TITLE: Sexual dimorphism in histamine regulation of striatal dopamine

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Abstract

Background

Many neuropsychiatric disorders show sex differences in prevalence and presentation. For example, Tourette's Syndrome (TS) is diagnosed 3-5 times more often in males. Dopamine modulation of the basal ganglia is implicated in numerous neuropsychiatric conditions, including TS. Motivated by an unexpected genetic finding in a family with TS, we previously characterized the modulation of striatal dopamine by histamine.

Methods

We used microdialysis to analyze striatal dopamine response to the targeted infusion of histamine and histamine agonists. siRNA knockdown of histamine receptors was used to identify the cellular mediators of observed effects.

Results

Intracerebroventricular histamine reduced striatal dopamine in male mice, replicating previous work. Unexpectedly, histamine increased striatal dopamine in females. Targeted infusion of selected agonists revealed that the effect in males depends on H2R receptors in the substantia nigra pars compacta (SNc). Knockdown of H2R in SNc GABAergic neurons abrogated the effect, identifying these cells as a key locus of histamine's regulation of dopamine in males. In females, in contrast, H2R had no role; instead, H3R agonists in the striatum increased striatal dopamine. Strikingly, the effect of histamine on dopamine in females was modulated by the estrous cycle, appearing in estrus/proestrus but not in metestrus/diestrus.

Conclusions

These findings confirm the regulation of striatal dopamine by histamine but identify marked sexual dimorphism in and estrous modulation of this effect. These findings may shed light on the mechanistic underpinnings of other sex differences in the striatal circuitry, perhaps including the marked sex differences seen in TS and related neuropsychiatric conditions.

Introduction

Sex differences are seen in both the prevalence and the clinical characteristics of many neuropsychiatric conditions, including Tourette syndrome (TS), autism, and attention deficit hyperactivity disorder (ADHD), in which the basal ganglia are implicated as a key contributor to pathophysiology. The basal ganglia are critically regulated by dopamine (DA) from the substantia nigra pars compacta (SNc), and there are sex differences in striatal DA levels, dynamics, and function. Women have been reported to have higher DA synaptic concentration (1), higher DA response to methylphenidate in the ventral striatum (2), higher D_2 DA receptor levels (3, 4), and higher levels of DA transporter (DAT), in some studies (5). Striatal reward processing also differs by sex – men show elevated DA synthesis in response to reward, while women show decreased synthesis in response to punishment (6). Many of these effects have been confirmed in animal models, with females showing elevated release amplitude (7, 8), denser SNc axonal fibers during development (9), higher DA uptake rates (10), and increased accumulation of DA in the absence of DAT clearance (11, 12). Estradiol potentiates striatal dopaminergic signaling – ovariectomy significantly decreases DA release and turnover, and estradiol enhances striatal DA responses specifically in females (13-18). Estrogen receptors are expressed on SNc dopaminergic neurons that project to the striatum (19-21).

TS is diagnosed 3-5 times more often in males than in females (22-28). Males typically have more complex and severe tics (29, 30), while females have later age of symptom onset and of diagnosis and different comorbidity patterns, with evidence

that the menstrual cycle can influence tic severity (31). TS is characterized by increased DA levels in the basal ganglia, as indicated by elevated DAT (32-36) and enhanced DA release (37-39). Genetic evidence suggests that some cases of TS are associated with abnormalities in histamine (40-43), and subsequent work in animal models has focused attention on the modulation of striatal activity and striatal DA by histaminergic neurons in the tuberomammillary nucleus (TMN) of the hypothalamus (44, 45) (46-48). Specifically, we have shown that intracerebroventricular (ICV) infusion of histamine reduces striatal DA levels (Castellan Baldan et al, 2014). Notably, these early experiments were performed exclusively in male mice.

Histaminergic neurons project from the TMN throughout the brain, including to both the striatum (49, 50) and SNc (51). Histamine binds to four G-protein-coupled receptors (H1R-H4R); the first three of these are expressed in both the striatum and SNc (52-54). H1R is coupled to $G_{\alpha}q$ and H2R is coupled to $G_{\alpha}s$; both generally increase the firing of neurons on which they are expressed (52, 55-57). H3R is coupled to $G_{\alpha}i$ and can act as an inhibitory autoreceptor on histaminergic terminals (58-61) as well as an inhibitory heteroreceptor on the axon terminals of a several other neuron types (58, 62-65). Additionally, postsynaptic H3Rs in the striatum interact with DA receptors to modulate intracellular signaling in striatal medium spiny neurons in complex ways (66-68).

Here, we sought to confirm and extend our earlier finding that histamine reduces striatal DA *in vivo* (44). We identified an unexpected sexual dimorphism in the effects of histamine on DA: it reduced DA levels in males, as seen before, but increased them in females. Mechanistic dissection using specific agonists and cell type-specific receptor knockdown identified H2Rs on GABAergic neurons of the SN as a key locus of the effect on DA in males, while H3Rs in the striatum are the key regulators in females. Strikingly, the effect in females varies with the estrous cycle. These results identify a new locus of sexually dimorphic effects in striatal DA dynamics. Characterization of sex differences in the neurochemistry and function of the basal ganglia may help elucidate the pathophysiological underpinnings of the marked sex differences seen in Tourette syndrome, and many other neuropsychiatric conditions.

Methods

Animals

All animal use followed protocols approved by Yale's Institutional Animal Care and Use Committee. Adult male and female wild-type C57BL/6 mice and Gad2-cre mice (8-10 weeks old) were group housed in our animal facility in self-ventilating cages, maintained on a 12hr light/dark cycle, and provided with food and water *ad libitum*. Gad2-cre mice were initially obtained from Jackson Laboratories (#010802) then bred in-house.

For guide cannulation, mice were anaesthetized using a combination of ketamine/xylazine (10mg/kg / 1mg/kg) until completely sedated as measured by lack of response to noxious stimuli. The skull was exposed, and an anchor screw was inserted between the two sites of cannula implantation for implant stability. Two holes were drilled – one for each cannula. Microdialysis guide canulae (CMA7) were implanted at AP+0.9 and ML+/-1.5 and at a depth of 1.0mm to allow the 2mm length microdialysis membrane when inserted to enter directly into the dorsal striatum (Figure 1A). Infusion guide cannulae were implanted at two different locations depending on the experiment. ICV guide cannulae were implanted at AP+0.4, ML+/-0.7, and DV+2.3 contralateral to the microdialysis cannula to hit the ventricle (Figure 1B). Targeted infusion guide cannula targeting the SN were implanted at AP-3.0, ML+/-1.5, and DV+4.5 ipsilateral to the microdialysis guide (Figure 1C). Guide cannulae were cemented in place using Metabond Dental cement (Parkell). Following surgery, mice recovered for at least one week prior to microdialysis experiments.

