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Estradiol influences on dopamine release from the nucleus accumbens shell: Sex differences and the role of selective estradiol receptor subtypes

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Abstract

Background and purpose

Females are more sensitive than males to both the acute and prolonged effects of psychomotor stimulants. In females, this is regulated by estradiol, which enhances dopamine (DA) release in the dorsal striatum. In this study we tested the acute effect of estradiol on DA release in the nucleus accumbens (NAc) shell after cocaine administration and investigated which estradiol receptors (ER) contribute to sex differences in the response to cocaine.

Experimental Approach

The ability of estradiol benzoate (EB) to acutely modulate the effect of cocaine on phasic DA release in the NAc shell was measured by fast-scan cyclic voltammetry in anesthetized male and female rats. In addition, the role of ER subtypes ERα and ERβ was determined through use of selective agonists.

Key Results

EB acutely enhanced the effect of cocaine on stimulated DA release from the NAc shell in females but not in male rats only at levels of stimulation that are expected to optimally saturate dopamine transporters. Enhanced DA release after cocaine administration was also observed in females after selective activation of ERβ but not ERα. EB attenuated the effect of cocaine on NAc shell DA reuptake in males but not females.

Conclusions and Implications

Estradiol acutely and rapidly regulates DA release in females and DA reuptake in males. In females, estradiol rapidly enhances the effect of cocaine on DA release, likely via activation of ERβ. The effect of estradiol in males is not seen with selective receptor subtype activation, a topic deserving of further study.

Linked Articles

Keywords: Estradiol, sex differences, cocaine, dopamine, fast-scan cyclic voltammetry, nucleus accumbens

Abbreviations

DA, dopamine; DPN, diarylpropionitrile; EB, estradiol benzoate; ER, estradiol receptor; Hz, hertz; NAc, nucleus accumbens; OVX, ovariectomy/ovariectomized; p, pulse; VTA, ventral tegmental area

Introduction

Sex differences in striatal circuitry affect both the initial response to psychomotor stimulants as well as the propensity to develop addiction-like behaviors after prolonged use (Becker and Koob, 2016). In clinical populations, women present for treatment earlier than men, and report taking more drug, even though they have been using for less time (Brady and Randall, 1999). After treatment, women have lower rates of sustained abstinence, which is attributed to a greater susceptibility to both cue and stress induced relapse (Potenza et al., 2012). Similar sex differences have been found in preclinical rodent models. Female rats acquire cocaine self-administration more rapidly, show greater escalation of cocaine self-administration, and maintain levels of cocaine intake at higher levels than males (Becker and Hu, 2008).

Sex differences in sensitivity to psychomotor stimulants are in part due to differences in the release of gonadal hormones, which also exert different effects on reward systems. Removal of gonadal hormones by ovariectomy (OVX) attenuates the behavioral response to cocaine in females, while removal of the testes has no effect on the response in males (Hu et al., 2004; Jackson et al., 2006). Estradiol replacement to OVX females results in enhanced responses to psychomotor stimulants, underscoring the importance of estradiol for mediating the effects of cocaine in females (Becker and Rudick, 1999a; Becker et al., 2001; Hu et al., 2004; Cummings et al., 2014). Importantly, estradiol treatment does not enhance cocaine self-administration or stimulant-induced behavior in males (Becker et al., 2001; Jackson et al., 2006), indicating a sex difference in the activational effects of estradiol and organizational sex differences in the neural systems mediating drug taking.

Estradiol enhancement of the behavioral responses to psychomotor stimulants has been linked to changes in striatal dopamine (DA) release. Within the dorsal striatum, estradiol acutely enhances DA release in females but not in males (Cummings et al., 2014; Shams et al., 2016). A substantial body of work has demonstrated that estradiol acts directly on GABAergic striatal tissue to disinhibit DA terminals and promote DA release within the striatum (Yoest et al., 2018). However, while the effects of estradiol in dorsal striatum have been well established, studies on the rapid effects of estradiol on DA release within the nucleus accumbens (NAc) have produced mixed results. Some studies have shown that estradiol is able to act directly on NAc circuitry to rapidly enhance stimulated DA release (Thompson and Moss, 1994), others have demonstrated an effect of estrous cycle but no effect of direct estradiol application (Calipari et al., 2017), and still others have failed to show an acute effect of estradiol at all (Cummings et al., 2014).

Of note, many of the studies that have seen an effect of ovarian hormones, either by direct application of estradiol or measurement of estradiol across the estrous cycle, have measured DA using fast-scan cyclic voltammetry, while studies that failed to show an effect used microdialysis. Fast-scan cyclic voltammetry measures phasic DA release on the scale of seconds within discrete subregions of the NAc, while experiments using microdialysis measures slower changes in tonic release within the NAc as whole. Estradiol may have opposite effects within the NAc core and shell, and decreased regional specificity in experiments using microdialysis likely contributes to the lack of effects in these studies (Peterson et al., 2015).

