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Fundamental Sex Differences in Cocaine-Induced Plasticity of Dopamine D1 Receptor– and D2 Receptor–Expressing Medium Spiny Neurons in the Mouse Nucleus Accumbens Shell

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ABSTRACT

BACKGROUND: Cocaine-induced plasticity in the nucleus accumbens shell of males occurs primarily in dopamine D_1 receptor–expressing medium spiny neurons (D1R-MSNs), with little if any impact on dopamine D_2 receptor–expressing medium spiny neurons (D2R-MSNs). In females, the effect of cocaine on accumbens shell D1R- and D2R-MSN neurophysiology has yet to be reported, nor have estrous cycle effects been accounted for.

METHODS: We used a 5-day locomotor sensitization paradigm followed by a 10- to 14-day drug-free abstinence period. We then obtained ex vivo whole-cell recordings from fluorescently labeled D1R-MSNs and D2R-MSNs in the nucleus accumbens shell of male and female mice during estrus and diestrus. We examined accumbens shell neuronal excitability as well as miniature excitatory postsynaptic currents (mEPSCs).

RESULTS: In females, we observed alterations in D1R-MSN excitability across the estrous cycle similar in magnitude to the effects of cocaine in males. Furthermore, cocaine shifted estrous cycle–dependent plasticity from intrinsic excitability changes in D1R-MSNs to D2R-MSNs. In males, cocaine treatment produced the anticipated drop in D1R-MSN excitability with no effect on D2R-MSN excitability. Cocaine increased mEPSC frequencies and amplitudes in D2R-MSNs from females in estrus and mEPSC amplitudes of D2R-MSNs from females in diestrus. In males, cocaine increased both D1R- and D2R-MSN mEPSC amplitudes with no effect on mEPSC frequencies.

CONCLUSIONS: Overall, while there are similar cocaine-induced disparities regarding the relative excitability of D1R-MSNs versus D2R-MSNs between the sexes, this is mediated through reduced D1R-MSN excitability in males, whereas it is due to heightened D2R-MSN excitability in females.

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Cocaine alters the neurophysiology of medium spiny neurons (MSNs) of the nucleus accumbens (NAc) (1–7), with enduring drug-induced plasticity observed in the NAc shell (NAcSh) subregion (2). In male rodents, cocaine reduces the firing rates of dopamine D_1 receptor–expressing MSNs (D1R-MSNs), with no effect on dopamine D_2 receptor–expressing MSNs (D2R-MSNs) (8–10). Regarding cocaine-mediated glutamatergic synaptic plasticity, various groups have reported some degree of change at D1R-MSNs characterized by alterations to miniature excitatory postsynaptic current (mEPSC) frequencies and/or amplitudes (8,11,12). Similar studies using females have been sparse, and data have often been pooled with data from males (11,13–15), most likely because it has been widely assumed that these fundamental effects of cocaine would not differ between the sexes. In subtype-unidentified NAcSh MSNs, glutamatergic synaptic plasticity following cocaine was reported to be sex independent, although the NAc core did show an effect of cocaine by sex (16).

Most studies that have examined cocaine-induced alterations to neurophysiology that included sex as a biological variable did not track the estrous cycle. However, the estrous cycle and sex hormones in particular have been shown to cause dynamic changes in MSN neuroplasticity in the accumbens core of rats (17–20). In addition, cocaine can cause changes in neuroplasticity in both the NAcSh and core (1,2). Estrous cycle–dependent neuroplasticity may interact with cocaine-induced neuroplasticity, creating a scenario where researchers may not be able to discern drug effects from the effects of female sex hormones if the two are not tracked pre- and post drug exposure.

It has been hypothesized that females and males seek drugs of abuse and relapse for different reasons. In males, drug seeking and relapse are often attributed to appetitive behaviors, whereas in females, they are usually attributed to negative affect (21–23). That said, female mice in estrus show enhanced cocaine conditioned place preference compared

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with diestrus females and males (24). In total, sex-dependent differences in cocaine-induced alterations to NAcSh D1R-MSN and D2R-MSN neurophysiology would offer a potential mechanism for behavioral differences in drug seeking and relapse. This is positing that D1R-MSNs drive appetitive behavior and D2R-MSNs drive aversive behavior (25,26), although it is recognized that the mechanisms that drive these disparate behaviors are more nuanced (27).

In this study, we tested whether there are sex differences in NAcSh MSN excitability, both across the estrous cycle and following cocaine exposure. Using transgenic Drd1a-tdTomato and Drd2-eGFP mice and with sufficient statistical power for comparisons between sexes, we found both estrous cycle effects in MSN excitability and major sex differences in response to cocaine.

METHODS AND MATERIALS

Animals

Animal procedures were performed at the University of Minnesota in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and in accordance with protocols approved by the University of Minnesota Institutional Animal Care and Use Committee, as well as the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male and female mice with a single copy of Drd1a-tdTomato and/or Drd2-eGFP bacterial artificial chromosome transgene were maintained on a C57BL/6J genetic background, obtained from the Rothwell lab (University of Minnesota) (28) and bred onsite. Mice aged 8 to 16 weeks were used in all experiments and were group housed and kept on a 14/10-hour light/dark cycle with food and water ad libitum. Seventy-seven animals were used in total, with 48 animals used for electrophysiology recordings (n = 29 females and n = 19 males). For neuronal excitability experiments, 22 animals were used (n = 14 females and n = 8 males). For mEPSC experiments, 26 animals were used (n = 15 females and n = 11 males).

Psychomotor Sensitization

All experiments were conducted between 12:00 PM and 5:00 PM, with houselights on at 6:00 AM and off at 8:00 PM. Animals were handled and habituated to locomotor chambers as well as subcutaneous injections 2 days prior to behavioral testing. On test days, animals were given either a subcutaneous injection of cocaine (15 mg/kg) daily for 5 days (2) or an equivalent volume of 0.9% saline and placed promptly into the behavioral testing chamber (18″ × 9″, with 8.5″ tall walls) with light levels of 250 ± 10 lx. Videos were recorded for 80 minutes using ANY-maze tracking software, and total distance traveled was analyzed offline.