For short hairpin RNA (shRNA) experiments, we used a custom adeno associated virus (AAV) generated by VectorBuilder expressing a shRNA against the murine *Hrh2* gene and an eGFP reporter within a cre-activated DIO cassette (Vector ID: VB200728-1049hdn) for controlled expression of the shRNA in cells expressing Cre. Two weeks prior to cannulation surgery,

mice were anesthetized as described above, skull exposed, and holes drilled bilaterally at AP-3.0, ML+/-1.5. A Hamilton syringe (1.0µl, 32ga, blunt) containing either the shRNA virus or a scramble GFP control was lowered to DV+4.5 and 0.5µl of virus was injected slowly over three minutes. The needle remained in place two minutes following injection to prevent aspiration of the virus as the needle is removed. Following AAV surgery, the scalp was sealed by VetBond and mice recovered for two weeks prior to further canulation surgery. Viral targeting and reduction of H2 were confirmed visually following experiments (Figure 3).

Microdialysis

Prior to microdialysis, mice were acclimated to the testing facility for 30 minutes. For estrous cycle testing, female mice were restrained prior to the acclimation period and 10µl of sterile saline was injected into the vaginal cavity using a blunt pipette tip, then quickly aspirated. Vaginal samples were then applied to a glass slide and visualized on a light microscope (AmScope). Estrous cycle stage was identified by cell density and composition, as previously described (69).

All microdialysis experiments were performed in the CMA120 System for freely moving animals (Harvard Apparatus). Infusion of ACSF (Harvard Apparatus, #597316) was performed using a CMA402 Microdialysis Syringe pump set at a rate of 2µl/min. At the start of the experiment, mice were briefly restrained, the dummy protective cannula was removed from the guide, and a CMA7 microdialysis probe (6 kDa, 2mm membrane length, Harvard Apparatus) was inserted gently. Mice were then placed into the apparatus and flow was turned on. Mice were allowed to acclimate and ACSF to flow through the tubing for 30 minutes. Following acclimation, samples were collected every ten minutes and put immediately on dry ice. After one hour of baseline, mice were given drug or saline, and samples were collected for another 90 minutes. Mice received one treatment per day on consecutive days; drug treatment order was randomized for each mouse. Some mice were unable to complete all drug treatments due to cannula malfunction; for this reason the N differs in some treatment groups.

For infusion of drug through infusion cannulae (ICV and SN), mice were briefly restrained and an infusion cannula matched to the length of the guide (2.3mm for ICV, 4.5mm for SN infusion) was inserted into the guide. Drugs were infused at a rate of 1µl/min for 2 minutes total. The infusion cannula was left in place for one minute following the end of drug infusion, to prevent backflow. For drug infusion into the striatum, reverse microdialysis was used and drug was infused through the microdialysis cannula at double the concentration used for direct infusions. Mice were briefly restrained prior to the initiation of reverse microdialysis infusion, such that the effects of restraint stress prior to drug were matched between groups. Drug doses were: histamine 20µg/mouse (44), 2-(3-trifluoromethylphenyl)histamine (FMPH) 6µg/mouse (70), Dimaprit 20µg/mouse (71), and RAMH 20µg/mouse (72). All drugs were prepared in sterile saline.

HPLC

Mouse striatal microdialysis samples were snap-frozen on dry ice and stored at -80° prior to quantification in batches using HPLC. DA concentration was measured on a DIONEX UltiMate3000 RS Electrochemical Detector (Thermo-Fisher) using a computer running Chromeleon 7 software (Thermo-Fisher). Samples were run through a Hypersil ODS C18 column with a 3µm particle size, 3mm diameter and 150mm length (Thermo-Fisher) and analyzed using a 6011RS ultra-analytical cell (Thermo-Fisher). Samples were run using MDTM mobile phase (Thermo-Fisher) at a rate of 0.5mL/min through the column and analyzed using electrode potentials at 500nm/-500nm with a 0.2s filter over a run time of 10 minutes. Using these parameters, DA standards reliably produced a peak even at low concentrations between 3:45 and 4:15 after injection; as such, peaks in our samples were analyzed within this time window. Once samples were run, we checked for abnormal background or false peaks (which can be caused by air bubbles in the sample) and rerun if errors were apparent. HPLC data was analyzed using Cobra Wizard (Thermo-Fisher) to measure peak heights against baseline.

To control for individual animal, day, and order variance, each animal was compared against its own baseline on each trial day. The first five samples collected prior to injection were averaged to produce a baseline. All further values were then analyzed as (value/baseline)*100. Sample values from 10-60 min post-infusion were averaged.

Behavior

Open field (OF) recordings were performed using the Fusion Sensor System and Experimentation Software (Omnitech Electronics, Inc.). Following 30 minutes acclimation to the behavioral room, mice were placed in the open field apparatus and baseline data collected for one hour. After one hour of baseline, mice were briefly returned to their home cage and administered drug as described above. Mice received one treatment per day, in a counterbalanced order. Following drug administration, mice were returned to the OF and their total activity was measured for another hour. All OF data was normalized to the same day's baseline to control for individual animal and day effects.

Immunohistochemistry

For histology, mice were anesthetized (ketamine 100mg/kg and xylazine 10mg/kg, followed by isoflurane inhalation until no response to noxious stimulus) followed by transcardial perfusion with 4% paraformaldehyde in phosphate buffered saline (PBS) (Thermo-Fisher) and brain extraction. Cryostat sections were cut at 30 µm and mounted on Diamond White Glass Slides (Globe). For immunostaining, slides were incubated overnight at room temperature with primary antibodies diluted in 0.1 M PBS containing 10% normal donkey serum (Abcam, ab7475) and 1% Triton-X 100 (AmericanBio). Primary antibodies were Rabbit anti-GFP (1:1000, Thermo-Fisher, A-11122) and goat anti-H2R (1:250, Everest Biotech, EB06905). Following incubation with primary antibody, slides were washed three times for ten minutes each in PBS with 0.1% Triton at room temperature. Detection was performed with appropriate secondary antibodies, again in PBS + 10% normal donkey serum and 1% Triton: polyclonal donkey anti-rabbit coupled to Alexa Fluor 488 (1:1000, Thermo-Fisher, A-21206) and polyclonal donkey anti-goat coupled to Alexa Fluor 568 (1:1000, Thermo-Fisher, A-11057). Following a two-hour incubation in secondary antibody, slides were rinsed three times at twenty minutes each in PBS and then mounted and coverslipped in ProLong Gold with DAPI (Thermo-Fisher, P36935). Immunostained coronal SNc sections (30 µm), identified following landmarks in the atlas of Paxinos (73), were visualized on a Zeiss Scope.A1 using a Plan-APOCHROMAT 10x objective and an Axiocam 503 Mono at 100x magnification.