Additionally, for studies using fast-scan cyclic voltammetry, differences in estradiol dose may account for discrepancies in findings. The range at which estradiol modulates cellular function is very narrow (Becker, 1990; Tanapat et al., 2005). Doses that are lower than this range fail to have an effect, and higher concentrations either have no effect or the opposite effect, presumably due to disruption of the cell membrane (Whiting et al., 2000). Therefore, we hypothesized that an

acute, physiological dose of estradiol would enhance phasic DA release specifically within the NAc shell.

Here, we sought to characterize the receptor mechanism by which estradiol could modulate DA release within the NAc shell. There are multiple estradiol receptor subtypes, the most well-studied of which are ERα and ERβ (Morissette et al., 2008). These receptors can be expressed either within the cytosol or bound to plasma membrane and are differentially expressed within striatal circuitry. While ERα is expressed on GABAergic interneurons and DAergic terminals within the NAc, ERβ has only be found within the ventral tegmental area (VTA; (Mitra et al., 2003; Almey et al., 2015). Therefore, the goal of the current study was to determine which estradiol receptor subtypes may mediate estradiol's ability to modulate stimulated DA release after systemic cocaine treatment.

Methods

Animals

Male (n=28) and female (n=29) Sprague Dawley rats (Charles River Breeding Laboratory, Portage, MI, RRID:RGD_734476) 50-55 days of age were maintained on 14:10 L:D cycle (lights on at 9:00 AM) and housed in same-sex pairs in standard laboratory cages with *ad libitum* access to water and phytoestrogen free rat chow (2017 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were carried out in accordance with the National Institutes of Health guidelines on laboratory animal use and care, using a protocol approved by University of Michigan Institutional Animal Care and Use Committee. Animals were gonadectomized as described previously (Cummings et al., 2014). Absence of estrous cycle in ovariectomized females was verified by daily vaginal lavage, and animals were left otherwise undisturbed in their home cage for two weeks following gonadectomy.

Materials

Estradiol benzoate (EB; Sigma Aldrich, St. Louis, MO, Cat No. E8515, CAS #50-50-0) was suspended in peanut oil at a concentration of 5μg per 0.1 ml and left on a stir plate overnight prior to use. EB was stored at room temperature for the duration of use. EB is an estradiol ester that is commonly used in hormone priming experiments due to its increased stability when compared to free estradiol. In previous work from the Becker laboratory we have found that plasma estradiol was significantly increased at 30 minutes and 1 hour following subcutaneous administration of EB (Hu et al., 2004). Additionally, we have repeatedly shown an effect of EB on stimulated DA in microdialysate within 30 minutes, indicating that this time is sufficient to induce an estrogenic effect on the DA response to psychomotor stimulants (Castner et al., 1993; Becker and Rudick, 1999b; Cummings et al., 2014). The ERα selective agonist propyl pyrazole triol (PPT; Tocris, Minneapolis, MN. Cat No. 1426, CAS #263717-53-9) and the ERβ selective agonist diarylpropionitrile (DPN, Tocris, Minneapolis, MN, Cat. No. 1494, CAS #1428-67-7) were suspended in gelatin at a concentration of 1mg/ml. Agonists were stored at 4^{o}C until the day of use, at which time syringes were prepared and brought to room temperature. All hormones and agonists were used within two weeks of preparation.

Fast-Scan Cyclic Voltammetry Surgery

On the day of surgery, animals were anesthetized with urethane (1.5g/kg, IP in 0.9% sterile saline; Sigma Aldrich, St. Louis, MO) and prepared in a stereotaxic frame (Kopf Instruments, Tujunga, CA). A glass-encased cylindrical carbon fiber electrode was lowered into the dorsal portion of the NAc shell (AP, +1.7; ML, ± 0.8; DV, -6) and a silver / silver chloride reference electrode was placed in the contralateral cortex (AP, -2.3; ML ±2.7). DA release was recorded by oxidation and reduction of DA in response to the application of a triangular waveform (oxidative scan, 0.4-1.3 V; reductive scan, 1.3-0.4 V; 400V/s) applied to the carbon fiber electrode. Prior to taking recordings of stimulated release, this waveform was repeatedly cycled at a rate of 60 Hertz (Hz) for 30 minutes, followed by another 10 minutes of cycling at 10 Hz. All recordings of

stimulated release were taken at 10 Hz. A bipolar stimulating electrode (AP, -5.2; ML, ±0.8) was lowered into the VTA incrementally starting at -7 DV while periodically checking for DA release using 60Hz 60 pulse (p) biphasic stimulations. Once release was optimized, three recordings were taken during application of a 20Hz 24p stimulation to the VTA in order to verify stability of DA response; recordings were considered stable if peak release during these recordings varied by less than 10%. Stability of the electrode was also verified periodically at the beginning and end of each stage of the experiment. After placement of the recording and stimulating electrodes, rats then received a subcutaneous intrascapular injection of either EB (5μg/0.1ml peanut oil) or vehicle (peanut oil; 0.1ml). The ERα selective agonist PPT (1mg/kg) or the ERβ selective agonist DPN (1 mg/kg) were used to determine the role of estradiol receptor subtypes. For all groups, baseline recordings were taken 30 minutes following treatment. The experimental timeline is outlined in Figure 1A.

