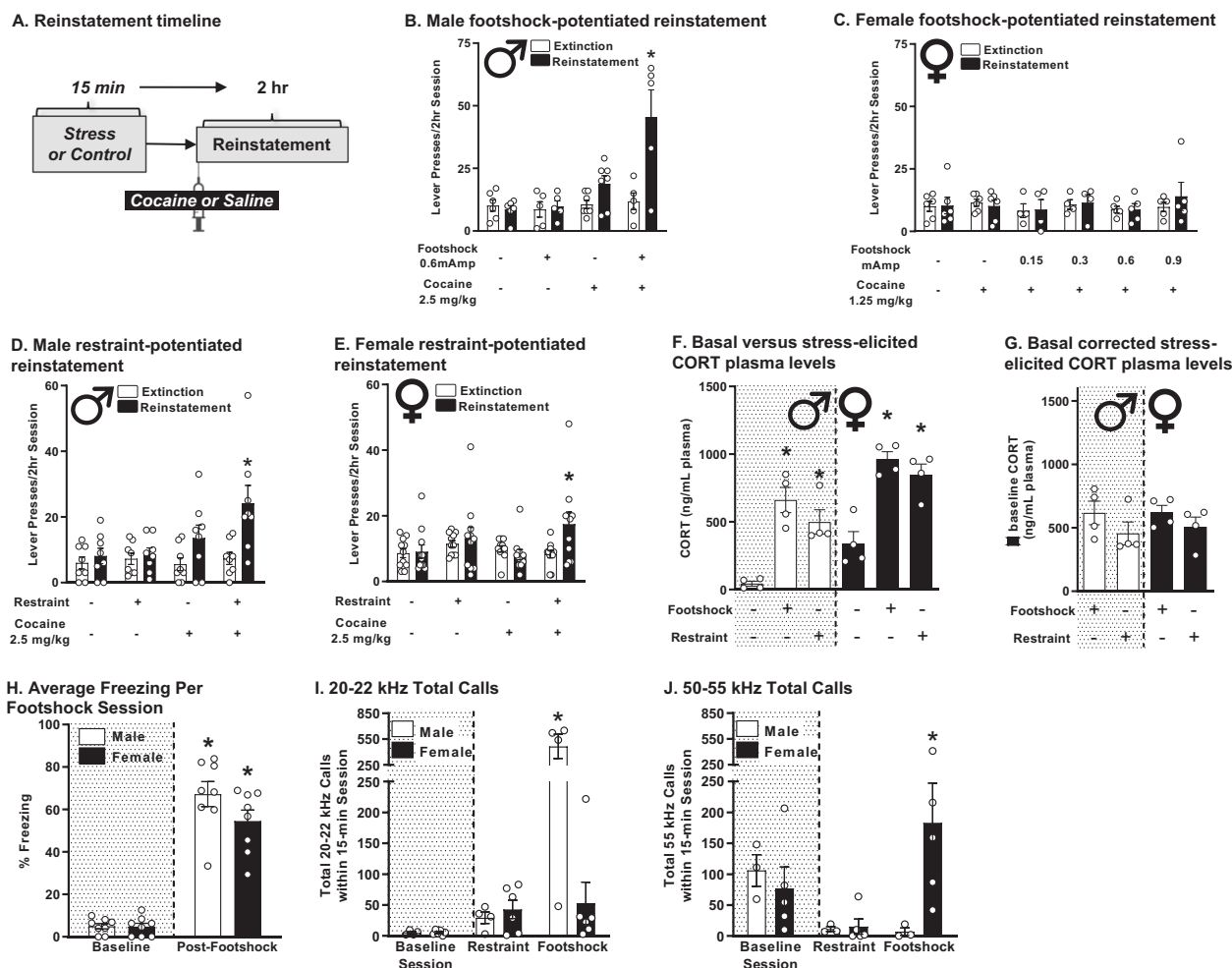


Fig. 2 Sex comparison of footshock and restraint stress effects on subthreshold cocaine-primed reinstatement, plasma CORT levels, defensive behavior, and ultrasonic vocalizations. Within x-axis labels, “+” symbols indicate administration of the variable of interest (e.g., cocaine), whereas “-” symbols indicate administration of the associated control condition (e.g., saline). **a** Timeline for stress-potentiated reinstatement. **b** Footshock (0.6 mA) potentiated reinstatement to 2.5 mg/kg cocaine in males ($n = 29$; $p < 0.05$ vs. prior extinction and each of the other reinstatement conditions). **c** Effects of footshock (0.15, 0.3, 0.6, and 0.9 mA) on reinstatement to 1.25 mg/kg cocaine in females ($n = 6$). **d** Immobilization restraint-potentiated reinstatement to 2.5 mg/kg cocaine in males ($n = 10$; $p < 0.05$ vs. prior extinction, no restraint/saline, and restraint/saline). **e** Immobilization restraint-potentiated reinstatement to 1.25 mg/kg cocaine in females ($n = 11$; $p < 0.05$ vs. prior extinction, no restraint/saline, and restraint/saline). **f** Sex comparison of restraint- and footshock-elicited total CORT plasma levels ($n = 4$ per condition; $p < 0.05$ vs. baseline). **g** Sex comparison of absolute total CORT plasma level increases from baseline ($n = 4$ per condition). **h** Sex comparison of average freezing during footshock session ($n = 8$ per condition; $p < 0.05$ vs. baseline). **i** Sex comparison of total 20–22 kHz ultrasonic vocalizations emitted during footshock and restraint ($n = 8$ per condition; $p < 0.05$ vs. females). **j** Sex comparison of total 50–55 kHz ultrasonic vocalizations emitted during footshock and restraint ($n = 8$ per condition; $p < 0.05$ vs. males). Data are represented as mean \pm SEM (**b**, **d**, **e**: $*p < 0.05$ vs. extinction; **f**, **h**: $*p < 0.05$ vs. baseline).