Statistics

All data were imported into Graph Pad Prism 10 (Graph Pad Software, LLC) or JASP (Eric-Jan Wagenmakers, University of Amsterdam) for statistical analysis. For analysis of changes in DA levels from baseline over time, a repeated measures ANOVA (RM-ANOVA) was used to look for effects of treatment at the time points from 10-60 minutes post injection. Collapsed averaged data over this time period was measured using an unpaired Student's T-Test (for the single comparison between histamine and saline) or a one-way ANOVA using post-hoc Tukey comparisons (for all other group analyses). Analysis of changes in DA levels in females in response to agonist and estrous was performed using a three-way ANOVA to account for estrous phase, brain region, and drug treatment with Post-Hoc Tukey comparisons between groups. Analysis of changes in DA in mice with shRNA block of H2 were analyzed using a three-way ANOVA to account for genotype, virus treatment, and drug with post-hoc Tukey comparisons between groups. Graphs were generated using Graph Pad 10.

Results

Histamine regulates striatal DA levels differently in male and female mice

We previously showed that histamine reduces DA levels in the striatum (44, 45), but these experiments were limited to males. We cannulated adult male and female mice and infused histamine or saline ICV while measuring striatal DA using

microdialysis (Figure 1A,B). Infusion of ICV histamine into male mice confirmed previous findings – striatal DA levels 10-60 minutes post-infusion were significantly decreased compared to saline controls both over time (Figure 1C; RM-ANOVA: main effect of group, $F[1.000,13.00]=5.668$, $p=0.033$) and when collapsed across all time points (Figure 1D; t-test: $t[29]=2.961$, $p=0.006$). Surprisingly, females showed a very different pattern: DA was significantly increased after ICV infusion of histamine, both over time (Figure 1E; RM-ANOVA: $F[1.000, 13.00]=5.633$, $p=0.034$) and collapsed over the full interval (Figure 1F; t-test: $t[17]=2.924$, $p=0.009$). Notably, the female data showed a high variance, with a small number of mice with very high DA levels following HA infusion appearing to drive the effect; we return to this point below.

H2Rs in the SNc suppress striatal DA in males

To determine which histamine receptor regulates the effects of histamine on striatal DA in males, we infused specific histamine receptor agonists ICV. Analysis of change of DA over the 60 minutes following infusion indicates a significant effect of treatment (Figure 2A; ANOVA, $F[3\ 39]=3.665$, $p=0.020$), and post-hoc analysis showed that infusion of the H2 agonist dimaprit was the only treatment that significantly decreased DA levels from baseline, relative to saline (Tukey's: Saline vs H2 Agonist $p=0.027$).

As HA has previously been shown to cause hypolocomotion (44) that may be associated with its effects on DA, we also tested male mice in the OF after HA receptor agonist infusion. In males, ICV infusion of the H2R agonist dimaprit produced a trend towards decreased locomotion (Figure 2B; ANOVA: $F(3, 25)=2.467$, $p=0.08$; post-hoc Tukey's $p=0.033$).

To determine the locus of the H2R effect in males, we infused H2 and H3 agonists (dimaprit and RAMH, respectively) into both the striatum and the SN (Figure 2C). Once again there was a significant effect of treatment (Figure 2D; ANOVA: $F[5,40]=2.976$, $p=0.0224$), with decreased striatal DA following infusion of dimaprit into the SNc, relative to saline (Tukey's, $p=0.0322$). Targeted infusion of dimaprit into the striatum and of the H3 agonist RAMH into either the SNc or the striatum had no effect.

H2Rs on GABAergic interneurons in the SNc mediate the H2-induced decrease of striatal DA in males

As the Gαs-coupled H2R typically has a net excitatory effect, we hypothesized that it may reduce striatal DA indirectly by activating SNc GABAergic interneurons. To test this, we specifically downregulated H2R in SNc GABAergic interneurons by infusing recombinant AAV expressing an anti-H2R shRNA in a cre-dependent fashion into the SNc of male GAD2-cre transgenic mice (Figure 3A-B). ICV HA infusion into these mice once again reduced striatal DA in wild-type mice and GAD2-cre mice receiving a negative control GFP-expressing virus, but this effect was absent in mice in which H2R was knocked down (Figure 3C; two-way ANOVA: condition x drug, $F(2,22)=3.584$, $p=0.044$, condition, $F(2,32)=8.207$, $p=0.001$; drug, $F(2,22)=3.584$, $p=0.001$). ICV Histamine infusion significantly decreased DA, relative to saline infusion, in both WT and GAD2-Cre-GFP controls (post-hoc Tukey's: WT $p=0.001$, GAD2-Cre-GFP $p=0.005$) but not in GAD-cre shH2 mice ($p=0.901$). Similar effects were seen after ICV infusion of the H2 agonist dimaprit (Figure 3D; 2-way ANOVA: interaction genotype x drug: $F(1,46)=4.068$, $p=0.0496$; Tukey's: wild type, $p=0.013$; shH2, $p=0.523$).

H3R activation increases striatal DA in females

We repeated this analysis in female mice, with strikingly different results. ICV infusion of histamine receptor agonists in females again showed significant effects 10-60 minute post-treatment (Figure 4A; ANOVA: $F[3,44]=3.417$, $p=0.025$). Here, post-hoc analysis did not show any effect of the H2 agonist dimaprit (Tukey's: $p>0.9$). Instead, infusion of the H3 agonist RAMH significantly increased DA levels, compared to saline ($p=0.045$). As after histamine infusion (Figure 1E-F), there were a small number of animals with very high DA levels following RAMH infusion. This variability in females suggests the possibility of an effect that varies with the estrous cycle.

While dimaprit did not cause any behavioral effects compared to saline, RAMH significantly elevated locomotor activity in females, paralleling effects on DA (Figure 4B; ANOVA: $F[1.9,13.3]=6.89$, $p=0.009$, post-hoc Tukey's H2 $p=0.92$, H3 $p=0.04$).

Histamine H3R modulation of DA is regulated by estrous cycle

Targeted infusion of dimaprit and RAMH into the striatum and SNc of females did not show a statistically significant effect (Figure 4C; $F[5,26]=1.486$, $p=0.229$). As before (Figure 1E,F; Figure 4A), several outlier female mice showed very high levels of DA, this time after RAMH infusion into the striatum.