Fast-Scan Cyclic Voltammetry Recordings

For recordings of stimulated release, ten recordings were taken for each stimulation parameter. Stimulations of 20Hz 24p, 20Hz 12p, 10Hz 24p, and 10Hz 12p were applied in order to determine whether the effect of estradiol on DA signaling after cocaine administration is dependent on the amount of DA release. The highest stimulation parameter, 20Hz 24p, was selected based on previous work demonstrating that stimulation within this range saturates transporters and leads to steady state overflow kinetics (Wightman et al., 1988; Wightman and Zimmerman, 1990). Representative current traces (Figure 1A, B) and color plots (Figure 1C, D) are presented in Figure 1. Following baseline recordings, animals received an IP injection of Cocaine HCl (10mg/kg, NIDA). After cocaine administration, a second set of recordings were taken using the same stimulation parameters. All recordings were taken by an experimenter blind to the animal treatment groups.

Data Processing and Analysis

Analysis of stimulated DA release and uptake was conducted using DEMON Voltammetry software provided courtesy of Dr. Sara R. Jones at Wake Forest University (Yorgason et al., 2011). Analysis was conducted without reference to animal treatment groups. From each recording, peak DA concentration was extracted as a measure of DA release, and Tau was calculated as a measure of DA reuptake. Tau is an exponential decay constant that has been recommended for quantifying DA reuptake after stimulated release (Yorgason et al., 2011). Tau is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. Therefore, treatment with cocaine, which blocks DA transporters (DAT) and prevents reuptake, leads to an increase in Tau. This method of quantifying DA release and reuptake is recommended when stimulation does not induce substantial synaptic overflow, and therefore does not fulfill the assumptions required for Michaelis-Menten modeling techniques. At lower levels of stimulation (10Hz 24p and 10Hz 12p), the DA signal was not sufficient to reliably extract release and reuptake values for all animals; such animals (10Hz 24p: n=2, 10Hz 12p: n=6) were excluded from further analysis for these parameters. At the 10Hz 12p level of stimulation, this resulted in less than five animals in one group (DPN females). As such, this data should be considered exploratory. The numbers of animals per group included in the final analysis at each stimulation parameter are included with the data in Table 1.

There is substantial individual variation in the amount of DA release measured both before and after cocaine administration (Table 1). This variability may be due to individual differences in DA signaling but may also be due to electrode performance or differences in the placement of both the recording and stimulating electrodes. In order to account for these factors, the percent change from baseline was used to analyze group differences in the effect of cocaine on NAc DA release. Average DA release and reuptake at each stimulation parameter was calculated for each animal, and the % change from baseline was obtained by dividing the post-cocaine values by the pre-cocaine values and multiplying by 100. Data are expressed as % change from baseline in

order to control for variability in the sensitivity of electrodes used or individual differences in baseline DA release and reuptake.

Experimental Design

Target group sizes (n=6) were calculated based on effect sizes found in previous studies from our lab (Cummings et al., 2014). Power analysis (α=0.05, 1-β=0.9) using an estimated effect size of 1 indicated a minimum necessary sample size of 6 animals per treatment group. Male and female animals were assigned to receive either EB (females: n=8, males: n=6), the ERα selective agonist PPT (females: n=8, males: n=8), the ERβ selective agonist DPN (females: n=6, males: n=7), or vehicle (females: n=7, males: n=7). Due to the high rate of detection failure in anesthetized voltammetry experiments due to missed probe sites, lack of recording stability, or premature mortality, animals were tested in pseudorandom order until minimally required group size was reached for each condition.

Statistical Analysis

The data and statistical analyses comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). Group comparisons were performed using GraphPad Prism v7.0a (GraphPad, San Diego, CA; RRID:SCR_002798). Data were analyzed using two-way ANOVA with Holm-Sidak post hoc tests to compare animals treated with hormones or agonists to controls within sex, as well as to determine if there were sex differences in the effect of each treatment. The accepted value for statistical significance was set to $p<0.05$, and post hoc tests were only run when initial F values reached this threshold.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to

PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18(Alexander et al., 2017).

