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ARTICLE

# Elevated prefrontal dopamine interferes with the stress-buffering properties of behavioral control in female rats

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Stress-linked disorders are more prevalent in women than in men and differ in their clinical presentation. Thus, investigating sex differences in factors that promote susceptibility or resilience to stress outcomes, and the circuit elements that mediate their effects, is important. In male rats, instrumental control over stressors engages a corticostriatal system involving the prelimbic cortex (PL) and dorsomedial striatum (DMS) that prevent many of the sequelae of stress exposure. Interestingly, control does not buffer against stress outcomes in females, and here, we provide evidence that the instrumental controlling response in females is supported instead by the dorsolateral striatum (DLS). Additionally, we used in vivo microdialysis, fluorescent in situ hybridization, and receptor subtype pharmacology to examine the contribution of prefrontal dopamine (DA) to the differential impact of behavioral control. Although both sexes preferentially expressed D1 receptor mRNA in PL GABAergic neurons, there were robust sex differences in the dynamic properties of prefrontal DA during controllable stress. Behavioral control potently attenuated stress-induced DA efflux in males, but not females, who showed a sustained DA increase throughout the entire stress session. Importantly, PL D1 receptor blockade (SCH 23390) shifted the proportion of striatal activity from the DLS to the DMS in females and produced the protective effects of behavioral control. These findings suggest a sex-selective mechanism in which elevated DA in the PL biases instrumental responding towards prefrontal-independent striatal circuitry, thereby eliminating the protective impact of coping with stress.

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## INTRODUCTION

Exposure to adverse life events is strongly linked to negative mental health outcomes, with prevalence often significantly higher in women than men [1–3]. Substantial evidence points to coping factors such as perceived self-efficacy [4] or actual ability to exert control over an adverse event [5] as potent modulators of the immediate and long-term impact of adversity. Therefore, examining how coping processes differ between the sexes may lead to the identification of novel mechanisms underlying the sex bias in disease prevalence. One experimental approach for investigating neural mechanisms of coping is to compare animals (typically rats) that receive physically identical stressors (tailshock), with one group having instrumental control over an aspect of the adverse event (its termination, escapable stress, ES) and the other group having no control (inescapable stress, IS). Each IS subject is paired ("yoked") with one member of the ES group, thus ensuring that subjects with and without control receive equivalent stressor exposure (shock duration, intensity, onset/offset) while differing in only the degree of behavioral control.

A consistent finding is that the impact of behavioral control is sex-specific. In males, numerous neurochemical and behavioral consequences that typically follow IS (social avoidance, enhanced freezing, impaired shuttle box escape, etc.) do not develop following physically identical ES (for review see [6]). Furthermore, an initial experience with ES buffers males against the behavioral outcomes of future IS and other uncontrollable stressors, such as social defeat [7, 8]. In contrast, recent studies report that both the immediate and enduring forms of ES protection are completely absent in females [9, 10].

It is somewhat surprising that behaviorally controllable stressors in females produce sequelae that are comparable to those of uncontrollable stress, given that females rapidly acquire the instrumental controlling response at a rate similar to that of males [9, 10]. One possibility is that male and female coping behavior is supported by separate circuitry. Efforts within the appetitive domain have identified two distinct learning systems by which instrumental responses can be acquired [11–14]. One that is sensitive to variations in outcome (goal-directed) and another that is characterized as inflexible and regulated by antecedent stimuli through the formation of stimulus-response associations (habit). Evidence suggests that goal-directed action requires activation of a corticostriatal loop involving the prelimbic region (PL) of the medial prefrontal cortex (mPFC) and dorsomedial striatum (DMS), while habit performance is mediated by a prefrontal-independent circuit that includes the sensorimotor cortex and the dorsolateral striatum (DLS). Behavioral control and instrumental learning are identical concepts [15, 16], albeit studied under very different circumstances, and prior research has implicated both the PL and DMS as necessary for control to be protective in males [17–19].

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In the present study, we investigated the neural substrates of behavioral control in females. Here, we demonstrate that, unlike males, female ES performance is supported by DLS rather than DMS activity. Additionally, we assessed sex differences in the static (RNAscope in situ hybridization) and dynamic (in vivo microdialysis) properties of prefrontal dopamine given that prior reports implicate stress-induced catecholamine levels in the mPFC as critical for switching from flexible to habitual responding [20, 21]. Last, we show that stress-induced PL DA interferes with the protective effects of control in females. Collectively, our findings highlight a sex-specific mechanism that determines the impact of operational coping with stress.

## MATERIALS AND METHODS

### Animals

Adult female and male Sprague–Dawley rats (Envigo) were pair-housed on a 12:12 light-dark cycle (lights on at 0700 h) with standard laboratory chow and water provided ad libitum. Rats were allowed to acclimate to colony conditions for at least one week prior to experimentation. Stress treatment and behavioral testing were conducted between 0900 and 1400 h. All animal procedures were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

### Wheel-turn ES/yoked IS procedure

For manipulation of stressor controllability, subjects were run in a triadic design as previously described [18]. Briefly, one subject of each triad received ES, a second received yoked IS, and a third received no tailshock (home cage, HC). Each ES and IS rat were placed in a Plexiglas box ($14 \times 11 \times 17$ cm) with a wheel mounted in the front. The tail was secured to a Plexiglas rod extending from the back of the box and affixed with two copper electrodes and electrode paste. The single stress session consisted of 100 tailshock trials (1.0 mA, 60-s variable interval schedule). Initially, the shock was terminated by a quarter turn of the wheel. When trials were completed in less than 5 s, the response requirement was increased by one-quarter turn of the wheel, up to a maximum of four full turns of the wheel. The requirement was reduced if the trial was not completed in less than 5 s. If the trial was not completed in 30 s, the shock was automatically terminated, and the requirement was reset to a one-quarter turn of the wheel. For yoked IS rats, the onset and offset of each tailshock were identical to those of its ES partner. A computer equipped with Graphic State 4 (Coulbourn Instruments) controlled the experimental events and recorded the wheel turn requirement and escape latency for each trial.

### Juvenile social exploration (JSE)

Twenty-four hours before stress treatment, subjects were removed from the colony and transferred to a novel procedural testing room (150 lux at the position of the animal) where a baseline interaction measure was taken, as previously described [22]. Each experimental adult rat was allocated to a separate plastic cage with a wire lid and bedding. After 1 h, a juvenile female (28–35-day-old Sprague-Dawley) was introduced to the cage. Investigative behaviors, including sniffing, pinning, chasing, and allogrooming, initiated by the adult rat were timed by an observer blind to group membership. Following the 3-min baseline, the adult rat was returned to its home cage and returned to the colony. An identical social interaction test was repeated 24 h following stress treatment. The total interaction time and percentage change of the test from baseline were calculated.

### Lesion/sham surgery

Ten to fourteen days prior to behavioral experimentation, subjects were secured in a stereotaxic apparatus and anesthetized with isoflurane (5% induction, 2% maintenance in 2.5 L/min $O_2$; Piramal Critical Care). Subjects received a lesion or sham surgery of the bilateral DLS. Ibotenic acid (Millipore Sigma) was dissolved in 0.1 M sterile phosphate-buffered saline (PBS, $Ca^{2+}$, and $Mg^{2+}$ free, pH 7.2) for a final concentration of 10.6 µg/µL [23]. A beveled needle attached to a 10 µl syringe (31 gauge; Hamilton Company) and a UMP3 microinjection pump (World Precision Instruments) were used to bilaterally infuse 0.5 µl of the excitotoxin or sterile PBS (rate of 0.1 µL/min) into the DLS (A/P: +0.7; M/L: ±3.6; D/V: −5.0 mm from the pial surface). The injection needle was left in place for 10 min to allow for the solution to diffuse away from the needle. After surgery, VetBond (3M) was used to seal the cranial incision, and subjects were given postoperative subcutaneous injections of a nonsteroidal anti-inflammatory (meloxicam, 0.5 mg/kg; Vetmedica) and an antibiotic (CombiPen-48, 0.25 mL/kg; Bimeda).