We therefore repeated the experiment with careful monitoring of estrous phase. This revealed clearer effects (Figure 4D). RAMH infusion produced a significant increase in striatal DA, but only in estrus/proestrus, and only when the infusion was into the striatum; there was no effect in metestrus/diestrus, and no effect of RAMH infusion into the SNc. A 3-Way ANOVA (drug vs saline, region, estrous phase) trended towards a 3-way interaction ($F(1,72)=3.079$, $p=0.083$), a significant interaction of treatment and brain region ($F(1,72)=6.340$, $p=0.014$) and treatment and estrus phase ($F(1,72)=4.909$, $p=0.0299$), significant effects of treatment ($F(1,72)=3.997$, $p=0.049$) and estrous phase ($F(1,72)=5.214$), and trended towards a significant interaction of estrus phase with brain region ($F(1, 72)=6.340$, $p=0.067$). Post-hoc analyses showed that infusion of RAMH into the striatum during proestrus/estrus was the only treatment to significantly elevate DA, compared to all other conditions (Fisher's LSD: H3 Striatum P/E vs SAL Striatum P/E $p<0.001$, vs SAL Striatum M/D $p<0.001$, vs H3 Striatum M/D $p<0.001$, vs H3 SN P/E $p=0.004$ vs H3 SN M/D $p<0.001$).

Infusion of the H2 agonist dimaprit produced more ambiguous effects. There was no 3-way interaction; there was an interaction of treatment and brain region (Figure 4E; 3-Way ANOVA: treatment x region, $F[1,61]=4.377$, $p=0.04$), but no interaction with estrous phase.

Discussion

Here, we replicate our previous findings that histamine decreases DA levels in the striatum in male mice (44, 45) and find a striking difference in females, in which histamine produces a marked elevation of striatal DA levels. The opposite effects of histamine on striatal DA seen in males and females may contribute to some of the sex differences seen in striatal DA dynamics (74), and perhaps to the sex differences seen in the prevalence and presentation of TS and related neuropsychiatric conditions.

Sexual dimorphism in the relationship between histamine and DA extends to the regional and receptor mechanisms revealed by our analysis. Both the striatum and the SN express H1R, H2R, and H3R (52, 75-79). In males, we show that activation of H2Rs on GABAergic neurons in the SN mediates histamine's effects. Histamine has previously been shown to activate these cells *ex vivo* (56, 80). H2R is a $G\alpha i$-coupled receptor that typically excites neurons in which it is expressed (52, 54, 78, 81-84). Our findings *in vivo* suggest that histamine activation of H2R on GABAergic interneurons in the SN activates them and thereby inhibits neighboring dopaminergic neurons, inhibiting their firing and thus their release of DA in the striatum – in males.

In females the effect is quite different. Infusion of dimaprit, either ICV or into the SN, doesn't effect striatal DA. Instead, the H3R agonist RAMH increases striatal DA (Figure 4), and this appears to be mediated in the striatum, not the SN. H3R has a complex role in the striatum, where it functions as a heteroreceptor, an autoreceptor, and a postsynaptic receptor (58, 59, 61-64, 66, 79). Presynaptic H3Rs on DA terminals are unlikely to be responsible for the increase in striatal DA levels, as H3R activity on these terminals would be predicted to decrease DA release (58). H3 heteroreceptors on GABAergic terminals can suppress GABA release (58, 85, 86); RAMH may inhibit GABAergic synapses onto dopaminergic terminals, disinhibiting DA release (87). However, H3R is also expressed postsynaptically on spiny projection neurons (SPNs), the GABAergic principal cells of the striatum, where it interacts with DA receptors and modulates downstream signaling in

complex ways (66-68). H3R on SPNs may modulate their activity and thereby regulate DA release, through either local mechanisms or indirectly, via projections to the SN. These mechanisms remain to be elucidated.

Strikingly, these effects in females are modulated by the estrous cycle, with the effects of H3R activation on striatal DA emerging only during proestrus/estrus. Some evidence shows that estrogen receptors colocalize with histamine receptors (88, 89) and that histamine-estrogen interactions may be important in regulation of hypothalamic neurons (90, 91). Whether similar interactions occur in the striatum is unclear. If H3-induced inhibition of GABA release, or modulation of SPN activity, is potentiated by estrogen, then more pronounced functional effects of the H3R agonist RAMH would be expected during phases of the estrous cycle when estrogen is highest – exactly what we see here. This could also explain the lack of an H3 effect in males. DA levels are known to fluctuate during the estrus cycle, including enhanced DA release during proestrus/estrus (15, 18, 74, 92, 93); these histamine-mediated effects may contribute to such fluctuations. It will be intriguing to explore whether HA modulation of DA contributes to cyclic changes in neuropsychiatric symptomatology.

Our investigations of striatal histamine are motivated by genetic evidence that histamine dysregulation contributes to the pathophysiology of TS (44, 48). We speculate that the differential effects of histamine on striatal DA in males and females may help explain the fact that TS and other tic disorders occur 3-5 more often in males (22-28). In males, histamine acting on H2R on GABAergic neurons in the SN reduces striatal dopaminergic tone (Figure 2, 3). If this mechanism importantly constrains DA levels at baseline, then reduced histamine, such as is in patients or in mice with a TS-associated mutation in the *histidine decarboxylase* gene, may disinhibit DA release (40, 44), leading to the elevation in striatal DA tone that has been inferred in TS patients (32-39). Since this mechanism is not operant in females, a similar loss of histamine in females would not be expected to lead to dysregulation of striatal DA, and may not produce tics as it does in males. As histamine increases striatal DA in females, a reduction in histamine tone might reduce striatal DA and could even be protective in a hyperdopaminergic condition like TS.

This study has limitations, of course, and there are important areas where future work is needed. The importance of these mechanisms in TS and other forms of neuropsychiatric pathophysiology remains to be resolved. How estrous phase influences histamine's effects on DA likewise remain to be elucidated, and it is unclear why the H2R in the SN does not regulate DA in the females as it does in the males. As this is the first time to our knowledge that such a marked sexual dimorphism in the effects of histamine has been identified, it will be of interest to characterize what other histamine-regulated processes also differ in males and females. Finally, it will be important to determine whether these effects are recapitulated in humans.

In sum, we have identified unexpected complexity in the regulation of striatal DA by histamine, with qualitatively different mechanisms and effects seen in male and female mice. It will be important to further characterize sex differences in the function of the basal ganglia, both in health and in disease states.