Results

Effect of estradiol on stimulated DA release after cocaine administration

The effect of estradiol on stimulated DA release after cocaine administration was assessed at four levels of stimulation. Stimulation of VTA with 20Hz 24p was used to promote maximal occupancy of DAT without exceeding a balance between release and reuptake. Lower levels of stimulation were used to assess changes in DA release and reuptake after cocaine, when release did not achieve maximal reuptake thresholds.

At the highest stimulation parameter (Figure 2A), there was a significant effect of sex ($F(1,49)=5.24$), but no significant effect of treatment or interaction between sex and treatment. In animals treated with vehicle, there was no sex difference in the effect of cocaine on DA release demonstrating that there were no pre-existing sex differences in the response to cocaine in gonadectomized animals. There was a sex difference, however, in animals treated with estradiol, where EB-treated females showed a significantly greater effect of cocaine on DA concentration compared to EB-treated males (Figure 2A). The cocaine induced increase in DA overflow was also greater in EB-treated females compared to vehicle treated females. Together, these results demonstrate that treatment with estradiol enhanced the effect of cocaine on stimulated DA release in females, but not in males, when stimulation parameters promoted maximal DAT occupancy.

At the 20Hz 12p stimulation level (Figure 2B), there was a significant main effect of sex ($F(1, 49)=4.71$) but no significant effect of treatment or interaction between sex and treatment. Multiple comparisons revealed no significant differences in any of the treatment groups, though

there was a trend toward significance where EB treated females showed slightly enhanced DA release compared to vehicle ($p<0.10$).

After 10Hz 24p stimulation (Figure 2C), there was a significant main effect of treatment on DA release ($F(3,47)=5.85$) but no effect of sex and no significant interaction. Subsequent analysis showed no significant differences between any of the individual treatment groups. There was also no significant effect of treatment or sex on cocaine enhanced DA release after 10Hz 12p stimulation of the VTA, nor a significant interaction between the two (Figure 2D).

Estradiol enhancement of cocaine's effect on DA release is mediated by ERβ

Treatment with the ERβ selective agonist DPN increased the effect of cocaine on stimulated DA release in females at the highest stimulation parameter (Figure 3). There was also a sex difference in animals treated with DPN, where DPN-treated females showed greater enhancement of DA release following cocaine compared to DPN-treated males when stimulation parameters promoted maximal DAT occupancy. There was no effect of DPN during sub-maximal stimulation of the VTA, although there was a trend toward a significant enhancement of DA release after DPN in females after 20Hz 12p stimulation of the VTA.

The ERα selective agonist PPT had no effect on DA release in either sex (Figure 3).

Effect of estradiol on DA reuptake after cocaine administration

After the 20Hz 24p stimulation, there was a significant interaction between sex and treatment on DA reuptake as indicated by an effect on Tau ($F(3, 49)= 3.34$; Figure 4). There was no preexisting sex difference in the effect of cocaine on DA reuptake. However, there was a sex difference in the effect of estradiol, where males showed reduced sensitivity to the effect of cocaine on DA reuptake compared to within-subject vehicle, while females did not (Figure 4). Neither the ERα selective agonist PPT or the ERβ selective agonist DPN had a significant effect

on reuptake in either sex (Figure 5) and there was no significant effect of treatment, sex, or interaction between treatment and sex at any of the lower stimulation parameters (Figure 4B-D & Figure 5).

Discussion

Estradiol increases DA release in response to cocaine in females by an ERβ mediated mechanism

Estradiol treatment rapidly enhanced the effect of cocaine on stimulated DA release in the NAc shell of female rats by an ERβ mediated mechanism. Estradiol increased DA reuptake in males, but the receptor mechanism was not identified. Both effects were only seen at the maximal level of stimulation used, and not at lower levels of stimulation. These data clarify the role of estradiol on ventral striatal DA release and provide a potential mechanism by which estradiol renders females more susceptible to drug abuse.

The effect of estradiol in females was only significant when release was elicited by stimulation of the VTA that was expected to saturate DAT, although the general direction of an effect of estradiol on DA release after cocaine administration was still apparent at lower levels of stimulation in females. These results suggest that the rapid effects of estradiol in the NAc shell of females are affecting the stimulated increase in DA release, since the effect is only seen when DAT is fully occupied. VTA DA neurons show both tonic (0.5-8Hz) and phasic (15-50Hz) patterns of activity (Zhang and Sulzer, 2004). Phasic burst firing of DA neurons has been linked to the detection and prediction of salient environmental events, while tonic DA is associated with changes in overall motivation and willingness to work (Schultz, 1998; Niv et al., 2007; Tsai et al., 2009; Hamid et al., 2016). Estradiol enhancement of cocaine's effects on phasic DA release only at this highest level of stimulation may indicate that estradiol heightens the ability of cocaine to enhance DA release in response to highly salient environmental events, without altering tonic release or phasic release elicited by less salient stimuli. It is important to note that

the VTA stimulation used here does not selectively stimulate DA neurons within the VTA, but rather non-selectively stimulates various classes of neurons within the VTA (Tsai et al., 2009). Therefore, any conclusions regarding the role of estradiol on physiological conditions of DA release drawn from these data are speculative, and future work using methods that selective stimulate different cell populations within the VTA (e.g. optical stimulation) or work in awake behaving animals will be necessary to understand the physiological relevance of estradiol modulation of cocaine's enhancement of NAc DA release.