### In vivo microdialysis

Under isoflurane anesthesia, a guide cannula was chronically implanted in one hemisphere of the PL (A/P: +2.6; M/L: 0.5; D/V: −1.8 mm from the pial surface) and fixed in place with stainless steel screws and acrylic cement. A screw cap of a 15-mL conical centrifuge tube (with the central portion removed) was affixed to the skull in an inverted orientation so that it encircled the guide cannula. This was done to protect the microdialysis guide cannula during subsequent exposure to tailshock. Seven to ten days later, a CMA 12 microdialysis probe (0.5 mm diameter, 2 mm length, 35 kD cut-off) was inserted through the cannula guide to the dorsal mPFC. A portion of a 15-mL conical tube was screwed onto the skull-mounted screw cap, through which the dialysis tubing, protected within a metal spring, was entered and attached to the probe. Each subject was first placed individually in a Plexiglas bowl and infused with artificial cerebrospinal fluid (pH 7.2; 145.0 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl, 1.0 mM $MgCl_2$) at a rate of 0.8 µL $min^{-1}$. After a 90-min stabilization period, four baseline samples were collected. Next, rats were placed in wheel-turn boxes for tailshock, and following the session, they were transferred back to the Plexiglas bowl, where three additional samples were collected. During the baseline, stress, and post-stress phases, dialysates were collected every 20 min and stored at −80 °C until subsequent processing. Microdialysis data are expressed as a percentage of baseline, defined as the mean of four consecutive samples collected prior to the stress phase.

### High-performance liquid chromatography (HPLC)

Catecholamine (NE and DA) concentrations were measured in 15 µL dialysate samples by HPLC coupled with an electrochemical detector. The system consisted of an online Shimadzu DGU-2045 degasser, an ESA 584 pump, a Dionex UltiMate 3000 RS electrochemical detector and autosampler, and an ESA 5020 guard cell. The column was an ESA HR-80 × 3.2 maintained at 38 °C, and the mobile phase was the ESA buffer MD-TM. The analytical cell potentials were kept at +220 mV, and the guard cell was kept at +250 mV. The system was calibrated using external standards (Sigma) dissolved in artificial cerebrospinal fluid.

### Immunohistochemistry (IHC)

Two hours after the final tailshock, female subjects were transcardially perfused with ice-cold physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed, post-fixed overnight in 4% paraformaldehyde, and then cryoprotected with 30% sucrose in 0.1 M PB. Dorsal striatal sections (30 µm) were collected in a −20 °C cryostat and stored in cryoprotectant solution at 4 °C until further processing. Standard IHC procedures were used to stain striatal sections for Fos as described previously [17]. Sections were incubated for 24 h at room temperature (RT) with anti-Fos primary antibody (1:7,500, rabbit polyclonal; Santa Cruz Biotechnology). Following primary antibody incubation, sections were then exposed to goat anti-rabbit biotinylated secondary antibody (1:200; Jackson ImmunoResearch) for 2 h at RT. This was followed by an avidin-biotin-horseradish peroxidase (ABC) enzymatic step for 1 h at RT and then incubation in a solution containing 3,3′-diaminobenzidine (DAB), cobalt chloride, nickel ammonium sulfate, ammonium chloride, and glucose oxidase in 0.1 M PB. The peroxidase reaction was initiated by the addition of a glucose solution that reacted with the tissue for ~8–10 min. Striatal tissue was mounted onto glass slides and coverslipped with Permount mounting medium (Fisher Scientific).

### Fluorescent in situ hybridization (FISH) using RNAscope

To fluorescently label DMS-projecting PL neurons, female and male subjects were first anesthetized, and bilateral craniotomies were created above the dorsal striatum. AAV2retro-eSyn-eGFP-T2A-iCre ($1.2 \times 10\text{^}13$ vg/mL; Vector BioLabs) was infused into the bilateral DMS (A/P: −0.2; M/L: ±2.1; D/V: −3.0 mm from pial surface). The total injection volume (1.0 µL) and flow rate (0.1 µL/min) were controlled with a microinjection pump, and the 31-gauge needle was left in place for an additional 10 min to allow diffusion. After waiting 1 month for viral expression, brains were rapidly extracted and flash frozen on dry ice. Serial sections (12 µm) containing the PL were collected directly onto Superfrost Plus slides (Fisher Scientific) using a cryostat maintained at −24 °C. An RNAscope Multiplex Fluorescent

Reagent v2 Kit was used according to the protocol provided by Advanced Cell Diagnostics for *eGFP* (Cat# 538851), *SLC32a1* (VGaT, Cat# 424541), *Drd2* (Cat# 315641-C3), and *Drd1* (Cat# 317031-C2) mRNA. Opal dyes 520, 570, and 650 nm (FP1487001KT, FP1488001KT, FP1496001KT; Akoya Biosciences) were matched to each probe. Slides were treated with DAPI (Advanced Cell Diagnostics) and coverslipped with antifade mountant (ProLong Diamond; ThermoFischer Scientific).

### Drug microinfusion

A dual guide cannula (26 gauge, 1.0 mm center-to-center distance; P1 Technologies) was targeted to the PL (A/P: +2.6; M/L: ± 0.5; D/V: −1.8 mm from the pial surface) and secured to the skull with stainless steel screws and acrylic cement. Internal guide cannulae were inserted to keep the cannula patent and held in place with a fitted dust cap (P1 Technologies). Ten to fourteen days later, subjects received stress treatment. One hour prior, microinfusions of SCH 23390 (D1 antagonist, Sigma) or eticlopride (D2 antagonist, Sigma) were made in bilateral PL (0.5 µL/hemisphere, 4.0 µg/µL in sterile saline). Doses were chosen based on previous reports in Sprague-Dawley PL [24, 25]. HC animals received drug or sterile saline at the same time. Dual 33-gauge microinjectors (P1 Technologies) attached to PE 50 tubing were inserted through the cannula guides, from which they protruded 1.0 mm. The other end of the tubing was connected to a 25-µl Hamilton syringe that was attached to a microinjection unit (Kopf Model 5000). Volumes were injected over a period of 30 s, and the injector was left in place for 90 s to allow diffusion.

### Microscopy and image quantification

*Histology*. At the end of the lesion and cannulation experiments, subjects were perfused transcardially, and brains were cryoprotected and sectioned as described above. Sections (35 µm) were then stained with cresyl violet and examined under a light microscope to verify cannula placement and the location and extent of cell loss produced by the excitotoxic lesion. Behavioral data from subjects with incorrect placement were not included in the statistical analysis.

*IHC*. Images were acquired using an Olympus BX61 microscope with an attached DP73 color digital camera and CellSens Dimension software (Olympus). Adjacent coronal tissue sections approximately corresponding to Bregma +0.36 to −0.36 mm were assessed for the number of Fos-immunoreactive cells in the DMS and DLS. Fos-stained nuclei were identified by dark black ovoid particles indicative of the DAB reaction product. The mean per-field count of Fos-positive neurons was determined for the DMS and DLS (three coronal sections for each subject) and subsequently converted into the number of counts per unit area ($mm^2$) for each region of interest.

*FISH*. Imaging of triple fluorescent in situ hybridization tissue was acquired with a NikonA1 laser scanning confocal microscope and NIS-Elements software. The entire PL region (three coronal sections for each subject) was imaged using a 20×/0.75NA objective with 405, 488, 561, and 647 nm laser lines. Capture settings and z-stack image depth were kept constant across subjects. ImageJ/Fiji software along with the Cell Counter plugin was used to quantify the total number of cells expressing each label and, if any, their co-labeling. For each image, clusters of pixels with intensities above the mean background value were designated as signal.

### Statistical analysis

Data analysis was performed using Prism software (GraphPad, RRID:SCR_002798). The effect of treatments was analyzed with two-tailed independent *t* tests (IHC, FISH), one-way (wheel-turn efficacy), two-way (JSE), repeated measures (in vivo microdialysis) or mixed-design (IHC) ANOVA. Main effects and interactions were considered significant if $p < 0.05$. When appropriate, post-hoc analyses were performed with Tukey's multiple comparison test. In all cases, data are expressed as the mean ± SEM. Figures 1A, 2A, 5A, H were created with BioRender.com.