between-subjects ANOVA revealed no significant interaction between sex (male vs. female), day (extinction vs. reinstatement), and treatment (vehicle/saline, CORT/saline, vehicle/cocaine, CORT/cocaine; $F_{(2,180)} = 1.013$, $p = 0.388$). In both sexes, two-way reinstatement \times treatment condition ANOVA revealed significant main effects of reinstatement ($F_{(1,33)} = 19.03$, $p < 0.0001$ in males; $F_{(1,52)} = 9.38$, $p < 0.01$ in females) and treatment ($F_{(3,33)} = 3.694$, $p < 0.05$ in males; $F_{(3,52)} = 4.67$, $p < 0.01$ in females), and significant interactions ($F_{(3,33)} = 4.484$, $p < 0.01$ in males; $F_{(3,52)} = 5.82$, $p < 0.01$ in females). In both cases, post-hoc testing showed that neither CORT nor cocaine (2.5 mg/kg in males and 1.25 mg/kg in females) alone produced reinstatement, but that significant reinstatement was observed in both sexes when CORT was administered prior to the subthreshold cocaine dose ($p < 0.05$ vs. extinction, vehicle/saline, vehicle/cocaine, and CORT/saline conditions). To further examine CORT-potentiated cocaine seeking, we also tested for effects of CORT on

reinstatement in combination with 1.25 mg/kg cocaine in males and 2.5 mg/kg cocaine in females (Fig. S3A, B). Dose–response testing indicated that CORT produces a leftward shift in the dose–response curve for cocaine-induced reinstatement in both sexes, consistent with increased cocaine sensitivity. A three-way between-subjects ANOVA revealed no significant interactions between sex (male vs. female), day (extinction vs. reinstatement), and treatment (vehicle/saline, vehicle/cocaine-1.25, vehicle/cocaine-2.5, CORT/saline, CORT/cocaine-1.25, CORT/cocaine-2.5; $F_{(5,238)} = 0.987$, $p = 0.426$).

Estrous phase-dependent effects of CORT. As the level of CORT-potentiation tended to be lower in females than in males, estrous cycle interactions with CORT-potentiated reinstatement were assessed. Overall, a three-way between-subjects ANOVA did not reveal a significant interaction between estrous phase (proestrus, estrus, metestrus, or diestrus), day (extinction vs. reinstatement),