The reinforcing properties of cocaine and other psychomotor stimulants are strongly linked to their ability to enhance striatal DA transmission. Blockade of D1 DA receptors in both the NAc and VTA reduces the reinforcing effects of cocaine and disrupts cocaine induced conditioned place preferences (Maldonado et al., 1993; Ranaldi and Wise, 2001; Nazarian et al., 2004). Sex differences in these behaviors are likely due to sex differences in the underlying DA response. Females show greater acquisition and escalation of drug taking and establish conditioned place preferences for stimulants at lower doses than do males (Russo et al., 2003; Hu et al., 2004). Importantly, these sex differences are dependent on circulating gonadal hormones, where estradiol enhances the effects of stimulant drugs in ovariectomized females but not in castrated males.

One model of how ovarian hormones enhance the effect of cocaine has proposed that shifts in the excitability of VTA DA neurons during estrus lead to increased expression of the phosphorylated form of DAT, to enhance cocaine binding efficacy (Calipari et al., 2017). It is possible that increased DAT activation could increase DA release. On the other hand, cocaine-induced increases in NAc DA release are still apparent in mice lacking DAT, so other mechanisms may regulate DA signaling within this brain area (Carboni et al., 2001). Importantly, DAT is not required for behavioral expression of cocaine reward. Mice lacking DAT will still acquire cocaine self-administration and show conditioned place preferences for cocaine (Rocha et al.,

1998; Sora et al., 1998). Furthermore, although DAT is not required for the effects of cocaine on DA release or cocaine reinforcement, it is still necessary for changes in DA reuptake after cocaine administration (Budygin et al., 2002). This underscores the importance of changes in DA release, and not reuptake, in drug taking behaviors. This is further supported by our findings that estradiol, which enhances drug taking in females but not males, alters DA release without an effect on reuptake in female rats.

Estradiol may also be able to rapidly modulate DA signaling by altering binding of D2 DA receptors. Within the dorsal striatum, estradiol rapidly decreases D2 DA receptor binding by altering the proportion of high vs low affinity D2 DA receptors (Lévesque and Di Paolo, 1988; Bazzett and Becker, 1994). Decreased D2 DA receptor binding may lead to reduced autoreceptor mediated suppression of DA release and subsequent increases in DA release (Schmitz et al., 2002). This may be particularly important considering how studies of both clinical populations and non-human primates have highlighted the role of D2 DA receptors in the reinforcing effects of psychomotor stimulants and the development of substance use disorders (Volkow et al., 1999, 2001; Nader et al., 2006).

Effects of estradiol on cocaine induced DA release are mediated by ERβ

We tested whether selective activation of ER subtypes would also modulate the effects of cocaine on DA release in male and female rats. Activation of ERβ alone was sufficient to enhance the effect of cocaine on stimulated DA release. The ERβ selective agonist, DPN, but not the ERα selective agonist, PPT, duplicated the effects of estradiol on cocaine-induced DA release in females, where selective activation of ERβ enhanced the effect of cocaine on DA release compared to both vehicle-treated females and DPN-treated males. This is not the first report that ERβ activation enhances the response to cocaine in females. ERβ has also been shown to mediate the effects of chronic estradiol on expression of D2 receptors and DAT within the NAc (Morissette et al., 2008). Activation of ERβ within the NAc also mediates estradiol's

enhancement of conditioned place preference to both cocaine and amphetamine (Silverman and Koenig, 2007; Satta et al., 2018). The findings presented here confirm the importance of ERβ in mediating sex differences in the response to cocaine and provide a potential mechanism for how estradiol enhances DA activity in females.