## RESULTS

### Behavioral control in females recruits the dorsolateral striatum

In male rats, instrumental control over stress (escape learning) induces robust Fos expression within the DMS, but not DLS, and results in protection against stressor outcomes [17]. To explore whether a similar pattern occurs in females, striatal tissue was collected from female rats given a single session of ES or yoked-IS in wheel-turn boxes or HC treatment (Fig. 1A). Fos immunolabeling was quantified in coronal sections with regions of interest corresponding to posterior DLS and DMS. Immunoreactivity was not detected in HC subjects ($n = 8$); therefore, HC data were not included in the statistical analysis, as they are at zero with zero standard error (Fig. 1C). A mixed-design ANOVA revealed main effects of stress ($F_{1,13} = 19.97$, $p < 0.001$), brain region ($F_{1,13} = 40.35$, $p < 0.001$), and a significant stress × brain region interaction ($F_{1,13} = 8.995$, $p = 0.010$) for striatal Fos. At the level of the DMS, both female ES ($n = 8$) and IS ($n = 7$) led to a similar level of Fos expression ($p = 0.291$). In contrast, ES robustly increased Fos immunoreactivity in the DLS ($p < 0.001$) compared to IS, indicating that behavioral control in females induces an opposite pattern of dorsal striatal activity compared to that in males (Fig. 1C).

Next, we addressed the role of the DLS in the lack of protection afforded by control in females by producing intra-DLS excitotoxic lesions, thereby eliminating the possibility of using the DLS to learn the wheel-turn escape response. Lesions were made 10–14 days prior to stress treatment. In all cases, the lesions were bilateral and localized predominantly to the DLS (Fig. 2A). A photomicrograph of a lesion to the DLS is contrasted with that of a sham lesion in Fig. 2B. To quantify wheel-turn escape performance, the number of trials to reach the maximum wheel-turn requirement and the latency to terminate each tailshock were recorded (Fig. 2C, D). Importantly, female ES subjects with DLS lesions performed the instrumental escape response as efficiently as sham ES subjects (trials to achieve max requirement: $t_{17} = 1.638$, $p = 0.120$; escape latency: $t_{17} = 1.829$, $p = 0.085$). Similar to prior findings in females [9], ES and IS sham-operated groups both exhibited reduced social exploration 24 h following stress. However, bilateral lesions to the DLS prevented stress-induced social avoidance in ES, whereas it had no effect in IS (stress: $F_{2,60} = 12.50$, $p < 0.001$; lesion: $F_{1,60} = 7.826$, $p = 0.007$; stress × lesion interaction: $F_{2,60} = 3.727$, $p = 0.030$; Fig. 2E).

### Behavioral control in females, but not males, leads to a sustained prefrontal dopamine response

Adverse events impair a number of processes central to goal-directed action in part by increasing catecholamine levels in the mPFC [20]. To examine whether the impact of mPFC DA and NE efflux differs in male and female ES subjects, a microdialysis probe was targeted to the PL, with the dialysis membrane extending into the ventrally adjacent infralimbic cortex (IL) as well (Fig. 3A). In both males and females, microdialysis samples obtained during 20-min collections showed a similar pattern for NE efflux (Fig. 3B). ES produced a large increase in extracellular NE that remained potentiated throughout the entire stress session (S1–S5; stress: $F_{1,20} = 43.193$, $p < 0.001$; sex: $F_{1,20} = 0.539$, $p = 0.471$; stress × sex interaction: $F_{1,20} = 0.009$, $p = 0.924$; $n = 5$–7/group). Male and female ES groups both differed from their respective HC groups in NE levels, but did not differ from one another at any time point during stress.

In contrast, male ES led to only an initial transient increase in DA, with DA decreasing to HC levels by the second sample collection (40 min following stress onset; Fig. 3D). The rapid return to baseline in DA in ES males is notable because tailshock trials continued for an additional 60 min. ES females showed a different pattern. DA efflux was potentiated throughout the entire stress session (S1–S5; stress: $F_{1,20} = 63.67$, $p < 0.001$; sex: $F_{1,20} = 14.83$, $p = 0.001$; stress × sex × time interaction: $F_{4,80} = 3.070$, $p = 0.021$) and subsequently remained elevated during the post-stress period (P1–P3; stress: $F_{1,20} = 23.35$, $p < 0.001$; sex: $F_{1,20} = 18.65$, $p < 0.001$; stress × sex interaction: $F_{1,20} = 21.02$, $p < 0.001$). Remarkably, post-hoc analyses revealed that mPFC DA efflux differed between male and female ES groups in almost all measurements taken following

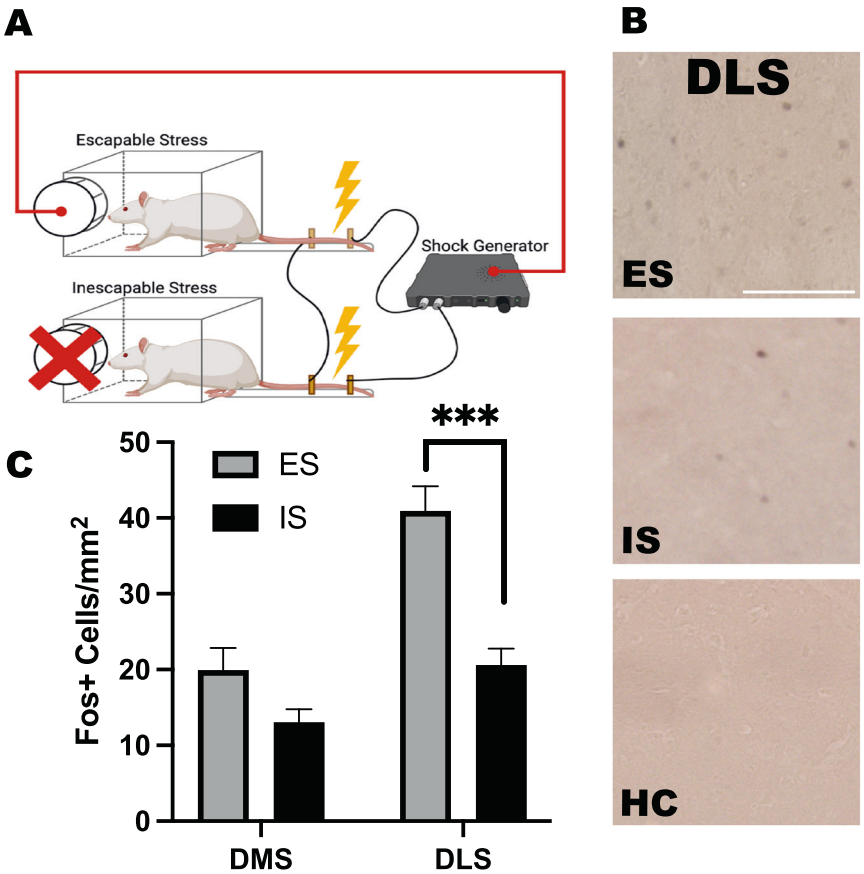


**Fig. 1 Instrumental control over stress recruits the dorsolateral striatum in females. A** Schematic diagram of the stressor controllability paradigm. Female rats are assigned to either escapable stress (ES; above), inescapable stress (IS; below) or no stress (home cage, HC; not shown). ES subjects receive a series of tailshocks (100) and can perform an instrumental wheel-turn response to terminate each shock. IS subjects are "yoked" to the ES subject such that shock is terminated for IS when the wheel-turn response criterion is achieved by the ES subject. Each subject in the pair receives physically equivalent tailshock. **B** Representative micrograph of Fos immunostaining of female dorsolateral striatum (DLS). Scale bar = 500 µm. **C** Quantification of Fos-immunoreactive cells per square millimeter in female dorsomedial striatum (DMS) and DLS. Bar graphs represent the mean ± SEM. ***$p < 0.001$, Tukey's.

shock onset (S2–P3). IS produced robust increases in mPFC NE (S1-S5; stress: $F_{1,20} = 61.56$, $p < 0.001$; sex: $F_{1,20} = 2.862$, $p = 0.106$; stress × sex interaction: $F_{1,20} = 0.660$, $p = 0.426$; $n = 5$–7/group) and DA (S1–S5; stress: $F_{1,20} = 68.80$, $p < 0.001$; sex: $F_{1,20} = 0.128$, $p = 0.724$; stress × sex interaction: $F_{1,20} = 0.0003$, $p = 0.986$) that persisted throughout the stress session but was independent of sex (Fig. 3C, E). Lastly, comparison of the mean area under the curve for stress-evoked catecholamine levels revealed that only DA, but not NE, differed between female and male ES groups (NE: $t_{12} = 0.129$, $p = 0.899$; DA: $t_{12} = 5.217$, $p < 0.001$; Fig. 3F, G).