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Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. MVZ has no competing interests. CP has consulted in the past year for Biohaven Pharmaceuticals, Freedom Biosciences, Ceruvia Life Sciences, Nobilis Therapeutics, Transcend Therapeutics, and F-Prime Capital Partners and has received research support from Biohaven, Freedom, and Transcend; none of these

relationships are related to the current work. Dr. Pittenger holds equity in Alco Therapeutics and Biohaven Pharmaceuticals and has pending patents related to the treatment of OCD, the actions of psychedelic drugs, and the role of specific antibodies in neuroimmune pathophysiology; again, none of these relationships are related to the current work.

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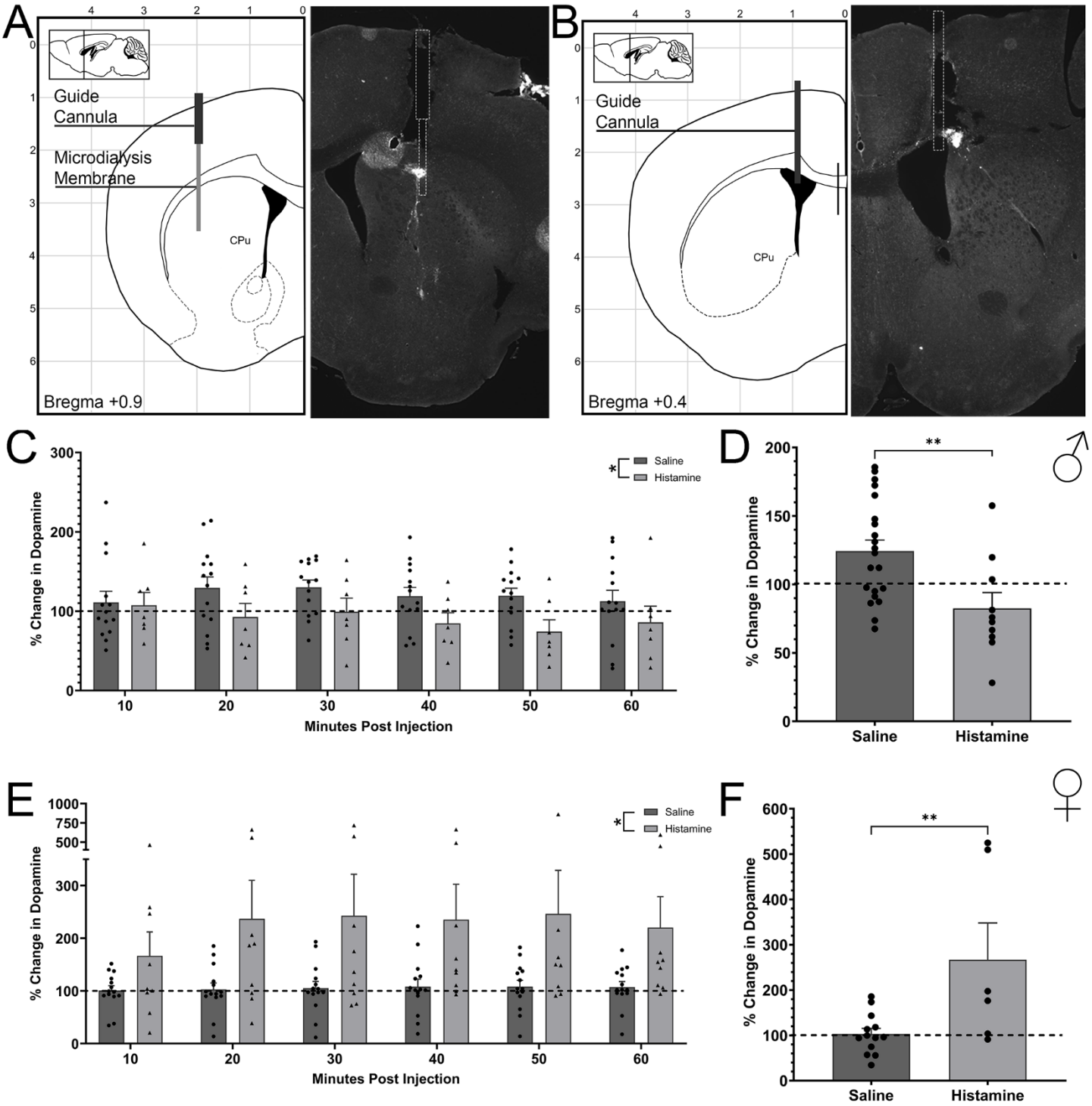
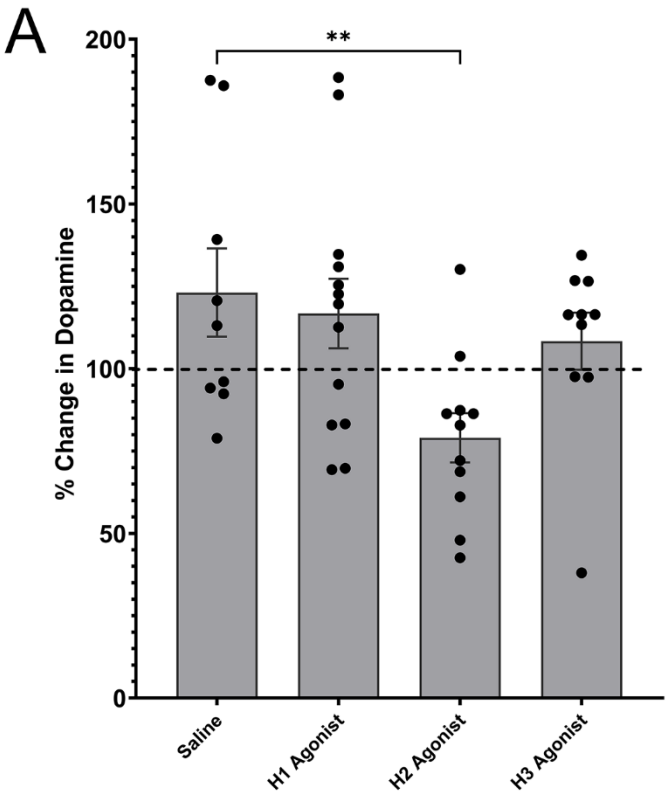