ERβ is not expressed locally within the NAc, but studies in mice have found ERβ within the VTA (Creutz and Kritzer, 2002). This would be consistent with the idea that estradiol enhances excitability of VTA DA neurons, rather than altering release within the NAc (Calipari et al., 2017; McHenry et al., 2017). However, this directly contradicts previous work showing rapid increases in K^+-stimulated DA release after direct application of estradiol to NAc (Thompson and Moss, 1994). The discrepancy in these results may indicate that estradiol alters DA release via multiple mechanisms. Importantly, studies using intact cycling females have produced results indicating that estradiol enhances excitability of VTA DA neurons, while experiments on the acute effect of exogenously applied estradiol have not. It is possible that estradiol acutely enhances DA release via direct actions on striatal circuitry, while slower changes in levels of estradiol and progesterone seen in intact cycling females leads to increased excitability of VTA DA neurons, perhaps via actions in hypothalamic nuclei that project to the striatum, including the MPOA (McHenry et al., 2017). The results reported here do not directly address where estradiol acts within striatal circuitry, particularly since we non-discriminately stimulated all neurons and fibers of passage within the VTA in order to induce NAc DA release.

However, the ability of selective activation of ERβ to enhance DA release after cocaine administration may provide further indication of the specific mechanism by which estradiol regulates DA release within this pathway. Although studies have not established the expression of ERβ within the NAc, there is indirect evidence for ERβ activity in this region. Knockdown of ERβ mRNA within the NAc prevented the effect of estradiol on cocaine-conditioned place preference, as well as estradiol enhancement of cocaine-induced NAc cFos (Satta et al., 2018).

Future work, utilizing site-specific microinjections to either the NAc or VTA, could clarify where estradiol is acting to alter the DA response to cocaine. It is also possible that ERs within hypothalamic or amygdala circuitry indirectly modulate DA release, leading to increased DA signaling in response to cocaine.

Estradiol modulates the effect of cocaine on DA reuptake after VTA stimulation in males

Consistent with previous research, we did not see an effect of estradiol on DA release in males. However, males treated with estradiol showed a decreased effect of cocaine on DA reuptake after electrical stimulation of the VTA. To our knowledge, this is the first evidence that estradiol rapidly modulates NAc DA transmission in males. Neither the ERβ or ERα selective agonist alone reduced changes in DA reuptake after cocaine. There are multiple potential explanations for this inconsistency. The decreased DA reuptake after estradiol administration, in the presence of systemic cocaine treatment, may require an interaction between ERα and ERβ. These receptors are known to interact and have potentially opposing effects to one another. In particular, ERβ often regulates the effects of ERα, which could explain the differences after selective and non-selective ER activation seen here. Alternatively, estradiol may act through another ER to attenuate the effect on DA reuptake seen after cocaine treatment. G-protein estradiol receptor-1 (GPER-1) is expressed in the NAc in female rats, and GPER-1 activation may be responsible for the attenuated changes in DA reuptake seen in EB treated males in this study.

Conclusion

These data add to the growing body of literature on sex differences in the neurochemical and behavioral responses to psychomotor stimulants. We provide further evidence of an effect of estradiol on DA release within the NAc mediated by ERβ and establish that this effect is absent in males. In addition, the findings reported here are the first to determine the estradiol receptor subtype responsible for the effects of estradiol on NAc DA release after cocaine. DA release

within the NAc is integral to the rewarding and addictive properties of cocaine, and sex differences in the cocaine induced DA release likely contributes to the greater vulnerability to addictive behaviors in females. As estradiol is known to regulate the DA response to drugs of abuse in females, anti-estradiol treatments have been proposed as a possible therapeutic for stimulant abuse (Mikelman et al., 2017). However, the significant side effects associated with these drugs, particularly side effects mediated by actions at ERα, are a barrier to their widespread use. Here we demonstrate that ERβ mediates the effects of estradiol on DA release within the NAc and thus may be a better target for development of pharmacotherapies for stimulant abuse in females. Future work characterizing how estradiol mediates acute responses to drugs of abuse should be prioritized in understanding sex differences in addiction. In particular, determining whether the estradiol similarly modulates DA release within the NAc core will be helpful in understanding how these distinct brain areas might uniquely contribute to sex differences in the response to cocaine.

Author Contributions

K.E.Y collected the data for Figures 3 & 5 and analyzed data for all experiments; J.A.C. collected data for Figures 2 & 4; J.A.C and J.B.B. designed the experiment; and K.E.Y, J.A.C, and J.B.B. wrote the manuscript.

Competing Interests

None

Bibliography

Alexander, S.P., Cidlowski, J.A., Kelly, E., Marrion, N.V., Peters, J.A., Faccenda, E., et al. (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Nuclear hormone receptors. Br. J. Pharmacol. *174 Suppl 1*: S208–S224.

Almey, A., Milner, T.A., and Brake, W.G. (2015). Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. Horm. Behav. *74*: 125–138.

Bazzett, T.J., and Becker, J.B. (1994). Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. Brain Res. *637*: 163–172.

Becker, J.B. (1990). Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. Synapse *5*: 157–164.

Becker, J.B., and Hu, M. (2008). Sex differences in drug abuse. Front Neuroendocrinol *29*: 36–47.