## Sex differences in dopamine receptor expression in select PL cell populations

Stress-induced changes in prefrontal extracellular DA may impact corticostriatal function by decreasing the output of DMS-projecting pyramidal neurons by direct DA innervation of these neurons or indirectly by driving feed-forward GABAergic inhibition [26–28]. Because sex differences in DA dynamics rapidly developed during the early phase of ES treatment (Fig. 3D, time point S2), we investigated whether males and females also differ in their constitutive expression of DA receptors on DMS-projecting PL neurons and PL GABAergic interneurons. PL neurons that project to the DMS were identified following bilateral delivery of a retrograde AAV virus expressing eGFP into the DMS [29]. Multiplex-fluorescence in situ hybridization was performed on PL sections for Drd1 and Drd2, along with VGaT (Fig. 4A–C) or viral-encoded eGFP (Fig. 4D–F). The proportion of VGaT-positive cells co-expressing D1 mRNA was greater than that expressing D2 mRNA, independent of sex (male: $t_6 = 3.489$, $p = 0.013$; female: $t_6 = 5.078$, $p = 0.002$; $n = 4$/group, Fig. 4A–C). EGFP mRNA expression, indicating PL neurons that project to the DMS, was largely restricted to layer 5, consistent with the notion that corticostriatal neurons are part of the intratelencephalic tract [30]. We found a sex difference, with females having a higher number of PL-to-DMS cells expressing D2 than D1 receptor mRNA ($t_6 = 5.518$, $p = 0.002$), while males showed equivalent levels of each receptor type on this pathway ($t_6 = 0.225$, $p = 0.829$, Fig. 4D–F).

## Blockade of PL D1 receptors in females establishes the stress-buffering properties of behavioral control and shifts striatal activation to the DMS

Next, we assessed the contribution of DA signaling through PL D1 and D2 receptors to the lack of protection afforded by control in females. ES, IS, and HC females received intra-PL microinfusions of either SCH 23390 (4.0 µg/µL; D1 antagonist), eticlopride (4.0 µg/µL; D2 antagonist), or saline vehicle 1 h prior to stress treatment [24, 25] (Fig. 5A). Blockade of D2 receptors with eticlopride in the PL interfered with the acquisition of the wheel-turn controlling response. A one-way ANOVA revealed main effects in the number of trials required to reach the maximum wheel-turn requirement ($F_{2,31} = 4.791$, $p = 0.015$, $n = 10$–13, Fig. 5C) and the latency to terminate shock ($F_{2,31} = 8.550$, $p = 0.001$, Fig. 5D). Both escape measures were significantly increased in eticlopride-treated

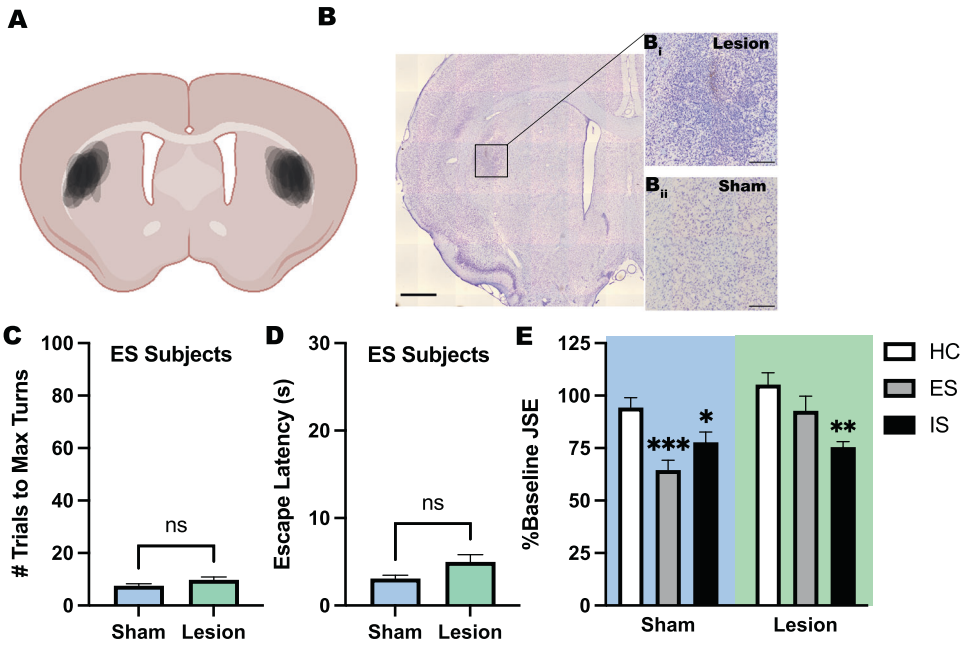


**Fig. 2 Targeted lesion to the dorsolateral striatum leads to behavioral control-induced protection in females. A** Extent and location of excitotoxic lesions. **B** Cresyl violet preparation of a coronal section from representative dorsolateral (DLS) lesion (**$B_i$**) and sham-operated (**$B_{ii}$**) subjects. Damage from the excitotoxin, indicated by the presence of gliosis, was centered in DLS. Scale bars = 1 mm and 100 μm (higher magnification). Efficiency of wheel-turn behavior as determined by **C** the mean number of trials needed to achieve the maximum response requirement and **D** the mean escape latency (seconds) across all 100 trials. **E** Twenty-four hours following escapable stress (ES), yoked-inescapable stress (IS) or no stress (home cage, HC), all female subjects were exposed to a 3-min juvenile social exploration (JSE; $n = 8$–15/group). Data are expressed as the percentage of baseline exploration. Bar graphs represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus HC, Tukey's.

subjects compared to vehicle-treated subjects ($ps = 0.014$ and 0.002, respectively); therefore, we did not assess its role in behavioral outcome. In contrast, efficiency in wheel-turn escape behavior was unaffected in SCH 23390 subjects. A social interaction test was performed 24 h after stress treatment. As is typical in females, vehicle-treated ES and IS groups showed reduced juvenile investigation. Importantly, SCH 23390 led ES to be protective with regard to social avoidance as it is in males. A two-way ANOVA identified main effects of stress ($F_{2,56} = 19.32$, $p < 0.001$), drug ($F_{1,56} = 11.63$, $p = 0.001$), and a significant stress × drug interaction ($F_{2,56} = 8.416$, $p < 0.001$; $n = 9$–11/group, Fig. 5E).

In addition to behavioral outcome, we also determined whether blockade of PL D1 would change the striatal Fos pattern in female ES. An additional cohort of female ES were given intra-PL SCH 23390 or saline vehicle prior to ES and then euthanized 2 h following the final tailshock. As expected, vehicle-treated ES subjects showed an increased number of Fos-positive cells in the DLS relative to DMS. However, blockade of D1 receptors in the PL led to the opposite pattern of Fos expression. Enhanced Fos expression now shifted to the DMS relative to DLS ($t_{11} = 2.28$, $p = 0.043$, $n = 6$–7/group, Fig. 5F, G) in SCH 23390-treated subjects.

## DISCUSSION

Previous research has shown that the presence of behavioral control blunts the neurochemical and behavioral impact of adverse events in male, but not female, rats. Evidence points to a corticostriatal circuit involving both the PL and the posterior DMS as critical structures for the detection of control in males [17, 31]. The fact that female ES subjects learn the controlling response with an efficiency comparable to males, yet are not protected, led us to provisionally hypothesize that instrumental control is supported by a separate neural process. The present study provides several lines of evidence that support this notion. First, the DLS rather than the DMS was selectively activated during the ES experience in females. This is the exact opposite pattern previously observed with ES in males. That is, behavioral control in males induces neural activity that leads to greater Fos expression in the DMS relative to the DLS, and this activity is necessary for the protective effects of control [17]. The foregoing suggests that females would be protected by control if the instrumental controlling response was supported by the DMS (goal-directed) rather than the DLS (habit) system. Consistent with this hypothesis, we demonstrate that bilateral lesions to the DLS now led female ES to protect against stress-induced social avoidance. The same manipulation was without effect in female IS subjects.