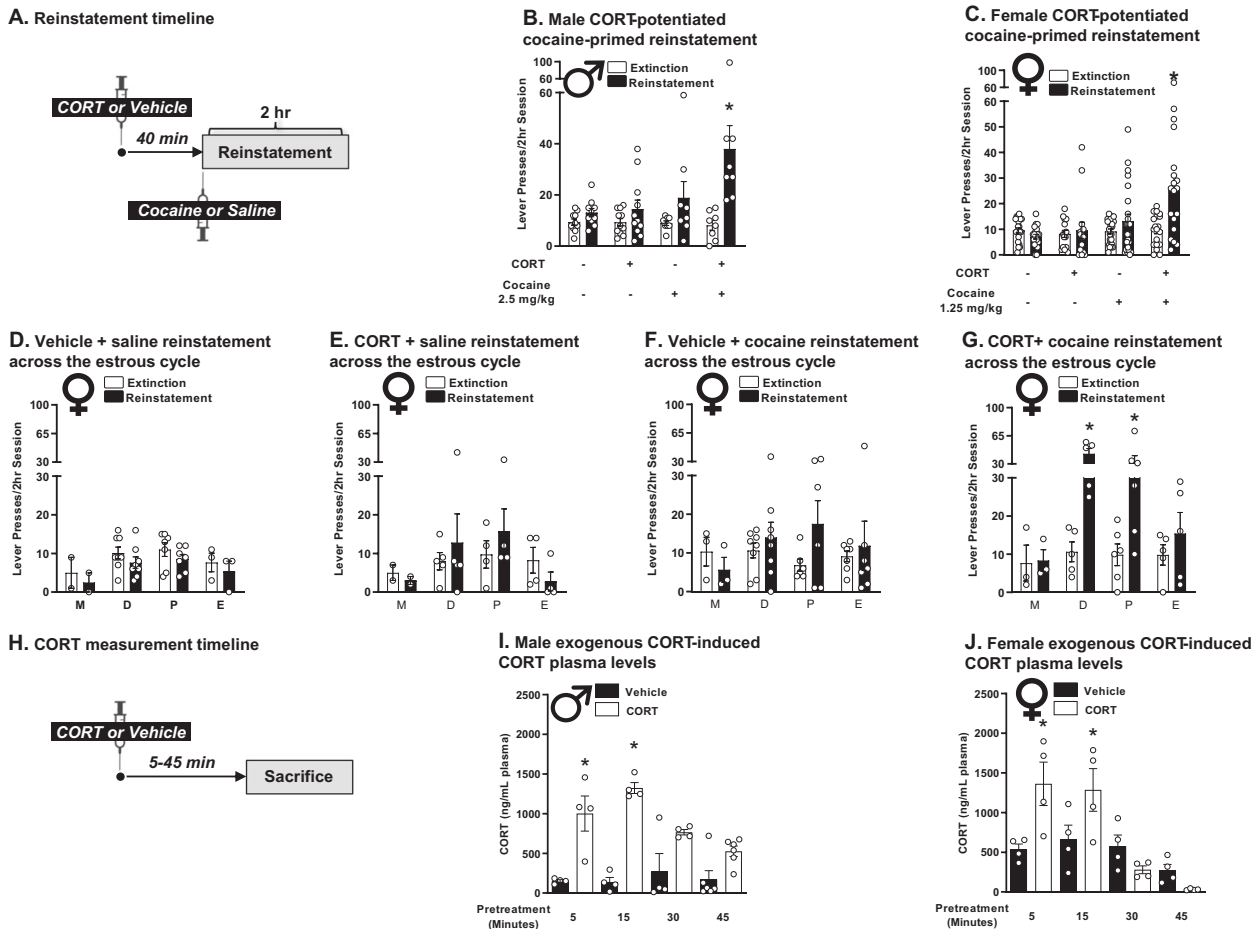


Fig. 3 Sex comparison of exogenous CORT effects on subthreshold cocaine-primed reinstatement and CORT plasma levels. Within x-axis labels, “+” symbols indicate administration of the variable of interest (e.g., cocaine), whereas “-” symbols indicate administration of the associated control condition (e.g., saline). **a** CORT-potentiated reinstatement timeline. **b** CORT- (2 mg/kg, ip) potentiated reinstatement in response to 2.5 mg/kg cocaine in males ($n = 12$; $p < 0.05$ vs. extinction). **c** CORT- (2 mg/kg, ip) potentiated reinstatement in response to 1.25 mg/kg cocaine in females ($n = 19$; $p < 0.05$ vs. extinction). **d** Reinstatement to vehicle and saline across the estrous cycle in females ($n = 20$). **e** Reinstatement to 2 mg/kg CORT and saline across the estrous cycle in females ($n = 15$). **f** Reinstatement to vehicle and 1.25 mg/kg cocaine across the estrous cycle in females ($n = 21$). **g** Reinstatement to 2 mg/kg CORT and 1.25 mg/kg cocaine across the estrous cycle in females ($n = 19$; $p < 0.05$ vs. diestrus vs. metestrus and estrus, proestrus vs. metestrus). **h** Timeline for plasma collection for CORT measurement. **i** Exogenous CORT-induced total CORT plasma levels 5, 15, 30, and 45 min following administration in males ($n = 4-6$ per condition; $p < 0.05$ vs. vehicle). **j** Exogenous CORT-induced total CORT plasma levels 5, 15, 30, and 45 min following administration in females ($n = 4$ per condition; $p < 0.05$ vs. vehicle). Data are represented as mean \pm SEM.

and treatment (vehicle/saline, CORT/saline, vehicle/cocaine, and CORT/cocaine; $F_{(6,130)} = 0.221$, $p = 0.969$). Furthermore, when analyzing estrous cycle variation in reinstatement (relative to extinction) in response to vehicle/saline (Fig. 3d), CORT/saline (Fig. 3e), or vehicle/1.25 mg/kg cocaine (Fig. 3f), no differences were observed. However, significant reinstatement was observed in response to CORT + 1.25 mg/kg cocaine, but only during diestrus and proestrus (Fig. 3g). A two-way independent-measures ANOVA revealed a main effect of reinstatement ($F_{(1,30)} = 13.43$, $p < 0.01$), treatment condition ($F_{(3,30)} = 3.839$, $p < 0.05$), and a significant interaction ($F_{(1,30)} = 3.028$, $p < 0.05$). Post-hoc testing revealed increased reinstatement in proestrus versus metestrus ($p < 0.05$) and during diestrus versus both metestrus and estrus ($ps < 0.01$).

Plasma CORT. To determine whether systemic CORT administration elicits similar blood levels in both sexes, a blood plasma level time-course sex comparison was made following administration of 2 mg/kg CORT (Fig. 3h). While a three-way ANOVA revealed no overall sex \times time (5, 15, 30, 45 min post injection) \times treatment (vehicle vs. CORT) interaction ($F_{(3,52)} = 1.296$, $p = 0.286$,

differences were observed. Basal (vehicle) CORT levels were higher in females at every timepoint ($F_{(1,26)} = 12.90$, $p < 0.01$) and plasma levels similarly peaked 15 min after CORT administration in both sexes (Fig. 3i, j; $F_{(3,26)} = 3.536$, $p < 0.05$). No sex differences were observed in CORT level elevations observed at 5 ($F_{(1,12)} = 0.004$, $p = 0.951$), or 15 min ($F_{(1,12)} = 2.747$, $p = 0.123$), but differences were observed at 30 ($F_{(1,12)} = 8.335$, $p < 0.05$) and 45 min ($F_{(1,17)} = 12.927$, $p < 0.01$). Males exhibited persistent CORT elevations relative to vehicle (Fig. 3i). A two-way repeated-measures treatment (vehicle versus CORT) \times time (5, 15, 30, or 45 min) ANOVA revealed main effects of time ($F_{(3,28)} = 3.760$, $p < 0.05$), treatment ($F_{(1,28)} = 69.83$, $p < 0.0001$) and a significant interaction ($F_{(3,28)} = 4.852$, $p < 0.01$), with CORT levels increased after 5 min, peak levels at 15 min, and reductions at 30 and 45 min relative to the peak ($p < 0.01$) close to levels observed following vehicle. By contrast, following the 15 min peak, CORT levels in females rapidly declined to the point where they were below vehicle levels (Fig. 3j). A two-way repeated-measures treatment \times time ANOVA revealed a main effect of time ($F_{(3,24)} = 12.21$, $p < 0.0001$) and a significant treatment \times time interaction ($F_{(3,24)} =$