Becker, J.B., and Koob, G.F. (2016). Sex differences in animal models: focus on addiction. Pharmacol. Rev. *68*: 242–263.

Becker, J.B., Molenda, H., and Hummer, D.L. (2001). Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. Ann. N. Y. Acad. Sci. *937*: 172–187.

Becker, J.B., and Rudick, C.N. (1999a). Rapid Effects of Estrogen or Progesterone on the Amphetamine-Induced Increase in Striatal Dopamine Are Enhanced by Estrogen Priming. Pharmacology Biochemistry and Behavior *64*: 53–57.

Becker, J.B., and Rudick, C.N. (1999b). Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. Pharmacol. Biochem. Behav. *64*: 53–57.

Brady, K.T., and Randall, C.L. (1999). Gender differences in substance use disorders. Psychiatr Clin North Am *22*: 241–252.

Budygin, E.A., John, C.E., Mateo, Y., and Jones, S.R. (2002). Lack of cocaine effect on dopamine clearance in the core and shell of the nucleus accumbens of dopamine transporter knock-out mice. J. Neurosci. *22*: RC222.

Calipari, E.S., Juarez, B., Morel, C., Walker, D.M., Cahill, M.E., Ribeiro, E., et al. (2017). Dopaminergic dynamics underlying sex-specific cocaine reward. Nat. Commun. *8*: 13877.

Carboni, E., Spielewoy, C., Vacca, C., Nosten-Bertrand, M., Giros, B., and Chiara, G. Di (2001). Cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens of mice lacking the dopamine transporter gene. J. Neurosci. *21*: RC141: 1–4.

Castner, S.A., Xiao, L., and Becker, J.B. (1993). Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. Brain Res. *610*: 127–134.

Creutz, L.M., and Kritzer, M.F. (2002). Estrogen receptor-beta immunoreactivity in the midbrain of adult rats: regional, subregional, and cellular localization in the A10, A9, and A8 dopamine cell groups. J. Comp. Neurol. *446*: 288–300.

Cummings, J.A., Jagannathan, L., Jackson, L.R., and Becker, J.B. (2014). Sex differences in the effects of estradiol in the nucleus accumbens and striatum on the response to cocaine: neurochemistry and behavior. Drug Alcohol Depend. *135*: 22–28.
Curtis, M.J., Bond, R.A., Spina, D., Ahluwalia, A., Alexander, S.P.A., Giembycz, M.A., et al. (2015). Experimental design and analysis and their reporting: new guidance for publication in BJP. Br. J. Pharmacol. *172*: 3461–3471.
Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Vander Weele, C.M., et al. (2016). Mesolimbic dopamine signals the value of work. Nat. Neurosci. *19*: 117–126.
Harding, S.D., Sharman, J.L., Faccenda, E., Southan, C., Pawson, A.J., Ireland, S., et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucleic Acids Res. *46*: D1091–D1106.
Hu, M., Crombag, H.S., Robinson, T.E., and Becker, J.B. (2004). Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology *29*: 81–85.
Jackson, L.R., Robinson, T.E., and Becker, J.B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology *31*: 129–138.
Lévesque, D., and Paolo, T. Di (1988). Rapid conversion of high into low striatal D2-dopamine receptor agonist binding states after an acute physiological dose of 17 beta-estradiol. Neurosci. Lett. *88*: 113–118.
Maldonado, R., Robledo, P., Chover, A.J., Caine, S.B., and Koob, G.F. (1993). D1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. Pharmacol. Biochem. Behav. *45*: 239–242.
McHenry, J.A., Otis, J.M., Rossi, M.A., Robinson, J.E., Kosyk, O., Miller, N.W., et al. (2017). Hormonal gain control of a medial preoptic area social reward circuit. Nat. Neurosci. *20*: 449–458.
Mikelman, S., Mardirossian, N., and Gnegy, M.E. (2017). Tamoxifen and amphetamine abuse: Are there therapeutic possibilities? J Chem Neuroanat *83-84*: 50–58.
Mitra, S.W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H.A., Hayashi, S., et al. (2003). Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. Endocrinology *144*: 2055–2067.
Morissette, M., Saux, M. Le, D'Astous, M., Jourdain, S., Sweidi, S. Al, Morin, N., et al. (2008). Contribution of estrogen receptors alpha and beta to the effects of estradiol in the brain. J. Steroid Biochem. Mol. Biol. *108*: 327–338.
Nader, M.A., Morgan, D., Gage, H.D., Nader, S.H., Calhoun, T.L., Buchheimer, N., et al. (2006). PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nat. Neurosci. *9*: 1050–1056.
Nazarian, A., Russo, S.J., Festa, E.D., Kraish, M., and Quinones-Jenab, V. (2004). The role of D1 and D2 receptors in the cocaine conditioned place preference of male and female rats. Brain Res. Bull. *63*: 295–299.
Niv, Y., Daw, N.D., Joel, D., and Dayan, P. (2007). Tonic dopamine: opportunity costs and the control of response vigor. Psychopharmacology *191*: 507–520.