Chemicals

All chemicals were obtained from Sigma-Aldrich except cocaine hydrochloride, which was obtained from Boynton Pharmacy (University of Minnesota).

Whole-Cell Recordings

Mice (8–16 weeks old) in late abstinence (10–14 days after the last behavioral day) were used for electrophysiology recordings. Animals were sacrificed between 9:00 AM and 5:00 PM. For females, prior to being anesthetized, estrous cycle was determined by vaginal cytology as previously described (29,30). Mice were anesthetized with isoflurane (3% in O_2) and decapitated. The brain was rapidly removed and chilled in ice-cold cutting solution containing (in mM) 228 sucrose, 2.5 KCl, 7 $MgSO_4$, 1.0 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, and 11 d-glucose with a pH 7.3 to 7.4 and continuously gassed with 95:5 O_2:CO_2 to maintain pH and pO_2. A brain block was cut including the NAcSh region and affixed to a vibrating microtome (Leica VT 1000S; Leica). Sagittal sections of 240 μm thickness were cut, and the slices were transferred to a holding container of artificial cerebrospinal fluid maintained at 30 °C, continuously gassed with 95:5 O_2:CO_2 containing (in mM) 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, 11 d-glucose, and 1.0 ascorbic acid (osmolality: 295–302 mosmol/L; pH 7.3–7.4) (4,31) and allowed to recover for 1 hour. Following recovery, slices were transferred to a glass-bottomed recording chamber and viewed through an upright microscope (Olympus) equipped with differential interference contrast optics, a SOLA SE light engine, appropriate fluorescent filters, an infrared (IR) filter, and a fluorescence/IR-sensitive video camera (Dage-MTI).

Slices transferred to the glass-bottomed recording chamber were continuously perfused with artificial cerebrospinal fluid, gassed with 95:5 O_2:CO_2, maintained at room temperature, and circulated at a flow of 2 mL/min. Patch electrodes were pulled (Flaming/Brown P-97; Sutter Instrument) from borosilicate glass capillaries with a tip resistance of 5 to 10 MΩ. Electrodes were filled with a solution containing (in mM) 135 K-gluconate, 10 HEPES, 0.1 EGTA, 1.0 $MgCl_2$, 1.0 NaCl, 2.0 Na_2ATP, and 0.5 Na_2GTP (osmolality: 280–285 mosmol/L; pH 7.3) (7). D1R-MSNs and D2R-MSNs were identified under epifluorescence and IR–differential interference contrast based on morphology and their hyperpolarizing membrane potential (−70 to −80 mV) and were voltage clamped at −80 mV using a Multiclamp 700B amplifier (Molecular Devices), with currents filtered at 2 kHz and digitized at 10 kHz. Holding potentials were not corrected for the liquid junction potential. Once a GΩ seal was obtained, slight suction was applied to break into whole-cell configuration, and the cell was allowed to stabilize, which was determined by monitoring capacitance, membrane resistance, access resistance, and resting membrane potential (V_m) (7,32,33). Cells that met the following criteria were included in the analysis: action potential amplitude ≥50 mV from threshold to peak, resting V_m negative to −66 mV, and <20% change in series resistance during the recording. Passive membrane properties, capacitance, and membrane resistance were measured from the membrane test in pCLAMP (Molecular Devices). The resting neuronal membrane potential shown in Table 1 was recorded immediately after breaking into whole-cell mode.

To measure NAcSh MSN neuronal excitability, V_m was adjusted to −80 mV by continuous negative current injection, and a series of square-wave current injections was delivered in steps of + 20 pA, each for a duration of 800 ms.

For mEPSC recordings, slices transferred to the glass-bottomed recording chamber were continuously perfused with artificial cerebrospinal fluid containing lidocaine (0.7 mM)

Table 1. Passive NAcSh MSN Membrane Properties

Experimental Group	Capacitance, pF	V_m, mV	R_m, MΩ
Male D1R-MSN Saline, *n* = 17	86.4 ± 3.5	−78.7 ± 0.9	104.1 ± 5.2
Male D2R-MSN Saline, *n* = 17	80.6 ± 4.7	−75.7 ± 1.1	116.7 ± 7.7
p Value	.325	.044	.185
Male D1R-MSN Cocaine, *n* = 20	91.2 ± 3.4	−77.9 ± 0.9	96.7 ± 4.9
Male D2R-MSN Cocaine, *n* = 18	84.6 ± 4.2	−75.9 ± 0.8	111.8 ± 6.6
p Value	.223	.111	.07
Female Estrus D1R-MSN Saline, *n* = 16	86.1 ± 3.5	−76.8 ± 0.6	94.1 ± 4.5
Female Estrus D2R-MSN Saline, *n* = 13	88.9 ± 4.2	−73.7 ± 1.0	95.8 ± 7.2
p Value	.608	.007	.838
Female Estrus D1R-MSN Cocaine, *n* = 14	78.2 ± 3.1	−75.6 ± 1.5	106.9 ± 4.6
Female Estrus D2R-MSN Cocaine, *n* = 15	74.6 ± 4.0	−73.5 ± 1.0	127.7 ± 8.2
p Value	.487	.252	.043
Female Diestrus D1R-MSN Saline, *n* = 13	80.6 ± 3.4	−76.2 ± 1.5	89.2 ± 4.8
Female Diestrus D2R-MSN Saline, *n* = 14	83.3 ± 3.1	−74.6 ± 1.1	101.4 ± 7.6
p Value	.603	.394	.193
Female Diestrus D1R-MSN Cocaine, *n* = 15	81.3 ± 4.0	−76.5 ± 1.0	92.9 ± 3.9
Female Diestrus D2R-MSN Cocaine, *n* = 14	72.5 ± 3.2	−73.5 ± 1.6	97.4 ± 7.9
p Value	.101	.124	.615