6.295, $p < 0.01$), with significantly lower CORT levels by 30 min and 45 min compared with 5 min and 15 min ($p < 0.001$ for each comparison), suggesting faster CORT clearance in females.

Experiment 4: Role of PrL-PFC CB1R activation in restraint-potentiated reinstatement. To test whether the effects of restraint on potentiated reinstatement require PrL-PFC CB1R activation in both sexes, as demonstrated with footshock in males [13], males and females underwent restraint-potential testing following intra-PrL CB1R antagonism (Fig. 4a; AM251, 300 ng/0.3 μ L/side, 15 min pretreatment). Both males and females exhibited CB1R-dependent restraint-potentiated reinstatement (Fig. 4b, c). A three-way between-subjects ANOVA did not reveal a significant interaction between sex (male vs. female), day (reinstatement vs. extinction), and treatment (intra-PrL vehicle/no restraint/saline, intra-PrL vehicle/restraint/saline, intra-PrL vehicle/no restraint/cocaine, intra-PrL vehicle/restraint/cocaine, PrL AM251/restraint/cocaine; ($F_{(4,172)} = 0.507$, $p = 0.731$)). In both sexes, two-way independent-measures ANOVAs revealed significant reinstatement \times treatment condition interactions ($F_{(4,74)} = 2.849$, $p < 0.05$ for males; $F_{(4,46)} = 1.496$, $p < 0.05$ for females). Post-hoc comparisons revealed that while reinstatement was not observed following restraint or subthreshold cocaine alone (2.5 mg/kg in males or 1.25 mg/kg in females), the cocaine/restraint combination produced reinstatement following intra-PrL vehicle ($p < 0.01$ in males vs. extinction, restraint/saline, no restraint/saline, and no restraint/cocaine and $p < 0.05$ in females vs.

extinction), no restraint/saline, and restraint/saline, but not intra-PrL AM251. Moreover, intra-PrL AM251 significantly reduced cocaine seeking relative to vehicle-pretreatment in both males ($p < 0.001$) and females ($p < 0.05$). Accuracy of cannula placements was confirmed (males, Fig. S1A, C; females, Fig. S4B, D).

Experiment 5: CORT and CB1R-dependent effects in the PrL-PFC in female rats. As reported in males [13], CORT-potentiated cocaine seeking was localized to the PrL-PFC in females. Intra-PrL delivery of HBC-CORT (50 ng/0.3 μ L/side, 15 min pretreatment) did not increase cocaine seeking on its own, but potentiated cocaine seeking when combined with 1.25 mg/kg cocaine (Fig. 5a, b). A two-way repeated-measures reinstatement \times treatment condition ANOVA revealed a main effect of reinstatement ($F_{(1,54)} = 4.660$, $p < 0.05$), and a significant interaction ($F_{(3,54)} = 3.240$, $p < 0.05$). Post-hoc comparison showed that, in contrast to intra-PrL CORT alone or sham treatment combined with cocaine, the intra-PrL CORT/cocaine combination resulted in significant reinstatement ($p < 0.05$ vs. extinction, sham/saline, and PrL CORT/saline). Histology and cannula placements are depicted in Figs. S5a, c.

PrL-PFC CB1R involvement in CORT-potentiated reinstatement. As demonstrated in males [13], intra-PrL CB1R antagonism (AM251, 300 ng/0.3 μ L/side, 15 min pretreatment) prevented CORT-potentiated reinstatement in females (Fig. 5c, d). A two-way independent-measures reinstatement \times treatment condition