Figure 1. Histamine regulates striatal dopamine differently in males and females. A. Schematic and representative image for placement of the microdialysis guide cannula in the striatum. Guide cannula placement was counterbalanced between left and right striatum. **B.** Schematic and representative image for guide cannula placement for ICV infusion. ICV guide cannulae were placed contralaterally to microdialysis guide cannulae. **C.** ICV infusion of HA significantly decreases striatal DA, compared to saline control (sal), in male mice (RM-ANOVA: main effect of treatment, F (1.000, 13.00) = 5.668, *p=0.033). **D.** Combining data over the 20-60 minute interval post infusion confirmed a significant decrease in striatal DA after HA relative to saline (Student's t-test (two-tailed): **p=0.006). **E.** ICV infusion of histamine significantly increased striatal DA, relative to saline-infused controls, in

female mice (RM-ANOVA: main effect of drug, F (1.000, 13.00) = 5.633, *p=0.034). **F.** This was confirmed when data were collapsed over 20-60 min post -infusion (Student's t-test (two-tailed): **p=0.009). Dashed lines indicate baseline dopamine levels, determined for each animal during the 50 min before histamine or saline infusion.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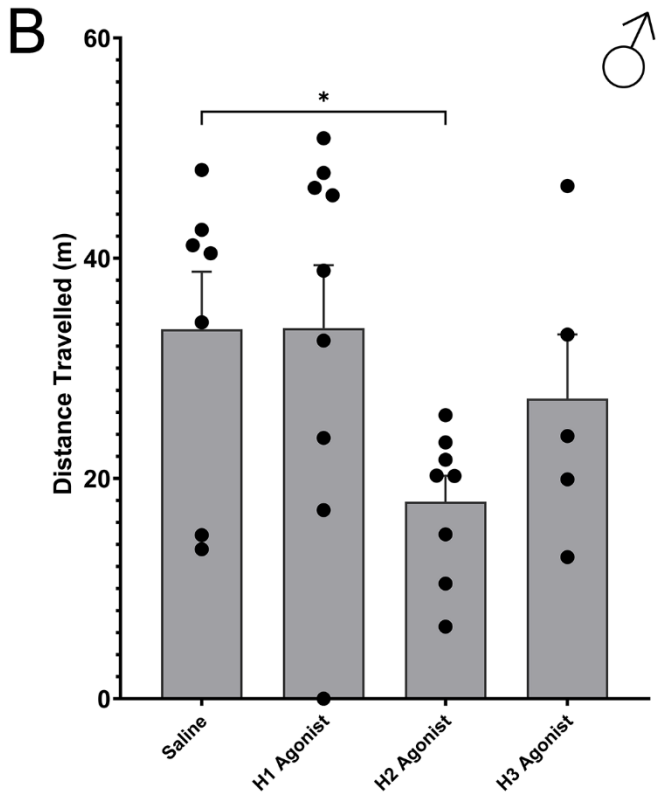
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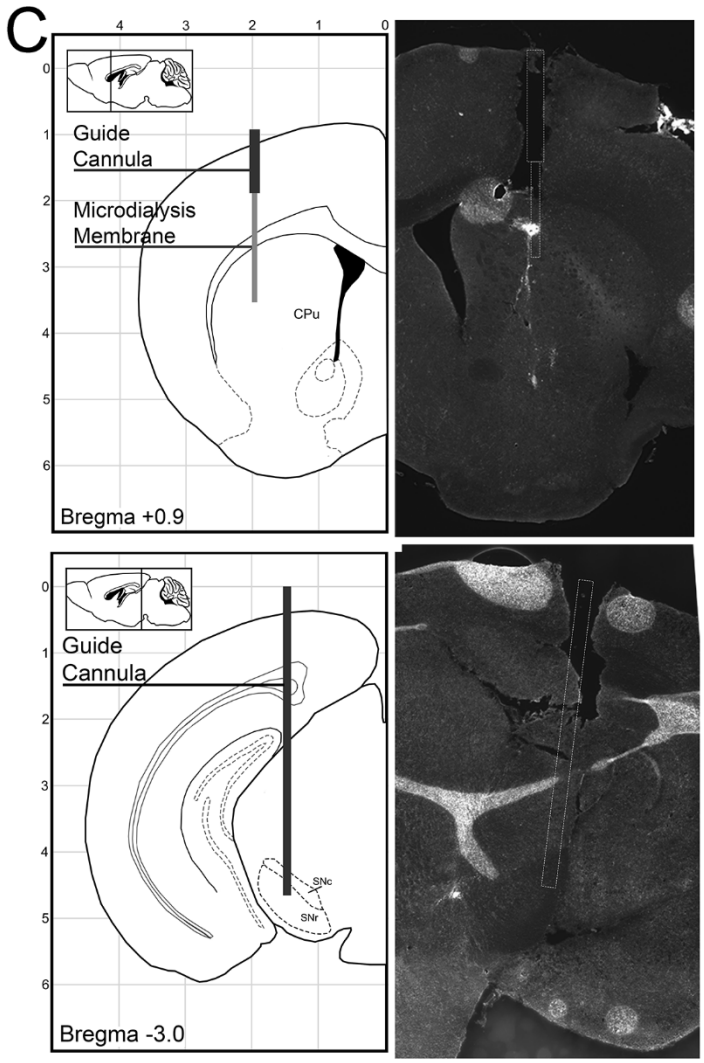
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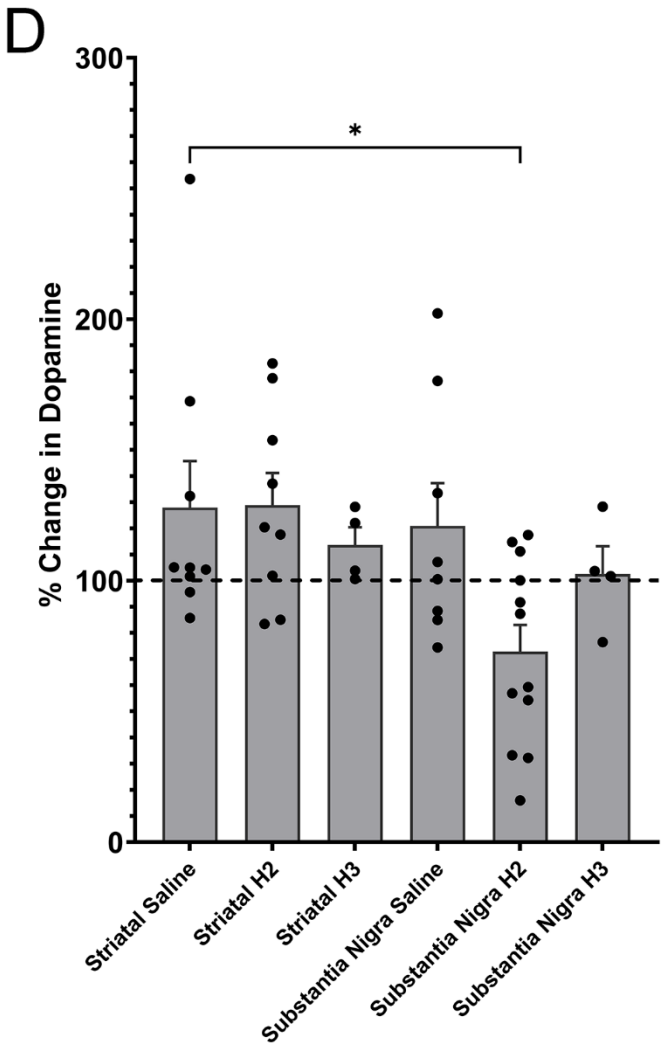
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Figure 2. Striatal dopamine is regulated by activation of histamine H2 receptors in the SNc of male mice. A. ICV infusion of the histamine H2R agonist dimaprit significantly reduces striatal DA in male mice, compared to saline controls and all other agonist treatments. (1-way ANOVA: F (3, 39) = 3.665, p=0.02; Post-Hoc Tukey's test: (sal vs dimaprit): **p=0.027). No effect of the H3R agonist RAMH was seen (Tukey's (sal vs RAMH): p=0.76) **B.** Locomotion in male mice following treatment with HA agonists was reduced by dimaprit relative to saline control (ANOVA: F (3, 25) = 2.467, p=0.08; Tukey's (sal vs dimaprit): p=0.033). **C.** Schematic and representative image for guide cannula placement of microdialysis guide cannula in the striatum and infusion guide cannula above the substantia nigra; drugs targeting the striatum in this experiment were delivered through the same cannula using reverse microdialysis. Guide placement was counterbalanced between left and right striatum. SN infusion guides were placed ipsilateral to microdialysis cannula. **D.** Infusion of dimaprit into the SN significantly reduces DA levels in the striatum, compared to saline controls and the H3R agonist RAMH (1-way ANOVA: F (5, 40) = 2.976, *p=0.022; Tukey's (Striatal sal v SN H2): *p=0.036, (Striatal H2 v SN H2): p=0.032).