The implication is that the exercise of behavioral control at a procedural level is not the critical factor in determining its impact; rather, this depends on the circuitry that is recruited during the acquisition of the controlling response. Our data are consistent with the rich literature on instrumental appetitive learning that shows that the performance of an action can be controlled by distinct associative processes involving distinct neural mechanisms in rodents and humans [16, 32–34]. One process, termed goal-directed, is demonstrably sensitive to the contingency between the instrumental response (R) and its consequence or outcome (O), an R-O association. Learning with the goal-directed system engages a circuit involving the PL, its projection to the DMS, and its reciprocal connectivity with the mediodorsal thalamus. Habit-based performance, in contrast, operates independently of R-O contingencies and instead is governed through established stimulus-response (S-R) associations. Stimulus-driven habits involve a circuitry that includes the sensorimotor cortex and the DLS [35].

The above is highlighted because the concepts of behavioral control and instrumental contingency learning are quite similar [15, 16]. Both are formally defined as a comparison between two conditional probabilities: the probability of reinforcement (e.g., obtaining a reward, escaping shock) given that a specific

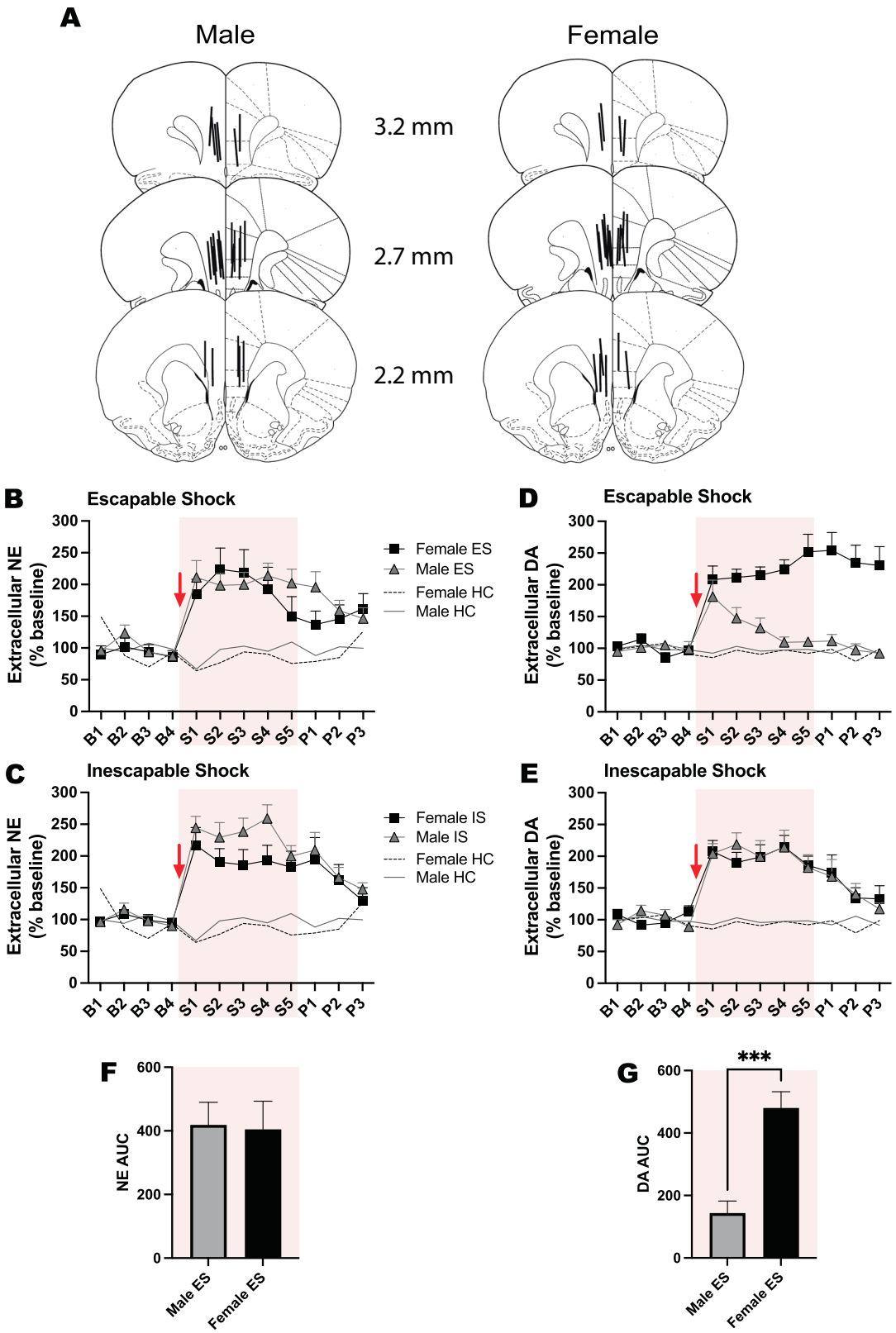


**Fig. 3 Sex differences in prefrontal catecholamine response to behavioral control. A** Male and female microdialysis probe membrane placement in the prefrontal cortex. Numbers indicate distance (mm) anterior to bregma. In vivo microdialysis measurement of prefrontal catecholamines before, during, and following a single session of escapable (ES), inescapable (IS), or no stress (HC). Samples were collected every 20 min during the baseline (B1–B4), stress (S1–S5), and post-stress (P1–P3) phases. Extracellular norepinephrine (NE) in ES (**B**) and IS (**C**) males and females. Extracellular dopamine (DA) in ES (**D**) and IS (**E**) males and females. The red arrow indicates initiation of differential treatment, and the pink shading represents the duration of stress exposure. All values are expressed as mean percent change from baseline ± SEM. **F**, **G** Histograms depicting the mean area under the curve (AUC, ± SEM) for extracellular NE and DA during male and female ES (S1–S5). ***$p < 0.001$, independent *t*-test.