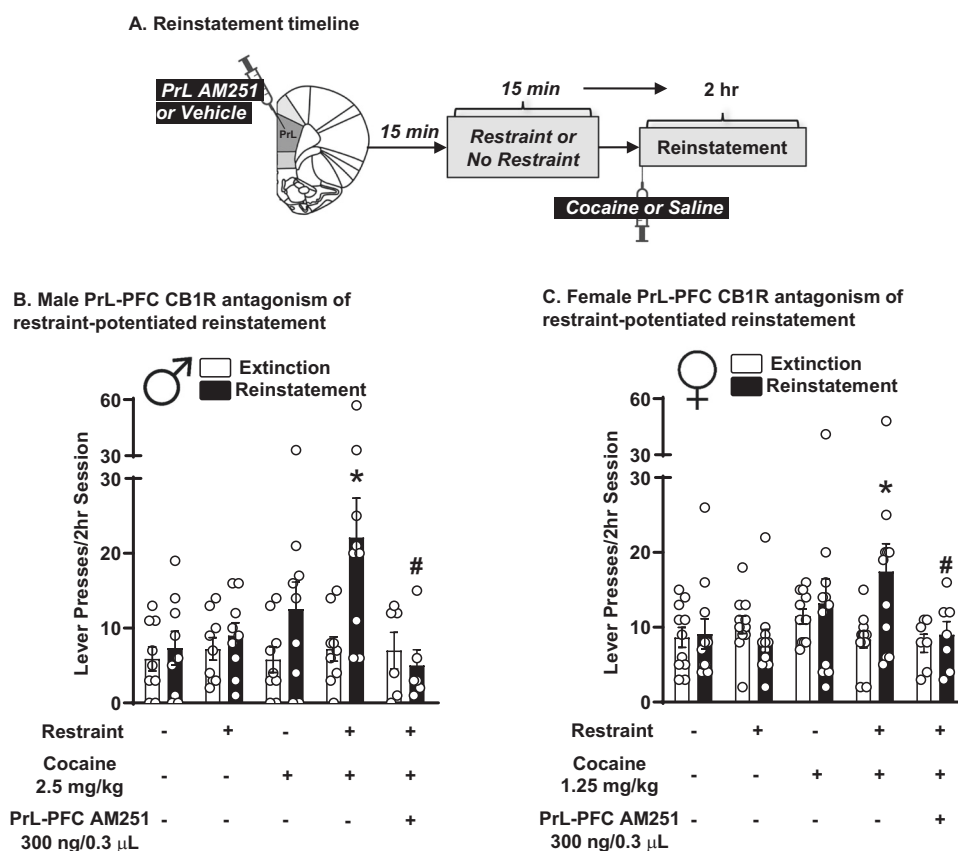
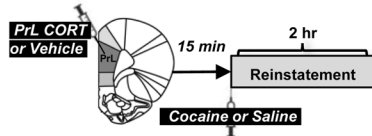


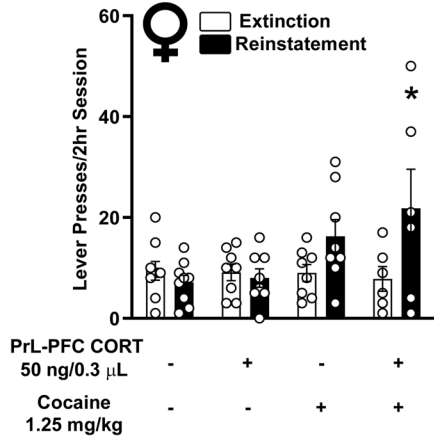
Fig. 4 Sex comparison of necessity for PrL-PFC CB1R activation in restraint-potentiated reinstatement. Within x-axis labels, “+” symbols indicate administration of the variable of interest (e.g., cocaine), whereas “-” symbols indicate administration of the associated control condition (e.g., saline). **a** Reinstatement timeline for examination of effects of PrL-PFC AM251 restraint-potentiated reinstatement. **b** Intra-PrL 300 ng/0.3 μ L AM251 effects on restraint-potentiated reinstatement to 2.5 mg/kg cocaine in males ($n = 7$; $*p < 0.05$ vs. extinction). Restraint-potentiated reinstatement was observed following intra-PrL vehicle injections ($*p < 0.05$ vs. extinction and each of the other reinstatement conditions) and was significantly attenuated by intra-PrL AM251 ($#p < 0.05$ vs. veh/restraint/cocaine). **c** Intra-PrL 300 ng/0.3 μ L AM251 also prevented restraint-potentiated reinstatement at the 1.25 mg/kg cocaine dose in females ($n = 7$; $*p < 0.05$ vs. extinction). Restraint-potentiated reinstatement was observed following intra-PrL vehicle injections ($*p < 0.05$ vs. extinction, no restraint/saline, and restraint/saline) and was significantly attenuated by intra-PrL AM251 ($#p < 0.05$ vs. veh/restraint/cocaine). Data are represented as mean \pm SEM.

Female PrL-PFC CORT is sufficient and CB1R activation is necessary for both potentiated reinstatement and suppression of pyramidal neuron inhibitory activity

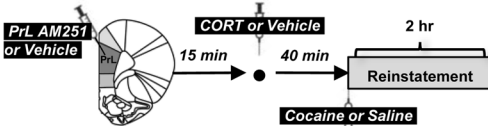
A. Reinstatement timeline



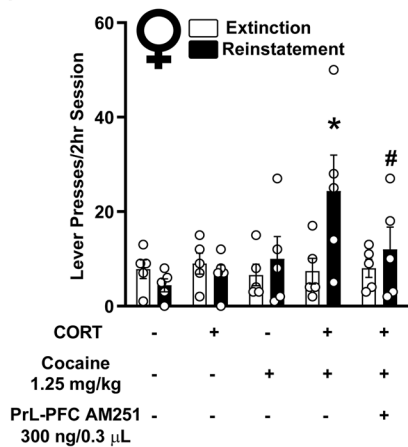
B. Female PrL-PFC CORT-potentiated reinstatement