D1R, D_1 receptor; D2R, D_2 receptor; MSN, medium spiny neuron; NAcSh, nucleus accumbens shell; R_m, membrane resistance; V_m, resting membrane potential.

to block voltage-gated sodium channels and picrotoxin (100 μM) to block $GABA_AR$ (gamma-aminobutyric acid A receptor), and was continuously gassed with 95:5 O_2:CO_2, maintained at room temperature, and circulated at a flow of 2 mL/min. Patch electrodes were pulled (Flaming/Brown P-97; Sutter Instrument) from borosilicate glass capillaries with a tip resistance of 5 to 10 MΩ, and whole-cell recordings were made. Electrodes were filled with a cesium methanesulfonate ($CsMeSO_4$) solution containing (in mM) 120 $CsMeSO_4$, 15 CsCl, 10 TEA-Cl, 10 HEPES, 0.4 EGTA, 8.0 NaCl, 2.0 Na_2ATP, and 0.3 Na_2GTP (osmolality: 280–285 mosmol/L; pH 7.3). D1R-MSNs and D2R-MSNs were identified under epifluorescence and IR–differential interference contrast based on morphology and their hyperpolarizing membrane potential (−70 to −80 mV) and were voltage clamped at −80 mV using a Multiclamp 700B amplifier (Molecular Devices), with currents filtered at 2 kHz and digitized at 10 kHz. Holding potentials were not corrected for the liquid junction potential. mEPSCs were recorded for 2 minutes and analyzed offline using Mini Analysis software (Synaptosoft) with an amplitude threshold set at 3 times the noise level.

Statistical Analysis

Data values were reported as mean ± SEM. All statistical analyses were performed with a commercially available statistical package (GraphPad Prism, version 9.4.1). Probabilities <5% were deemed significant a priori. Depending on the experiments, group means were compared using a paired Student's *t* test or a 1-way or 2-way repeated-measures analysis of variance (ANOVA). When differences were found, Bonferroni post hoc tests were used for multiple pairwise comparisons. For analyses collapsed across animals, D1R-MSN versus D2R-MSN comparisons were performed using a within-subject design whereas saline versus cocaine comparisons were performed using a between-subject design.

RESULTS

Cocaine Psychomotor Sensitization Does Not Differ by Sex in Drd1a-tdTomato and/or Drd2-eGFP Mice

Various genetic backgrounds can produce large sex differences in cocaine-mediated locomotor sensitization (34). Here, we used a mouse line where the actions of cocaine on male and female locomotion were extremely similar, and thus potential differences in NAcSh D1R-MSN and D2R-MSN physiology could not be attributed to differences in behavior. Studies followed previous methods, using a standard psychomotor sensitization protocol followed by 10 to 14 days of abstinence and then ex vivo whole-cell current- or voltage-clamp recordings of the NAcSh (Figure 1A) (2,34). The estrous cycle was also monitored prior to electrophysiology recordings to compare responses during diestrus (before hormonal surges) and estrus (after)—periods within the estrous cycle when the largest differences in MSN physiology (20) and behavior (24) have been reported.

Following 2 days of habituation during which all mice (*n* = 77) received saline in the behavioral testing chamber (Figure 1B), mice were subdivided, receiving either daily cocaine (15 mg/kg) or saline for the next 5 days (Figure 1C). This dose of cocaine has been used previously by our lab for psychomotor sensitization and examination of NAcSh neuroplasticity (2,4). This dosing has also been used in follow-up studies to examine the effects of cocaine on D1R- and D2R-MSN excitability in the NAcSh of male mice (9). Thus, we have attempted to maintain consistent comparisons in both cocaine psychomotor sensitization and neuroplasticity.