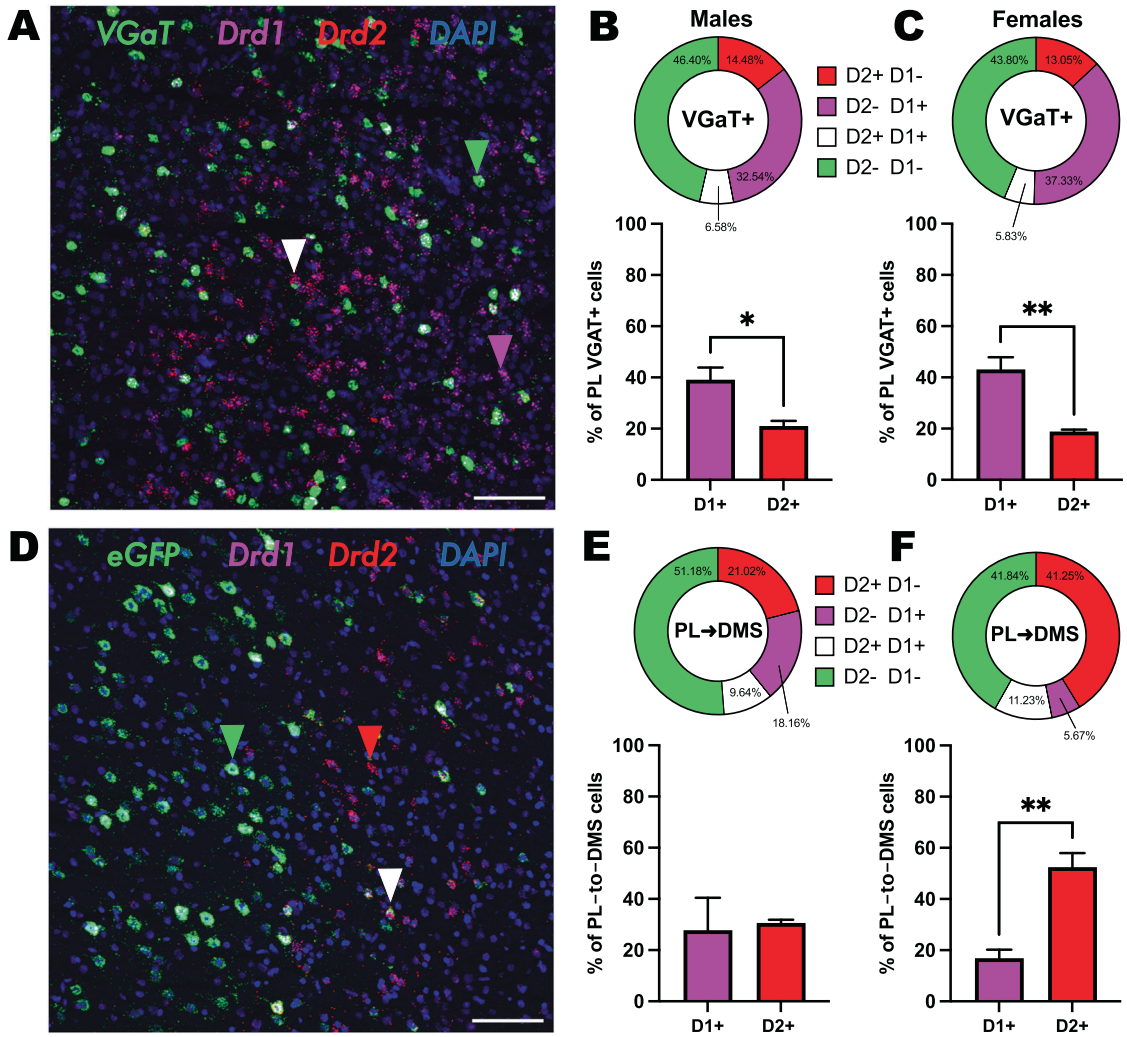


**Fig. 4 Analysis of sex differences in constitutive dopamine receptor expression in select prelimbic subpopulations. A** Fluorescent in situ hybridization labeling of *Drd1* (magenta) and *Drd2* (red) in prelimbic (PL) GABA interneurons. Quantification of dopamine receptor co-expression in PL *VGaT*-positive neurons in male (**B**) and female (**C**) rats. *Top*, sectors indicate the proportion of *VGaT*-positive neurons co-expressing *Drd1* only (magenta), *Drd2* only (red), both *Drd1* and *Drd2* (white), or neither label (green). *Bottom*, histogram depicting percentage of *VGaT* cells expressing *Drd1* or *Drd2*. **D** Fluorescent in situ hybridization labeling of *Drd1* (magenta) and *Drd2* (red) in PL neurons that project to the dorsomedial striatum (DMS). Quantification of dopamine receptor co-expression in the PL-to-DMS pathway in male (**E**) and female (**F**) rats. *Top*, sectors indicate the proportion of DMS-projecting PL neurons co-expressing *Drd1* only (magenta), *Drd2* only (red), both *Drd1* and *Drd2* (white), or neither label (green). *Bottom*, histogram depicting percentage of PL-to-DMS cells expressing *Drd1* or *Drd2*. Scale bars = 100 µm. All values represent the mean ± SEM. $*p < 0.05$, $**p < 0.01$, independent *t*-test.

instrumental response is performed (e.g., lever press, wheel turn) and the probability of reinforcement in the absence of the instrumental response. When the two conditional probabilities are unequal, some degree of instrumental control is present, whereas in circumstances when the two probabilities are equal, control is absent. It is the goal-directed, not the habit, system that is sensitive to contingency, and the present data suggest that female ES performance, unlike males, engages structures that support habit learning. Our data do not address whether females use the DLS habit system from the very beginning of the stress session or alternatively initially recruit the DMS during acquisition of the controlling response and then shift to the DLS for its maintenance. Recent work from Schoenberg et al. [36, 37] demonstrates that as instrumental appetitive learning progresses, the emergence of habitual behavior occurs at a faster rate in female rats compared to males. Whether this sex-dependent facilitation is present or even more pronounced within the aversive/stress domain [38] is unknown, but future work should establish which striatal system is engaged by females during the early acquisition of control.

An additional line of evidence that supports the notion that behavioral control is mediated by a neural process that differs between females and males concerns the mPFC. MPFC input to the striatum is critical for goal-directed processes [39–41], and here we investigated its potential contribution in biasing females to use the DLS during the wheel turn escape task. A number of mPFC-dependent cognitive functions are facilitated by catecholamines, however excessive levels, such as those driven by stress or psychostimulants, can lead to their impairment [42–45] while simultaneously strengthening habit formation and affective responding [46, 47]. The molecular and cellular mechanisms that coordinate the switch from flexible to reflexive responding are generally not well understood, but prefrontal D1 and α−1-adrenoreceptors have been implicated [21], with recent emphasis specifically on D1 [48]. Furthermore, stress-induced catecholamine levels are often elevated in