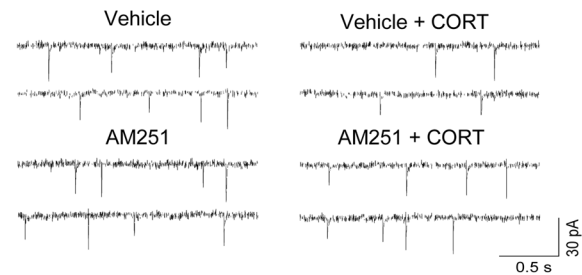
C. Reinstatement timeline



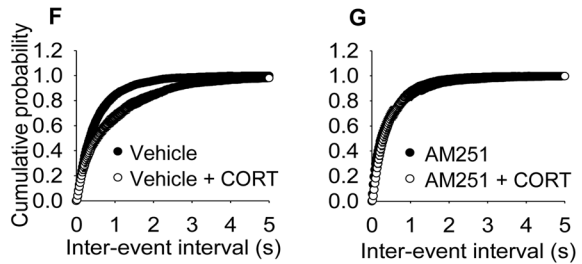
D. Female PrL-PFC CB1R antagonism of CORT-potentiated reinstatement



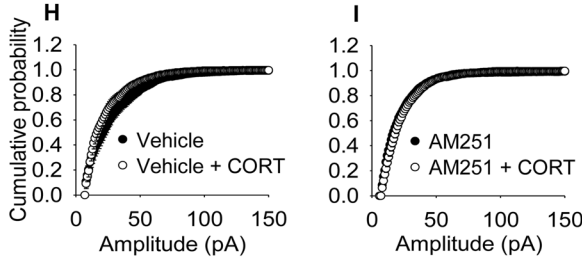
E. Representative Traces



F-G. PrL-PFC CB1R-dependent CORT effects on inter-event interval cumulative probability



H-I. PrL-PFC CB1R-dependent CORT effects on amplitude cumulative probability



J-K. PrL-PFC CB1R-dependent CORT effects on mIPSC frequency and amplitude

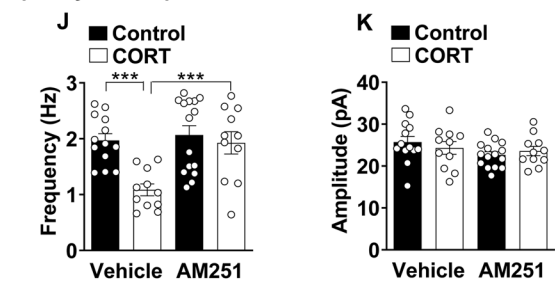


Fig. 5 PrL-PFC CORT is sufficient and CB1R activation is necessary for both potentiated reinstatement and suppression of pyramidal neuron inhibitory activity in females. Within x-axis labels, “+” symbols indicate administration of the variable of interest (e.g., cocaine), whereas “-” symbols indicate administration of the associated control condition (e.g., saline). **a** Reinstatement timeline for PrL-PFC CORT-potentiated reinstatement in females. **b** Female intra-PrL CORT (50 ng/0.3 µL) potentiates reinstatement to 1.25 mg/kg cocaine ($n = 13$; $*p < 0.05$ vs. extinction). **c** Reinstatement timeline for examination of effects of PrL-PFC AM251 on CORT-potentiated reinstatement in females. **d** Female intra-PrL AM251 (300 ng/0.3 µL) prevents ip CORT-potential of reinstatement to 1.25 mg/kg cocaine ($n = 7$; $*p < 0.05$ vs. extinction). CORT-potentiated reinstatement was observed following PrL sham injections ($*p < 0.05$ vs. extinction and each of the other reinstatement conditions) and was significantly attenuated by intra-PrL AM251 ($#p < 0.05$ vs. veh/CORT/cocaine). **e** Representative traces for recordings from female PrL-PFC layer V/VI pyramidal neurons following CORT and AM251. **f** PrL-PFC CORT suppression of inter-event interval cumulative probability. **g** PrL-PFC AM251 inhibition of CORT suppression of inter-event interval cumulative probability. **h** PrL-PFC CORT effects on amplitude cumulative probability. **i** PrL-PFC AM251 inhibition of CORT effects on amplitude cumulative probability. **j** PrL-PFC AM251 inhibition of CORT suppression of mIPSC frequency ($***p < 0.05$ vs. Cort or Vehicle). **k** PrL-PFC AM251 inhibition of CORT effects on mIPSC amplitude. Data are represented as mean \pm SEM.

ANOVA revealed a main effect of reinstatement ($F_{(1,20)} = 4.671$, $p < 0.05$) and a significant interaction ($F_{(4,20)} = 4.333$, $p < 0.05$). Post-hoc testing revealed that, while reinstatement was not observed following CORT/saline or vehicle/1.25 mg/kg cocaine, the combination of CORT and cocaine produced reinstatement following intra-PrL vehicle ($p < 0.01$ vs. PrL sham/vehicle/saline; sham/CORT/saline; sham/vehicle/1.25 mg/kg cocaine and extinction). Moreover, potentiated cocaine seeking was blocked by intra-PrL AM251 ($p < 0.05$ vs. intra-PrL sham/CORT/cocaine). Histology and cannula placements are depicted in Fig. S5b, d.