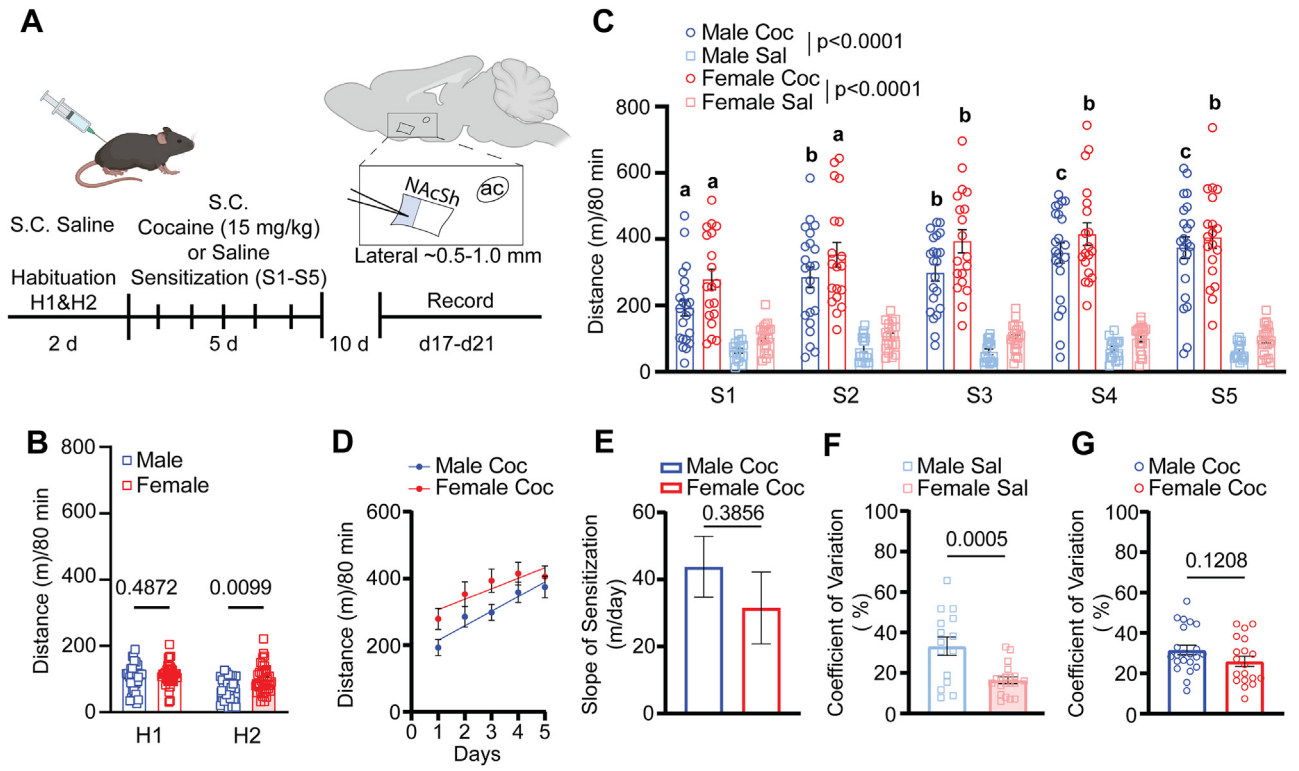

Figure 1. Cocaine psychomotor sensitization in male and female mice. **(A)** Experimental timeline and recording area of the NAcSh highlighted in light blue. **(B)** Two-day saline habituation (H1 and H2) summary data of grouped male (n = 37) and female (n = 40) mice. **(C)** Psychomotor sensitization, letter symbol (b) significantly different from (a) within sex and (c) significantly different from (a) and (b) within sex (cocaine: male n = 22, female n = 20; saline: male n = 15, female n = 20). **(D)** Linear regression of cocaine psychomotor sensitization. **(E)** Slope of locomotor sensitization. **(F)** Coefficient of variation for saline-treated animals. **(G)** Coefficient of variation for cocaine-treated animals. ac, anterior commissure; Coc, cocaine; NAcSh, nucleus accumbens shell; Sal, saline; S.C., subcutaneous.

Cocaine increased the total distance traveled in both male (n = 22) (2-way ANOVA, $F_{1,35}$ = 54.15, $p < .0001$) and female (n = 20) (2-way ANOVA, $F_{1,37}$ = 75.66, $p < .0001$) mice compared with their saline (n = 15 male, n = 20 female) counterparts. Sensitization to cocaine was observed in males starting on day 2 of cocaine (1-way repeated-measures ANOVA, $F_{4,66}$ = 27.38, $p < .0001$) treatment and starting on day 3 for females (1-way repeated-measures ANOVA, $F_{4,52}$ = 9.72, $p < .0001$). By day 5 of cocaine administration, both males and females were exhibiting similar locomotor responses. Notably, on day 2 of habituation, females displayed a small but significant increase in total distance traveled compared with their male counterparts when given saline (2-way ANOVA with Bonferroni post hoc test, p = .0099), a trend that continued in the saline-treated controls throughout subsequent days of testing. We also examined whether there were any sex differences in cocaine sensitization by analyzing the slope of total distance traveled on days 1 through 5 (Figure 1D). This analysis represented the average increase in distance traveled per day within sex compared with the previous day (34). We found no difference in the magnitude of sensitization between males and females (unpaired t test, p = .3856) (Figure 1E), again indicating minimal sex differences in the psychomotor response to cocaine in this line of transgenic mice.

For the last component of the behavioral analysis, we measured the coefficient of variation for each animal across the 5-day behavioral testing with respect to distance traveled and compared the coefficient across sexes. In saline-treated animals, females had a reduced coefficient of variation compared with their male counterparts (unpaired t test, p = .0005) (Figure 1F). This finding is consistent with a previous report showing that exploratory behavior was more consistent among female than male mice, regardless of estrous cycle (35), and that females did not increase behavioral variability (36). Interestingly, cocaine exposure normalized the coefficient of variation between sexes (Figure 1G), a finding consistent with what we observed in various other mouse strains (34). Overall, the data indicate similar locomotor sensitization following cocaine in these male and female mice.

The Estrous Cycle and Late Abstinence From Cocaine Alters NAcSh D1R- and D2R-MSN Excitability in a Sex-Dependent Manner

Next, we investigated cocaine-induced changes to excitability in the NAcSh among D1R-MSNs and D2R-MSNs during late abstinence (2). We recorded from a total of 186 neurons in the medial portion of the NAcSh. As others have reported (8–10), D1R-MSN excitability was reduced relative to D2R-MSN

excitability in saline-treated males (2-way ANOVA, $F_{1,32} = 6.70$, $p = .014$) (Figure 2A). Cocaine exposure followed by drug abstinence led to a more pronounced reduction in the firing frequency of D1R-MSNs in males (2-way ANOVA, $F_{1,35} = 9.76$, $p = .004$) (Figure 2B). No change was observed in male D2R-MSN excitability following cocaine treatment (2-way ANOVA, $F_{1,33} = 3.9 \times 10^{-3}$, $p = .95$) (Figure 2C). The net effect of cocaine in males is an augmented gap between D1R-MSN and D2R-MSN excitability, driven by reduced D1R-MSN activity (2-way ANOVA, $F_{1,36} = 55.70$, $p < .0001$) (Figure 2D, E).

For females in diestrus, neuronal excitability differences under baseline conditions were similar (if not greater) to the pattern seen in male animals, i.e., reduced D1R-MSN excitability compared with D2R-MSNs (2-way ANOVA, $F_{1,25} = 15.07$, $p = .001$) (Figure 2F). Interestingly, during estrus, the excitability of D1R-MSNs increased to the extent that there were no differences between D1R-MSNs and D2R-MSNs (2-way ANOVA, $F_{1,27} = 0.06$, $p = .804$) (Figure 2K). Notably, the magnitude of change in D1R-MSN excitability across the 4- to 5-day estrous cycle was comparable to the effect of cocaine in males (Figure 2B, K).