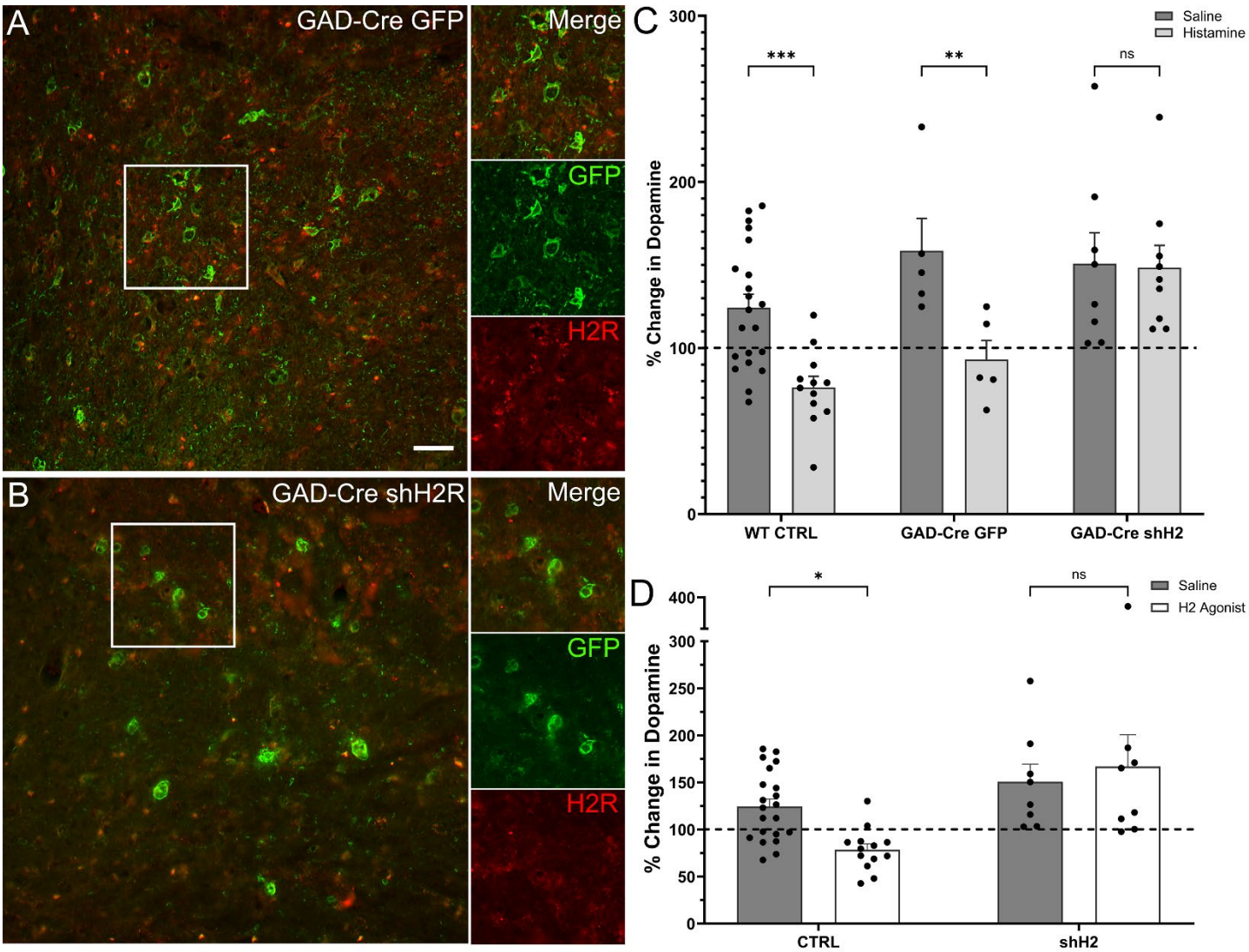

Figure 3. GABAergic interneurons in the substantia nigra mediate the effects of H2 activation on striatal DA in males. A, B. Representative image of GFP expressing GABAergic interneurons (green) and H2R (red) in the SN of a mouse that received control scramble shRNA AAV (A) or AAV-shH2 (B). Higher resolution images of selected regions demonstrate reduced expression of H2R on GFP-expressing GABAergic interneurons. Scalebars: 20µm. **C.** H2R knockdown in GABAergic interneurons in the SN blocks the reduction of striatal DA after ICV HA infusion [2

Way ANOVA: genotype: F (2, 32) = 8.207, p=0.0013, drug: F (1, 22) = 15.37, interaction: F (2, 22) = 3.584; Post-Hoc Tukey's: Wild Type CTRL (sal vs HA) p<0.001, Gad-Cre GFP CTRL (sal vs HA), p=0.005, Gad-Cre shH2 (saline vs histamine), p=0.910]. **D.** H2R knockdown in GABAergic interneurons in the SN blocks the effects of ICV dimaprit infusion on striatal DA levels (2 Way ANOVA: genotype x drug: F (1, 46) = 4.068, p=0.0496; Tukey's: Wild Type CTRL (sal vs dimaprit: *p=0.013), shH2 (sal vs dimaprit): p=0.523). Dashed lines indicate baseline striatal DA levels, prior to drug vs saline infusion.