CB1R-dependent effects of CORT on inhibitory synaptic transmission in the PrL-PFC. As in males [13], CORT produced a CB1R-dependent reduction in inhibitory currents in PrL-PFC pyramidal neurons in females. Whole-cell recordings were taken from layer V pyramidal neurons, and miniature IPSCs (mIPSCs) were assessed upon bath perfusion of vehicle, CORT (1 μ M), AM251 (2 μ M), or CORT + AM251 for 20–60 min (see Fig. 5e for representative traces). CORT ($F_{(1,49)} = 10.685$, $p = 0.002$) and AM251 ($F_{(1,49)} = 8.965$, $p = 0.004$) had a significant effect on mean mIPSC frequency, and there was a significant CORT \times AM251 interaction ($F_{(1,49)} = 5.653$, $p = 0.022$). The Tukey's post-hoc and K-S tests indicated CORT increased inter-event intervals of mIPSCs ($p < 0.01$; Fig. 5f) and decreased mean mIPSC frequency ($p < 0.001$, $n = 11$ –13; Fig. 5j); both effects were blocked by AM251 (Fig. 5g, j; $p < 0.001$, $n = 11$ –15). CORT and AM251 had no significant effects on the cumulative probability plot for mIPSC amplitude distribution ($p > 0.05$; Fig. 5h, i) or mean mIPSC amplitude (Fig. 5k), indicating that CB1R-mediated CORT effects are specific to mIPSC frequency.

DISCUSSION

Heightened susceptibility to cocaine-primed reinstatement in females

Our data indicate that females are more sensitive than males to cocaine-primed reinstatement. Despite no differences in acquisition, maintenance, or extinction of cocaine seeking, females display a leftward shift in the dose–response curve for cocaine-primed reinstatement. The observed lack of sex differences in self-administration using this cocaine dose (0.2 mg/0.2 mL infusion) and in responding during extinction aligns with previous reports [34, 35] and eliminates potential confounds when comparing subsequent reinstatement responding. These findings align with prior reports of enhanced relapse vulnerability in females compared with males [36, 37].

Sex comparison of stress-potentiated reinstatement

To determine if there are sex differences in stress-potentiated cocaine seeking, we compared the effects of footshock on cocaine seeking between males and females. Surprisingly, in contrast to males, footshock failed to potentiate cocaine seeking in females across a wide range of shock intensities. To determine whether this sex difference either reflects a general lack of capacity for stressors to potentiate cocaine seeking in females, or if footshock specifically produces divergent effects, we tested both males and females for the ability of another inescapable stressor, immobilization restraint, to potentiate cocaine seeking. Restraint-potentiated cocaine seeking was observed in both sexes, and the magnitude of reinstatement was similar. Thus, some stressors can promote relapse in both males and females. However, a key difference is that, in females, stressor effects are superimposed on a higher underlying sensitivity to reinstatement, thereby resulting in an increased cumulative risk for drug seeking. Whether sex differences in vulnerability, or resiliency, are observed with other stressors warrants further investigation. However, these observations are generally consistent with clinical reports that cocaine cue-induced craving is increased in the context of stress in women [38, 39].

Sex-divergent responses to footshock

The lack of footshock-potentiated cocaine seeking in females was unexpected and could be attributed to several factors. First, females may be differentially sensitive to the effects of footshock on cocaine seeking (e.g., nociception). To explore this possibility, we tested females for reinstatement across a wide range of shock intensities (0.15–0.90 mA) but did not observe increased drug seeking at any amperage. In addition, all males and females exhibited flinching responses to footshock, indicating both sexes perceived shocks (data not shown). Moreover, as with restraint, the magnitude of the plasma CORT response relative to baseline was comparable between the sexes, although overall CORT levels were higher in females as previously reported [40, 41]. In addition, there were no differences in physical reactivity or defensive behaviors, including latency to freeze, percent freezing throughout the footshock session, and defensive darting, though there was a trend for more darting in females as previously reported [33]. Sex differences were observed in “affective state” during footshock as indicated by USV measurements. Males displayed a dramatic increase in the number of 22 kHz calls and a decrease in 55 kHz calls. Females displayed only a modest increase in 22 kHz calls, comparable to that seen during restraint in either sex, with no change in the number of 55 kHz calls. This suggests that, despite no observable differences in hormonal or defensive responses, there are stressor-specific sex differences in emotional processing that could influence motivational state and therefore drug seeking. Consistent with this interpretation, comparison of prior studies on social interaction [42], maternal separation [43], and predator odor [44] indicates different stimuli can produce sex-dependent differences in USV “signatures” with respect to both total call number and sonographic characteristics. Though studies assessing appetitive stimuli are lacking, no sex differences in 55 kHz USVs are observed when rats exhibit either an amphetamine- or morphine-associated conditioned place preference [45]. Whether stressor-induced USVs vary with estrous phase, or are affected by CORT or CB1R manipulations, will require further investigation.

In contrast to footshock, which only potentiated cocaine seeking in males, restraint-potentiated cocaine seeking in both sexes. This finding was surprising considering that footshock and restraint produced comparable increases in CORT levels, and that CORT-potentiated cocaine seeking, which was observed in both males and females, involves the same PrL-PFC mechanism (i.e., CB1R activation). We conclude that shock differentially recruits other signaling mechanisms and/or pathways in females that compete with or dampen those promoting drug seeking. Clues to which processes are sex-specifically recruited may be provided by differences in the features of restraint vs. footshock and the conditions under which they were tested (e.g., physical/nociceptive nature, ability to express active coping strategies, delivery within or outside of the operant conditioning chamber). Notably, differences between the two stressors can be observed at the level of PFC Fos expression. Females have been reported to show greater restraint-induced increases in PFC c-fos [46] and reduced footshock-induced PFC c-fos increases [47] compared with males.