Cocaine administration to females produced additional unanticipated results. First, cocaine did not alter D1R-MSN (2-way ANOVA, $F_{1,26} = 1.01$, $p = .324$) (Figure 2G) or D2R-MSN (2-way ANOVA, $F_{1,26} = 0.01$, $p = .908$) (Figure 2H) excitability in females when measured during diestrus (Figure 2J). Second, during estrus, cocaine arrested D1R-MSN plasticity (2-way ANOVA, $F_{1,28} = 2.36$, $p = .136$) (Figure 2L) while simultaneously initiating D2R-MSN plasticity (2-way ANOVA, $F_{1,26} = 13.92$, $p = .001$) (Figure 2M). Specifically, there was an emergent increase in D2R-MSN excitability (Figure 2O). In total, cocaine flipped the effect of the estrous cycle on D1R-MSN/D2R-MSN excitability. Under drug-naïve conditions, the estrous cycle balances D1R-MSN/D2R-MSN activity through increased D1R-MSN excitability (Figure 2K), whereas following cocaine administration, the estrous cycle potentiated the discrepancy between the 2 subtypes of MSNs by enhancing D2R-MSN excitability (2-way ANOVA, $F_{1,27} = 33.92$, $p < .0001$) (Figure 2N). As an additional analysis, we also made comparisons across animals rather than across individual neurons. As shown in Table S1, the same comparisons were significant.

The passive membrane properties of D1R-MSNs and D2R-MSNs across groups are shown in Table 1. As previously reported (37,38), resting membrane potentials for D2R-MSNs tended to be more depolarized than those for D1R-MSNs, although this only reached significance in male and estrous females treated with saline. Data collapsed across all animals again indicated a significant effect in females, with a strong trend in males (Table S2).

Late Abstinence From Cocaine Differentially Impacts mEPSCs in a Sex-Dependent Manner

Given previous reports that cocaine can modulate glutamatergic synaptic activity in the NAcSh (8,11,12), we next tested the effect of cocaine on mEPSCs. As noted above, in the medial portion of the NAcSh, we recorded from 116 neurons. In males, we found enhancement of glutamatergic synaptic activity in cocaine-treated mice (Figure 3A–C). This was driven by increased mEPSC amplitude in both D1R-MSNs (unpaired t test, $p = .0193$) and D2R-MSNs (unpaired t test, $p = .0045$) (Figure 3C). For females, there were no estrous cycle effects in either mEPSC frequency or amplitude in saline-treated animals. For females recorded in diestrus (Figure 3D–F), cocaine caused enhancement of glutamatergic synaptic activity solely through an increase in D2R-MSN mEPSC amplitude (unpaired t test, $p = .0342$) (Figure 3F), although there was a strong trend for an increase in D2R-MSN frequency (unpaired t test, $p = .0561$) (Figure 3E) as well. For females recorded in estrus, cocaine also enhanced glutamatergic synaptic activity (Figure 3G–I). This was produced by an increase in mEPSC frequency (unpaired t test, $p = .0322$) (Figure 3H) and amplitude (unpaired t test, $p = .0018$) (Figure 3I), but again, only in D2R-MSNs. Finally, following cocaine treatment, an estrous cycle–dependent change developed in D1R-MSN mEPSC amplitude (Figure 3F, I: hatched bars), with greater amplitudes (unpaired t test, $p = .0082$) during estrus. Consistent with analyses between neurons, when comparing across animals, there were significant differences in mEPSC amplitudes on male MSNs following cocaine treatment, as well as an increase in mEPSC amplitude on female D2R-MSNs (Table S3).

DISCUSSION

This study is the first to explore the effects of cocaine on D1R-MSN versus D2R-MSN excitability of the NAcSh across sexes, with attention to the effects of estrous cycle before and after drug treatment. We have replicated previous findings that cocaine exposure leads to neuroadaptive effects in the NAcSh of male mice, primarily driven by an effect at D1R-MSN (8–11,13,39–42). This cocaine-induced D1R-MSN–driven plasticity is thought to be the primary contributor to the rewarding effects of the drug (43) as well as the locomotor response (13). In male mice, optogenetic stimulation of D1R-MSNs of the NAc mimicked cocaine reward, consistent with results in the dorsomedial striatum, where D1R-MSN optogenetic stimulation was deemed rewarding (25,26). It is notable that in contrast, D2R-MSN stimulation in these same studies was found to be aversive.

Data from female animals implicate alternative hypotheses regarding the role of D1R-MSNs and D2R-MSNs in cocaine action or possibly alternative rationales for female versus male drug responsiveness. As some investigators have hypothesized, it may be the gap or imbalance generated between D1R-MSN versus D2R-MSN activity in the NAc (13) that has a greater impact on the behavioral and rewarding effects of cocaine than strict D1R-MSN modulation alone. Even with opposite adaptations (decreased D1R-MSN excitability in males, increased D2R-MSN excitability in females), the resulting disparity between D1R-MSN and D2R-MSN excitability following cocaine is quite similar. Alternatively, if D2R-MSN activation is aversive in females as was observed in males, females might exhibit responses to cocaine more to minimize activation of D2R-MSNs, a neuronal population that exhibits heightened excitability. This could be contrasted with males, which may be motivated to drive D1R-MSN activation (which appears to be appetitive) from a population of neurons that exhibit decreased excitability.