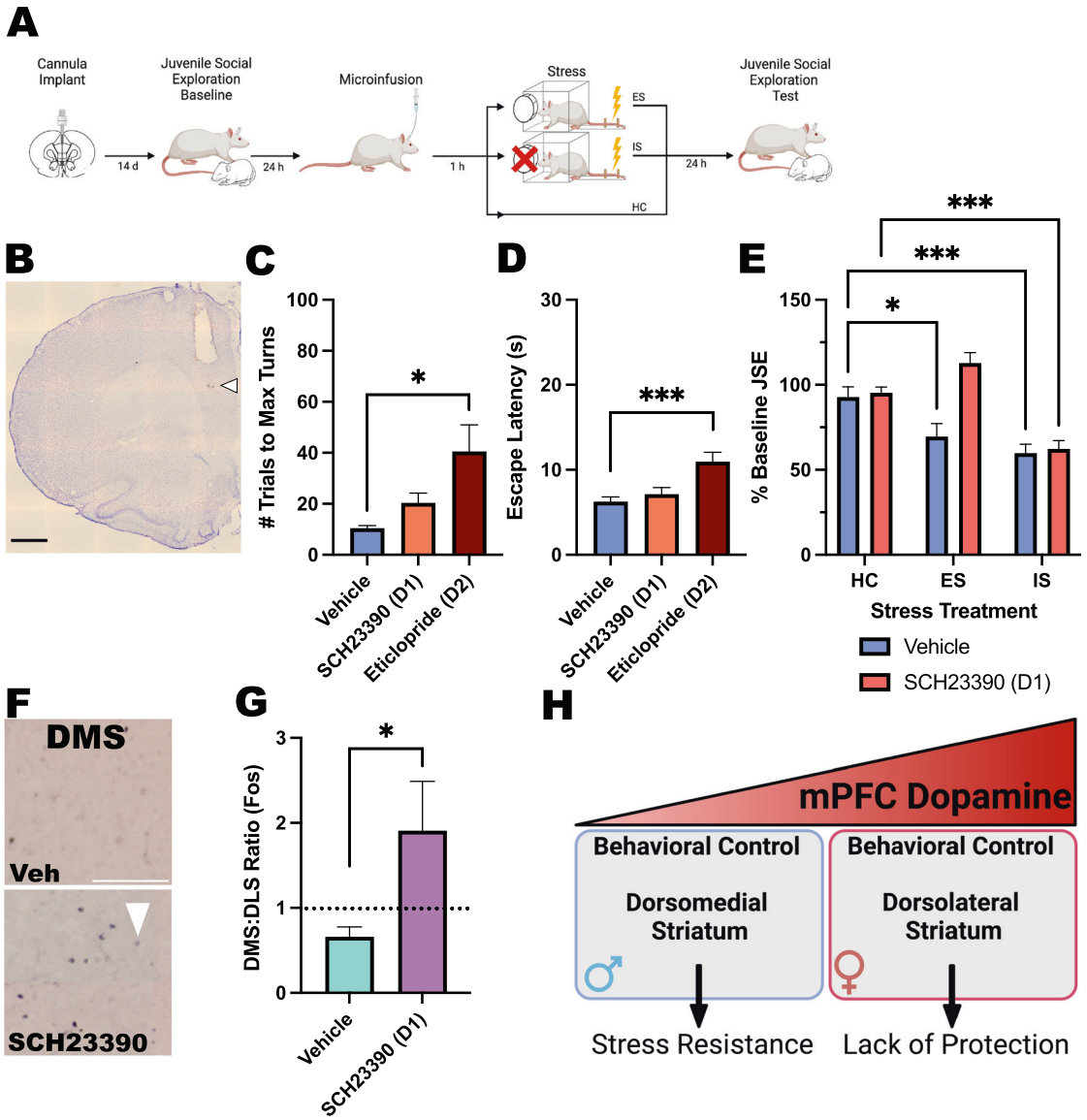


**Fig. 5 Effect of prelimbic dopamine receptor subtype blockade on stressor controllability outcome in females. A** Schematic illustration of the experimental timeline. **B** Photomicrograph of cresyl violet-stained tissue section depicting placement of cannula in prelimbic cortex. White arrow indicates the location of the injection site. Scale bar = 1 mm. Comparison of wheel-turn acquisition in female escapable stress (ES) subjects that received antagonists SCH 233390 (D1), eticlopride (D2), or vehicle 1 h prior to stress treatment. Mean number of trials to attain the maximum escape requirement (**C**) and mean escape latency (**D**). **E** A 3-min juvenile social exploration (JSE) test was given 24 h after stress treatment. Data are expressed as the percentage of baseline exploration. $*p < 0.05$, $***p < 0.001$ versus HC, Tukey's. **F** Representative micrograph of Fos immunostaining of dorsomedial striatum (DMS) of females that received SCH 233390 or vehicle 1 h prior to stress treatment. Scale bar = 500 µm. **G** Ratio of the number of DMS to dorsolateral (DLS) Fos-positive cells per $mm^2$ following wheel-turn escape performance. Values represent the mean ± SEM. $*p < 0.05$, independent *t*-test. **H** Proposed model of the relationship between medial prefrontal cortex (mPFC) dopamine levels and striatal system recruitment by behavioral control.

females relative to males across a variety of experimental contexts [49, 50]. Thus, we examined whether either NE or DA in the mPFC would be elevated during ES in females relative to males. NE efflux increased during ES in both males and females and remained elevated throughout the stressor session. This is consistent with data showing that locus coeruleus activity does not differ between ES and IS in males [51]. Moreover, the increases were of comparable magnitude. The pattern with regard to DA was quite different. In males, mPFC DA initially rose rapidly during ES but quickly returned to basal levels even as the tailshocks continued. In contrast, DA during ES in females remained elevated for the entire session as well as thereafter. Thus, the level of DA over time (area under the curve) during ES was dramatically greater in females than in males.

At present, it is unclear what active process drives the persistent stress-evoked DA levels in females. Sex differences in the regulation of synaptic catecholamine levels have been widely reported, such as those that involve dopamine transporter expression levels, rate of clearance from the synapse, and repackaging mechanisms for re-release [52, 53]. Additionally, a comparison of the neurochemical makeup of mesocortical neurons projecting to the PL, its main source of dopaminergic input, revealed large differences between male and female rats [54]. In females, the proportions of constituent dopaminergic cells were significantly higher than those in males. Thus, for a given level of activation, PL DA efflux would be expected to be potentiated in females during shock. One speculative hypothesis is that the goal-directed and habit systems "compete" for the

encoding of instrumental learning, with elevated prefrontal DA inhibiting the corticostriatal goal-directed system and shifting acquisition towards the dorsolateral striatal habit system (Fig. 5H). The present in situ hybridization study, along with prior reports [55], indicates that both female and male rats preferentially express the D1 receptor subtype on PL GABA interneurons. There is substantial evidence that part of the inhibitory action of DA on pyramidal cell output is due to an enhancement of local GABAergic transmission [26, 56–58]. In addition, D1 and D2 receptor subtypes have low and high affinities for endogenous DA, respectively, such that D1-mediated signaling may predominate during conditions of higher rates of release [59, 60]. As would be predicted from the above framework, blockade of PL D1 receptors with SCH 23390 shifted Fos expression from the DLS to the DMS, thereby enabling protection in females with behavioral control. We also found that a greater number of DMS-projecting PL neurons were colocalized with the D2 receptor subtype compared to D1 and that this expression pattern was specific to females. Somewhat surprising, D2 receptor blockade interfered with performing the escape response. If instrumental action is initially goal-directed in females, as appetitive studies suggest [36], then D2-mediated signaling on the PL-to-DMS pathway may be critical for some aspect of its acquisition.

Although not directly tested here, the IL cortex represents an additional structure by which DA may bias the use of the dorsolateral habit system. Direct connections between the IL and DLS are absent [61], however evidence from appetitive studies suggests that coordinated neural activity between the IL and DLS is critical for the emergence of habits [62]. Inactivation of the IL delays the development of habit responding [62, 63], and once habitual behavior is formed, disruption of IL activity [64–66] or intra-IL infusion of a D1 antagonist [67] can restore outcome-sensitivity in behavior. In the present microdialysis study, probe placement did not discriminate between the PL and IL, thus both subregions likely contributed to the increase in DA levels produced by female ES. Future studies should address whether intra-IL DA signaling through the D1 receptor is also responsible for recruiting the DLS in females.

## CONCLUSIONS

The current results demonstrate that the operation of behavioral control over adverse events, a key aspect of coping, recruits an instrumental learning system in females that differs from males. Our data provide novel insight into the influence of mPFC DA on striatal activity during instrumental learning involving aversive stimuli and its impact on behavioral control outcome. Furthermore, our findings may have significant implications for understanding the sex- and circuit-specific determinants by which coping experiences are translated into resilience against future adversity.

## REFERENCES

1. Rubinow DR, Schmidt PJ. Sex differences and the neurobiology of affective disorders. Neuropsychopharmacology. 2019;44:111–28.
2. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry. 1994;51:8–19.
3. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. Psychol Bull. 2006;132:959–92.
4. Bandura A. Self-efficacy: The exercise of control. New York, NY, US: W H Freeman/Times Books/Henry Holt & Co; 1997.
5. Chorpita BF, Barlow DH. The development of anxiety: the role of control in the early environment. Psychol Bull. 1998;124:3–21.
6. Maier SF, Watkins LR. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev. 2005;29:829–41.
7. Williams JL, Maier SF. Transituational immunization and therapy of learned helplessness in the rat. J Exp Psychol Anim Behav Process. 1977;3:240–52.
8. Amat J, Aleksejev RM, Paul E, Watkins LR, Maier SF. Behavioral control over shock blocks behavioral and neurochemical effects of later social defeat. Neuroscience. 2010;165:1031–8.
9. Baratta MV, Leslie NR, Fallon IP, Dolzani SD, Chun LE, Tamalunas AM, et al. Behavioural and neural sequelae of stressor exposure are not modulated by controllability in females. Eur J Neurosci. 2018;47:959–67.
10. Baratta MV, Gruene TM, Dolzani SD, Chun LE, Maier SF, Shansky RM. Controllable stress elicits circuit-specific patterns of prefrontal plasticity in males, but not females. Brain Struct Funct. 2019;224:1831–43.
11. Balleine BW, Liljeholm M, Ostlund SB. The integrative function of the basal ganglia in instrumental conditioning. Behav Brain Res. 2009;199:43–52.
12. Dickinson A, Balleine B. Motivational control of goal-directed action. Anim Learn Behav. 1994;22:1–18.
13. Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci. 2004;19:181–9.
14. Smith KS, Graybiel AM. Habit formation. Dialogues Clin Neurosci. 2016;18:33–43.
15. Maier S, Seligman MEP, Solomon RL. Pavlovian fear conditioning and learned helplessness: Effects on escape and avoidance behavior of (a) the CS-US contingency, and (b) the independence of the US and voluntary responding. Punishment and aversive behavior. New York, NY: Appleton-Century-Crofts; 1969. p. 299–342.
16. Liljeholm M, Tricomi E, O'Doherty JP, Balleine BW. Neural correlates of instrumental contingency learning: differential effects of action–reward conjunction and disjunction. J Neurosci. 2011;31:2474–80.
17. Amat J, Christianson JP, Aleksejev RM, Kim J, Richeson KR, Watkins LR, et al. Control over a stressor involves the posterior dorsal striatum and the act/outcome circuit. Eur J Neurosci. 2014;40:2352–8.
18. Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nat Neurosci. 2005;8:365–71.
19. Amat J, Paul E, Zarza C, Watkins LR, Maier SF. Previous experience with behavioral control over stress blocks the behavioral and dorsal raphe nucleus activating effects of later uncontrollable stress: role of the ventral medial prefrontal cortex. J Neurosci. 2006;26:13264–72.
20. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci. 2009;10:410–22.
21. Arnsten AFT. Stress weakens prefrontal networks: molecular insults to higher cognition. Nat Neurosci. 2015;18:1376–85.
22. Christianson JP, Benison AM, Jennings J, Sandsmark EK, Amat J, Kaufman RD, et al. The sensory insular cortex mediates the stress-buffering effects of safety signals but not behavioral control. J Neurosci. 2008;28:13703–11.
23. Farovik A, Dupont LM, Arce M, Eichenbaum H. Medial prefrontal cortex supports recollection, but not familiarity, in the rat. J Neurosci. 2008;28:13428–34.
24. Sun W, Rebec GV. The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. Psychopharmacology. 2005;177:315–23.
25. Hall DA, Powers JP, Gulley JM. Blockade of D1 dopamine receptors in the medial prefrontal cortex attenuates amphetamine- and methamphetamine-induced locomotor activity in the rat. Brain Res. 2009;1300:51–57.
26. Gulledge AT, Jaffe DB. Multiple effects of dopamine on layer V pyramidal cell excitability in rat prefrontal cortex. J Neurophysiol. 2001;86:586–95.
27. De Mei C, Ramos M, Iitaka C, Borrelli E. Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. Curr Opin Pharm. 2009;9:53–58.
28. Gulledge AT, Jaffe DB. Dopamine decreases the excitability of layer V pyramidal cells in the rat prefrontal cortex. J Neurosci. 1998;18:9139–51.
29. Tervo DGR, Hwang B-Y, Viswanathan S, Gaj T, Lavzin M, Ritola KD, et al. A designer AAV variant permits efficient retrograde access to projection neurons. Neuron. 2016;92:372–82.
30. Shepherd GMG. Corticostriatal connectivity and its role in disease. Nat Rev Neurosci. 2013;14:278–91.
31. Maier SF. Behavioral control blunts reactions to contemporaneous and future adverse events: medial prefrontal cortex plasticity and a corticostriatal network. Neurobiol Stress. 2015;1:12–22.
32. Balleine BW, O'Doherty JP. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology. 2010;35:48–69.
33. Morris RW, Dezfouli A, Griffiths KR, Le Pelley ME, Balleine BW. The neural bases of action-outcome learning in humans. J Neurosci. 2022;42:3636–47.
34. Corbit LH, Muir JL, Balleine BW. Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. Eur J Neurosci. 2003;18:1286–94.
35. Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. Nat Rev Neurosci. 2006;7:464–76.