CORT-potentiated reinstatement: sex comparison, contribution of estrous cycle, sex-dependent pharmacokinetics

We previously reported in males that elevated CORT is necessary for shock-potentiated reinstatement, and that CORT, at a dose that recapitulates stress levels, potentiates cocaine seeking [12]. Here, we report a similar effect in females. Potentiated reinstatement is observed following administration of the same CORT dose (2 mg/kg) at the same pretreatment time (40 min), albeit in combination with a lower subthreshold cocaine priming dose (1.25 mg/kg, vs. 2.5 mg/kg in males). Although robust CORT-potentiated reinstatement was observed in both sexes, reinstatement tended to be slightly lower in females.

Since ovarian hormones can influence drug seeking [48], we hypothesized that CORT effects may have covaried with estrous phase. In support of this possibility, CORT-potentiated cocaine seeking was only observed during diestrus and proestrus. This observation is consistent with a previous report that the ability of the pharmacological “stressor”, yohimbine, to potentiate reinstatement in response to cocaine-associated cues is most pronounced during diestrus and proestrus [7]. Future studies will determine if the ability of restraint and/or footshock to potentiate cocaine seeking shows similar variation across the estrous cycle.

Unexpectedly, in contrast to our own previous finding that reinstatement in response to 1.25 mg/kg cocaine alone is heightened during proestrus [26] and results from others who have reported increased cocaine-primed reinstatement during estrus (albeit at a higher priming dose [49–51]) we did not observe significant reinstatement in response to 1.25 mg/kg cocaine during any estrous phase or significant variation in reinstatement following this cocaine dose across the estrous cycle. Future experiments are needed to clarify how reinstatement in response to higher cocaine priming doses is influenced by the estrous cycle.

In the present study, CORT-potentiated reinstatement was only observed during diestrus, when both progesterone and 17 β -estradiol levels are relatively low and during proestrus when levels of 17 β -estradiol are relatively high. As cocaine-primed reinstatement is generally enhanced during either heightened 17 β -estradiol [7, 26, 49, 50] or lower progesterone phases [26, 50, 52, 53], we speculate that while heightened 17 β -estradiol mediates increased susceptibility to CORT-potentiated reinstatement during proestrus, low progesterone may contribute to heightened potentiated reinstatement during diestrus. This is consistent with the observation that endogenous progesterone levels negatively correlate with reactivity to yohimbine and cocaine cues in cocaine-dependent women [38].

To confirm that CORT administration recapitulated stressor-induced levels in both sexes, plasma levels following CORT injections were compared. Peak total CORT levels following administration were similar in males and females. Consistent with prior reports [40, 54], peak levels following either stressor were higher in females, an effect attributable to higher basal levels [41, 54]. Unexpectedly, CORT clearance was faster in females. In males, total CORT remained elevated 30 min after injection, while in females total CORT levels not only returned to baseline by 30 min but were reduced relative to vehicle controls (likely due to feedback inhibition of the HPA axis by administered CORT), a finding that aligns with earlier reports [55, 56]. Interestingly, despite sex differences in pharmacokinetics, the ability of CORT to potentiate cocaine seeking did not vary between sexes. This is surprising, as plasma CORT, at the time of testing 40 min post injection, was no longer elevated in females. One explanation is that blood CORT levels do not parallel brain levels, which peak ~20 min later [57] and may differ in a sex-dependent manner across brain regions [58]. In addition, once CORT is elevated, it may produce persistent signaling that continues to promote cocaine seeking even after CORT elevations dissipate. Notably, while the present findings demonstrate that “stress-level” CORT potentiates cocaine seeking in both males and females, sex differences in CORT sensitivity were not assessed.

A common PrL-PFC mechanism for CORT-potentiated cocaine seeking in males and females

We previously found that reinstatement-potentiating effects of CORT can be localized to the PrL-PFC in males [13]. Here we report that CORT also acts within the female PrL-PFC to potentiate reinstatement. Consistent with observations in males, effects of CORT within the PrL-PFC are CB1R-dependent in females [13], as intra-PrL delivery of AM251 blocks potentiation of reinstatement following systemic CORT administration. Although we cannot rule out a potential contribution of non-specific behavioral

suppression by intra-PrL AM251 to the observed effects on potentiated drug seeking, we previously showed that a systemic AM251 dose that prevents CORT-potentiated cocaine seeking has no effects on primed reinstatement in response to a higher cocaine dose [59, 60]. As in males [13, 19], CORT produces a CB1R-dependent suppression of inhibitory synaptic regulation of layer V PrL-PFC pyramidal neurons in females. These observations support a mechanistic model in which stressor-induced increases in CORT promote CB1R-dependent suppression of GABAergic constraint of PrL-PFC pyramidal neurons, thereby removing inhibition of output pathways that mediate drug seeking. Further, in contrast to the sexually dimorphic estrogenic endocannabinoid signaling regulation observed in hippocampus [61], our findings suggest that CORT regulation of endocannabinoid signaling in the PrL-PFC involves a similar mechanism in males and females. While not assessed here in females, we previously found that CORT produces an AM251-dependent increase in paired-pulse ratio in PrL-PFC layer V pyramidal neurons in males, supporting a mechanism that involves a CB1R-mediated inhibition of GABA release [13].

CONCLUSION

Our findings suggest that, by adding to an already heightened sensitivity to cocaine seeking, stress via CORT signaling can further promote cocaine seeking and thus create greater cumulative risk for relapse in females. Understanding the underlying mechanisms may reveal sex-specific relapse interventions and guide treatment.

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ADDITIONAL INFORMATION

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