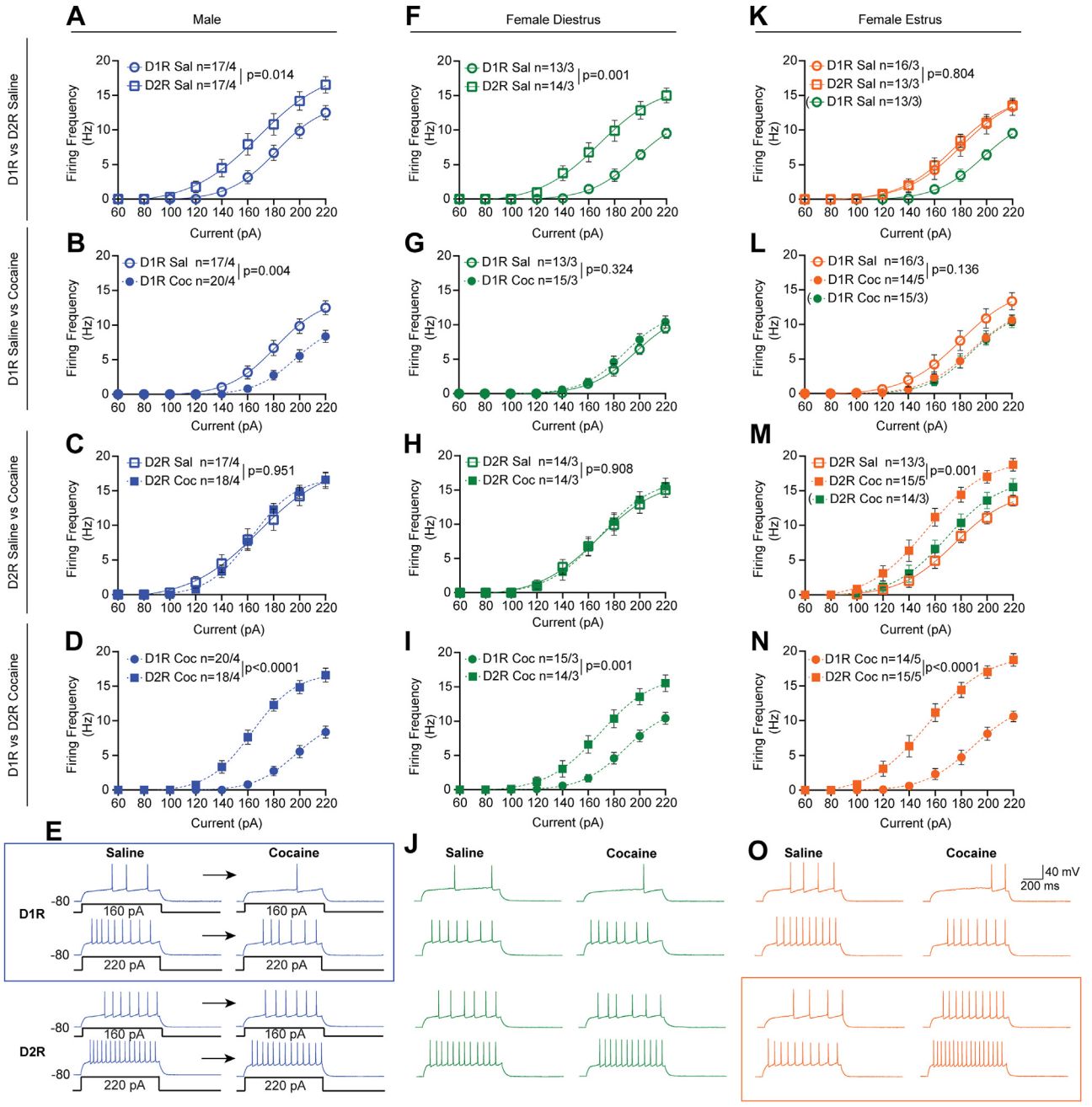

Figure 2. NAcSh neuronal excitability in male and female mice. **(A)** Summary data for saline-treated male D1R-MSN vs. D2R-MSN neuronal excitability. **(B)** Summary data for saline- vs. cocaine-treated male D1R-MSN neuronal excitability. **(C)** Summary data for saline- vs. cocaine-treated male D2R-MSN neuronal excitability. **(D)** Summary data for cocaine-treated male D1R-MSN vs. D2R-MSN neuronal excitability. **(E)** Representative raw traces from saline-treated and cocaine-treated male D1R-MSNs (top) and D2R-MSNs (bottom) from the NAcSh. **(F)** Summary data for saline-treated female D1R-MSN vs. D2R-MSN neuronal excitability recorded during diestrus. **(G)** Summary data for saline- vs. cocaine-treated female D1R-MSN neuronal excitability recorded during diestrus. **(H)** Summary data for saline- vs. cocaine-treated female D2R-MSN neuronal excitability recorded during diestrus. **(I)** Summary data for cocaine-treated female D1R-MSN vs. D2R-MSN neuronal excitability recorded during diestrus. **(J)** Representative raw traces from saline- and cocaine-treated female D1R-MSNs (top) and D2R-MSNs (bottom) from the NAcSh recorded during diestrus. **(K)** Summary data for saline-treated female D1R-MSN vs. D2R-MSN neuronal excitability recorded in estrus. Green overlay of D1R-MSN neuronal excitability from saline-treated females recorded in diestrus. **(L)** Summary data for saline- vs. cocaine-treated female D1R-MSN neuronal excitability recorded in estrus. Green overlay of D1R-MSN neuronal excitability from cocaine-treated females recorded in diestrus. **(M)** Summary data for saline- vs. cocaine-treated female D2R-MSN neuronal excitability recorded in estrus. Green overlay of D2R-MSN neuronal excitability from cocaine-treated females recorded in diestrus. **(N)** Summary data for cocaine-treated female D1R-MSN vs. D2R-MSN neuronal excitability recorded in estrus. **(O)** Representative raw traces from saline- and cocaine-treated female D1R-MSNs (top) and D2R-MSNs (bottom) from the NAcSh recorded during estrus. The number of neurons and mice per group is denoted as n = x/y, where x is neurons and y are mice. Each group consisted of 3 to 5 mice. Coc, cocaine; D1R, D_1 receptor; D2R, D_2 receptor; MSN, medium spiny neuron; NAcSh, nucleus accumbens shell; Sal, saline.