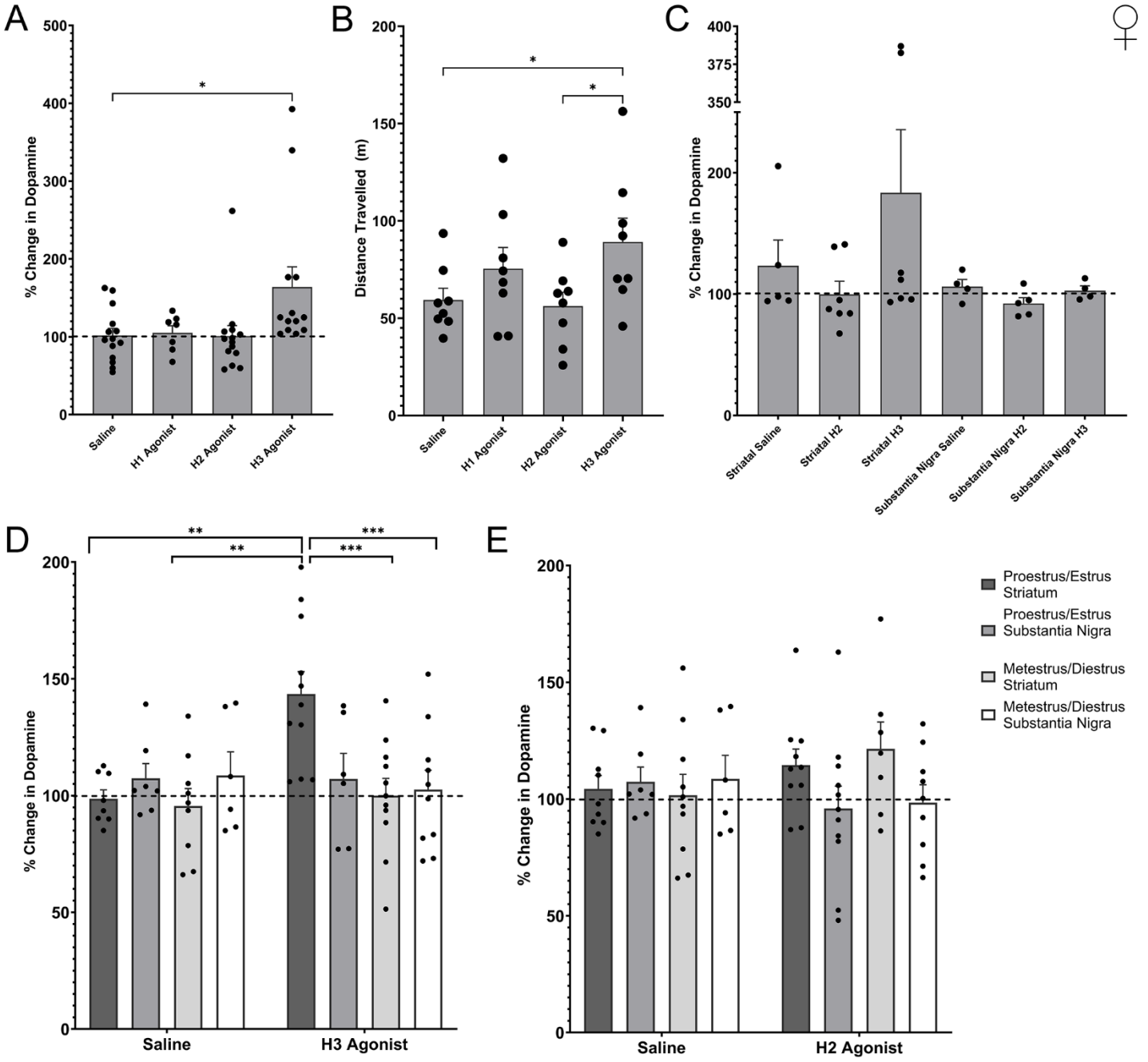

Figure 4. H3R activation in the striatum increases striatal DA in females, but only during the estrus phase of the estrous cycle. A. ICV infusion of the H3R agonist RAMH increased striatal DA in female mice, compared to saline controls and all other treatments. (1-way ANOVA: F (3, 44) = 3.417, p=0.025; Post-Hoc Tukey's (sal vs RAMH), p=0.045). In contrast to what we see in males, there is no effect of dimaprit infusion in females (Tukey's: p=1.00). **B.** In females, there was a significant increase in locomotion following RAMH infusion compared to saline control (1-

way ANOVA: F (1.903, 13.32) = 6.896, p=0.009; Tukey's (sal vs RAMH): p=0.042, (RAMH vs dimaprit): p=0.027). In contrast to what we see in males, there is no effect of ICV dimaprit infusion [Tukey's (sal vs H2): p=0.921]. **C.** Infusion of RAMH into the striatum appears to increase levels of striatal dopamine in females, though the effect is driven by outlier points and fails to reach statistical significance (1-way ANOVA: F (5, 26) = 1.486, p=0.229). **D.** When mice are separated into the proestrus/estrus or metestrus/diestrus phases of the estrous cycle, infusion of RAMH into the striatum increases striatal DA only during the proestrus/estrus (3 Way ANOVA: 3-way interaction, F (1, 72) = 3.079, p=0.083; Treatment x Estrus Phase, F (1, 72) = 4.909, p=0.0299, Treatment x Brain Region, F (1, 72) = 6.340, p=0.014, Estrus Phase x Brain Region F (1, 72) = 3.481, p=0.066, Treatment, F (1, 72) = 3.997, p=0.049, Estrus Phase, F (1, 72) = 5.214, p=0.025. Post-Hoc Fisher's LSD (P/E RAMH striatum vs P/E sal striatum) p<0.0001, (vs M/E sal SN) p=0.003, (vs M/D sal striatum) p<0.0001, (vs M/D sal SN) p=0.006, (vs M/D RAMH striatum) p<0.0001, (vs P/E RAMH SN) p=0.005, (vs M/D RAMH SN) p=0.0003). **E.** Infusion of dimaprit into the striatum during different phases of the estrous cycle did not significantly alter levels of striatal DA; there was if anything a suggestion of an increase in striatal DA after striatal dimaprit infusion (3 Way ANOVA: 3-way interaction N.S.; treatment x brain region: F (1, 61) = 4.377, p=0.041).