36. Schoenberg HL, Sola EX, Seyller E, Kelberman M, Toufexis DJ. Female rats express habitual behavior earlier in operant training than males. Behav Neurosci. 2019;133:110–20.
37. Schoenberg HL, Bremer GP, Carasi-Schwartz F, VonDoepp S, Arntsen C, Anacker AMJ, et al. Cyclic estrogen and progesterone during instrumental acquisition contributes to habit formation in female rats. Horm Behav. 2022;142:105172.
38. Wingard JC, Packard MG. The amygdala and emotional modulation of competition between cognitive and habit memory. Behav Brain Res. 2008;193:126–31.
39. Shiflett MW, Balleine BW. Molecular substrates of action control in cortico-striatal circuits. Prog Neurobiol. 2011;95:1–13.
40. Hart G, Bradfield LA, Balleine BW. Prefrontal corticostriatal disconnection blocks the acquisition of goal-directed action. J Neurosci. 2018;38:1311–22.
41. Hart G, Bradfield LA, Fok SY, Chieng B, Balleine BW. The bilateral prefronto-striatal pathway is necessary for learning new goal-directed actions. Curr Biol. 2018;28:2218–29.e7.
42. Murphy BL, Arnsten AFT, Jentsch JD, Roth RH. Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment. J Neurosci. 1996;16:7768–75.
43. Murphy BL, Arnsten AF, Goldman-Rakic PS, Roth RH. Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. Proc Natl Acad Sci. 1996;93:1325–9.
44. Arnsten AF, Dudley AG. Methylphenidate improves prefrontal cortical cognitive function through α2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. Behav Brain Funct. 2005;1:2.
45. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci. 2005;28:403–50.
46. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, et al. Chronic stress causes frontostriatal reorganization and affects decision-making. Science. 2009;325:621–5.
47. Taylor SB, Anglin JM, Paode PR, Riggert AG, Olive MF, Conrad CD. Chronic stress may facilitate the recruitment of habit- and addiction-related neurocircuitries through neuronal restructuring of the striatum. Neuroscience. 2014;280:231–42.
48. Gamo NJ, Lur G, Higley MJ, Wang M, Paspalas CD, Vijayraghavan S, et al. Stress impairs prefrontal cortical function via D1 dopamine receptor interactions with hyperpolarization-activated cyclic nucleotide-gated channels. Biol Psychiatry. 2015;78:860–70.
49. Mitsushima D, Yamada K, Takase K, Funabashi T, Kimura F. Sex differences in the basolateral amygdala: the extracellular levels of serotonin and dopamine, and their responses to restraint stress in rats. Eur J Neurosci. 2006;24:3245–54.
50. Heinsbroek RPW, Van Haaren F, Feenstra MGP, Endert E, Van de Poll NE. Sex- and time-dependent changes in neurochemical and hormonal variables induced by predictable and unpredictable footshock. Physiol Behav. 1991;49:1251–6.
51. McDevitt RA, Szot P, Baratta MV, Bland ST, White SS, Maier SF, et al. Stress-induced activity in the locus coeruleus is not sensitive to stressor controllability. Brain Res. 2009;1285:109–18.
52. Rivera-Garcia MT, McCane AM, Chowdhury TG, Wallin-Miller KG, Moghaddam B. Sex and strain differences in dynamic and static properties of the mesolimbic dopamine system. Neuropsychopharmacology. 2020;45:2079–86.
53. Zachry JE, Nolan SO, Brady LJ, Kelly SJ, Siciliano CA, Calipari ES. Sex differences in dopamine release regulation in the striatum. Neuropsychopharmacology. 2021;46:491–9.
54. Kritzer MF, Creutz LM. Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. J Neurosci. 2008;28:9525–35.
55. Santana N, Mengod G, Artigas F. Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cereb Cortex. 2009;19:849–60.
56. Gorelova N, Seamans JK, Yang CR. Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. J Neurophysiol. 2002;88:3150–66.
57. Seamans JK, Gorelova N, Durstewitz D, Yang CR. Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. J Neurosci. 2001;21:3628–38.
58. Tseng KY, O'Donnell P. Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci. 2004;24:5131–9.
59. Dreyer JK, Herrik KF, Berg RW, Hounsgaard JD. Influence of phasic and tonic dopamine release on receptor activation. J Neurosci. 2010;30:14273–83.
60. Martel JC, Gatti McArthur S. Dopamine receptor subtypes, physiology and pharmacology: new ligands and concepts in schizophrenia. Front Pharmacol. 2020;11:1003.
61. McGeorge AJ, Faull RL. The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience. 1989;29:503–37.
62. Smith KS, Graybiel AM. A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron. 2013;79:361–74.
63. Killcross S, Coutureau E. Coordination of actions and habits in the medial prefrontal cortex of rats. Cereb Cortex. 2003;13:400–8.
64. Barker JM, Glen WB, Linsenbardt DN, Lapish CC, Chandler LJ. Habitual behavior is mediated by a shift in response-outcome encoding by infralimbic cortex. ENeuro. 2017;4:ENEURO.0337-17.2017.
65. Coutureau E, Killcross S. Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. Behav Brain Res. 2003;146:167–74.
66. Smith KS, Virkud A, Deisseroth K, Graybiel AM. Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. Proc Natl Acad Sci. 2012;109:18932–7.
67. Barker JM, Torregrossa MM, Taylor JR. Bidirectional modulation of infralimbic dopamine D1 and D2 receptor activity regulates flexible reward seeking. Front Neurosci. 2013;7:126.

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## AUTHOR CONTRIBUTIONS

SFM and MVB conceived and designed the experiments. CJM, IPF, JA, RJS, NRL, DHR, and MVB performed the experiments and analyzed the data. CJM and MVB wrote the manuscript. All authors provided feedback on the manuscript.

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## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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