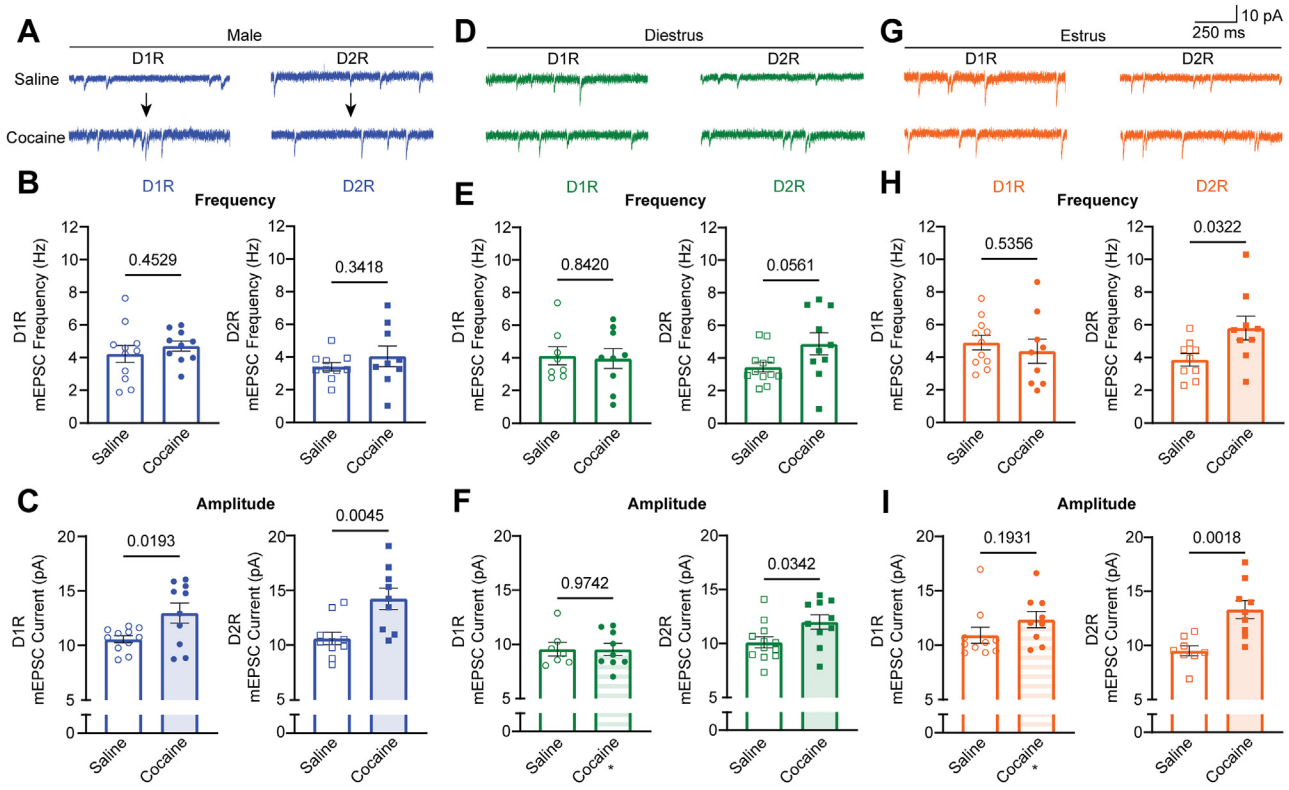

Figure 3. NAcSh mEPSCs in male and female mice. **(A)** Representative mEPSC traces from NAcSh MSNs for saline- and cocaine-treated male animals. **(B)** Summary NAcSh MSN D1R-MSN and D2R-MSN mEPSC frequencies for male animals treated with saline and cocaine. **(C)** Summary NAcSh D1R-MSN and D2R-MSN mEPSC amplitudes for male animals treated with saline and cocaine. **(D)** Representative mEPSC traces from NAcSh MSNs for saline- and cocaine-treated female animals recorded during diestrus. **(E)** Summary NAcSh D1R-MSN and D2R-MSN mEPSC frequencies for female animals treated with saline and cocaine, recorded during diestrus. **(F)** Summary NAcSh D1R-MSN and D2R-MSN mEPSC amplitudes for female animals treated with saline and cocaine, recorded during diestrus, $^*p < .05$ vs. **(I)**. **(G)** Representative mEPSC traces from NAcSh MSNs for saline- and cocaine-treated female animals recorded during estrus. **(H)** Summary NAcSh D1R-MSN and D2R-MSN mEPSC frequencies for female animals treated with saline and cocaine, recorded during estrus. **(I)** Summary NAcSh D1R-MSN and D2R-MSN mEPSC amplitudes for female animals treated with saline and cocaine, recorded during estrus. Neuronal recordings came from a minimum of 3 mice per treatment group (3–6 mice per group). D1R, D_1 receptor; D2R, D_2 receptor; mEPSC, miniature excitatory postsynaptic current; MSN, medium spiny neuron; NAcSh, nucleus accumbens shell.

It was hypothesized in a recent review that males are more likely to engage in drug usage for sensation seeking (i.e., positive reinforcement), whereas females are more likely to take drugs for self-medication (i.e., negative reinforcement), downward spirals that are exacerbated with continued use (22). It is provocative to consider that the sex differences reported here provide the neurophysiological underpinnings to support this hypothesis. Future experiments will further examine behavioral and physiological correlates related to sex differences and drug-taking behavior to test this theory.

Another important finding is that D1R-MSN plasticity is a normal process across the estrous cycle. Remarkably, the magnitude of change in D1R-MSN excitability across the estrous cycle was comparable to the effect of cocaine in males (see Figure 2B, K). This basic neurophysiological finding may help explain several behavioral phenomena across the female estrous cycle, such as reproductive behavior (e.g., seeking out a mate, copulation, etc.). Increased D1R-MSN neuronal excitability during the estrus phase of the estrous cycle should theoretically drive increased appetitive behaviors, including reproductive behavior and sexual receptivity (44). This could be contrasted with diestrus, when D1R-MSN excitability is lower, and females are not sexually receptive (44). These estrous cycle–dependent neurophysiological findings in the NAcSh may also be related to estrous cycle–dependent feeding habits. Feeding behavior in rats was lowest during proestrus/estrus and greatest during metestrus/diestrus (45). Furthermore, metestrus/diestrus animals exhibited greater motivation to work for sucrose pellets than proestrus/estrus females (45). In diestrus, we found that NAcSh D1R-MSN excitability was lower, which may drive diestrus female motivation for sucrose pellets (e.g., to increase D1R-MSN excitability). Again, this can be contrasted with what we found in the NAcSh during estrus, where D1R-MSNs are already excitable compared with diestrus animals.

In females, cocaine arrested D1R-MSN plasticity while simultaneously initiating estrous cycle–dependent changes in D2R-MSN. The implications of these results are numerous. One, if D1R-MSN and D2R-MSN reflect appetitive and aversive responses as described above, motivational fluctuations across the estrous cycle are based on entirely different processes in drug-naïve versus drug-treated individuals. Alterations in

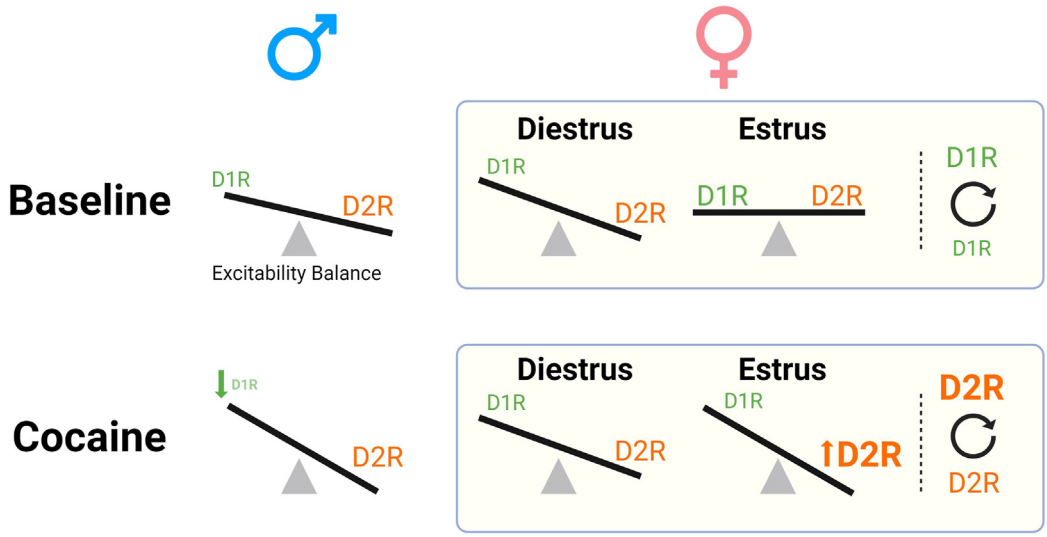

Figure 4. Sex differences in D1R- and D2R-MSN excitability in response to cocaine. In males, cocaine decreases D1R-MSN (D1R) excitability, furthering the baseline discrepancy between D1R- and D2R-MSN (D2R) excitability. For females, estrous cycle–dependent fluctuations in D1R-MSN excitability under baseline conditions results in a relative balance of activity during estrus. Following cocaine, changes in D1R-MSN plasticity are arrested, and an estrous cycle–dependent D2R-MSN plasticity emerges, exacerbating the discrepancy in excitability between these neuronal subtypes during estrus. Note that females in diestrus have virtually identical D1R- and D2R-MSN neuronal excitability with and without cocaine. D1R, D_1 receptor; D2R, D_2 receptor; MSN, medium spiny neuron.

NAcSh physiology following cocaine administration are also enduring, at least in males (2,7). It will be important to determine whether this shift in D1R-MSN to D2R-MSN plasticity in females across the estrous cycle endures not only for weeks, but also possibly for months or years. Furthermore, it will be important to determine the timing of when this switch occurs (the first day or last day of cocaine exposure or during the abstinence period) and whether it is mediated by NAc membrane estrogen receptors (46) or by estrous cycle–dependent ventral tegmental area–generated dopamine (24,47). In addition, while the mechanism by which cocaine produces these changes is currently unknown, it may also play a role in enhancement of cocaine conditioned place preference during estrus over diestrus (24), the time when D2R-MSNs exhibit heighted excitability.

Future studies will also need to determine whether other drugs of abuse produce similar sex-specific changes within the nervous system. Because the rodent estrous cycle repeats every 4 to 5 days, emphasis should be placed in understanding how the hypothalamus-pituitary-gonadal axis produces and/or aromatase activity in the NAc (48,49) contributes to these dynamic changes in neuronal activity. By having a greater understanding of the mechanisms by which the estrous cycle produces MSN plasticity (Figure 4), we may better identify treatments to reverse long-term drug-induced changes within these same populations of neurons across both sexes.

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ADC, CAN, PPJ and C-MHP performed experiments; ADC and CAN analyzed data; and ADC, CAN, MJT, and PGM prepared figures, drafted the manuscript, and interpreted the results of the experiments. All authors edited and revised the manuscript.

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