Peterson, B.M., Mermelstein, P.G., and Meisel, R.L. (2015). Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. Brain Struct. Funct. *220*: 2415–2422.
Potenza, M.N., Hong, K.A., Lacadie, C.M., Fulbright, R.K., Tuit, K.L., and Sinha, R. (2012). Neural correlates of stress-induced and cue-induced drug craving: influences of sex and cocaine dependence. Am. J. Psychiatry *169*: 406–414.
Ranaldi, R., and Wise, R.A. (2001). Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. J. Neurosci. *21*: 5841–5846.
Rocha, B.A., Fumagalli, F., Gainetdinov, R.R., Jones, S.R., Ator, R., Giros, B., et al. (1998). Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci. *1*: 132–137.
Russo, S.J., Jenab, S., Fabian, S.J., Festa, E.D., Kemen, L.M., and Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. Brain Res. *970*: 214–220.
Satta, R., Certa, B., He, D., and Lasek, A.W. (2018). Estrogen receptor β in the nucleus accumbens regulates the rewarding properties of cocaine in female mice. Int. J. Neuropsychopharmacol. *21*: 382–392.
Schmitz, Y., Schmauss, C., and Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. J. Neurosci. *22*: 8002–8009.
Schultz, W. (1998). Predictive reward signal of dopamine neurons. J. Neurophysiol. *80*: 1–27.
Shams, W.M., Sanio, C., Quinlan, M.G., and Brake, W.G. (2016). 17β-Estradiol infusions into the dorsal striatum rapidly increase dorsal striatal dopamine release in vivo. Neuroscience *330*: 162–170.
Silverman, J.L., and Koenig, J.I. (2007). Evidence for the involvement of ERbeta and RGS9-2 in 17-beta estradiol enhancement of amphetamine-induced place preference behavior. Horm. Behav. *52*: 146–155.
Sora, I., Wichems, C., Takahashi, N., Li, X.F., Zeng, Z., Revay, R., et al. (1998). Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. USA *95*: 7699–7704.
Tanapat, P., Hastings, N.B., and Gould, E. (2005). Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. J. Comp. Neurol. *481*: 252–265.
Thompson, T.L., and Moss, R.L. (1994). Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. J. Neurochem. *62*: 1750–1756.
Tsai, H.-C., Zhang, F., Adamantidis, A., Stuber, G.D., Bonci, A., de Lecea, L., et al. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science *324*: 1080–1084.
Volkow, N.D., Chang, L., Wang, G.J., Fowler, J.S., Ding, Y.S., Sedler, M., et al. (2001). Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am. J. Psychiatry *158*: 2015–2021.

Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gatley, S.J., Wong, C., et al. (1999). Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. J. Pharmacol. Exp. Ther. *291*: 409–415.
Whiting, K.P., Restall, C.J., and Brain, P.F. (2000). Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. Life Sci. *67*: 743–757.
Wightman, R.M., Amatore, C., Engstrom, R.C., Hale, P.D., Kristensen, E.W., Kuhr, W.G., et al. (1988). Real-time characterization of dopamine overflow and uptake in the rat striatum. Neuroscience *25*: 513–523.
Wightman, R.M., and Zimmerman, J.B. (1990). Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. Brain Res Brain Res Rev *15*: 135–144.
Yoest, K.E., Quigley, J.A., and Becker, J.B. (2018). Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. Horm. Behav.
Yorgason, J.T., España, R.A., and Jones, S.R. (2011). Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. J. Neurosci. Methods *202*: 158–164.
Zhang, H., and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. Nat. Neurosci. *7*: 581–582.

Table 1. Average stimulated DA release during baseline collections and after administration of cocaine. Values shown are mean ± SD after conversion to micromolar DA concentrations.

Figure 1. Measurement of stimulated DA release by fast scan cyclic voltammetry. Experimental timeline for voltammetry collection (A). Animals are gonadectomized at least two weeks prior to being anesthetized and implanted with a glass encased carbon fiber recording electrode aimed at the NAc shell and a bipolar stimulating electrode aimed at the VTA. Representative current over time traces for females (B) and males (D) show evoked release before (gray) and after (black) administration of 10mg/kg Cocaine HCl. Representative false color plots for females (D) and males (E) before (top rows) and after (bottom rows) administration of Cocaine HCl. Coronal diagrams displaying the placement of recording electrodes in the NAc shell (F) and VTA (G). NAc, nucleus accumbens; VTA, ventral tegmental area; EB, estradiol benzoate; PPT, propyl pyrazole triol; DPN, diarylpropionitrile

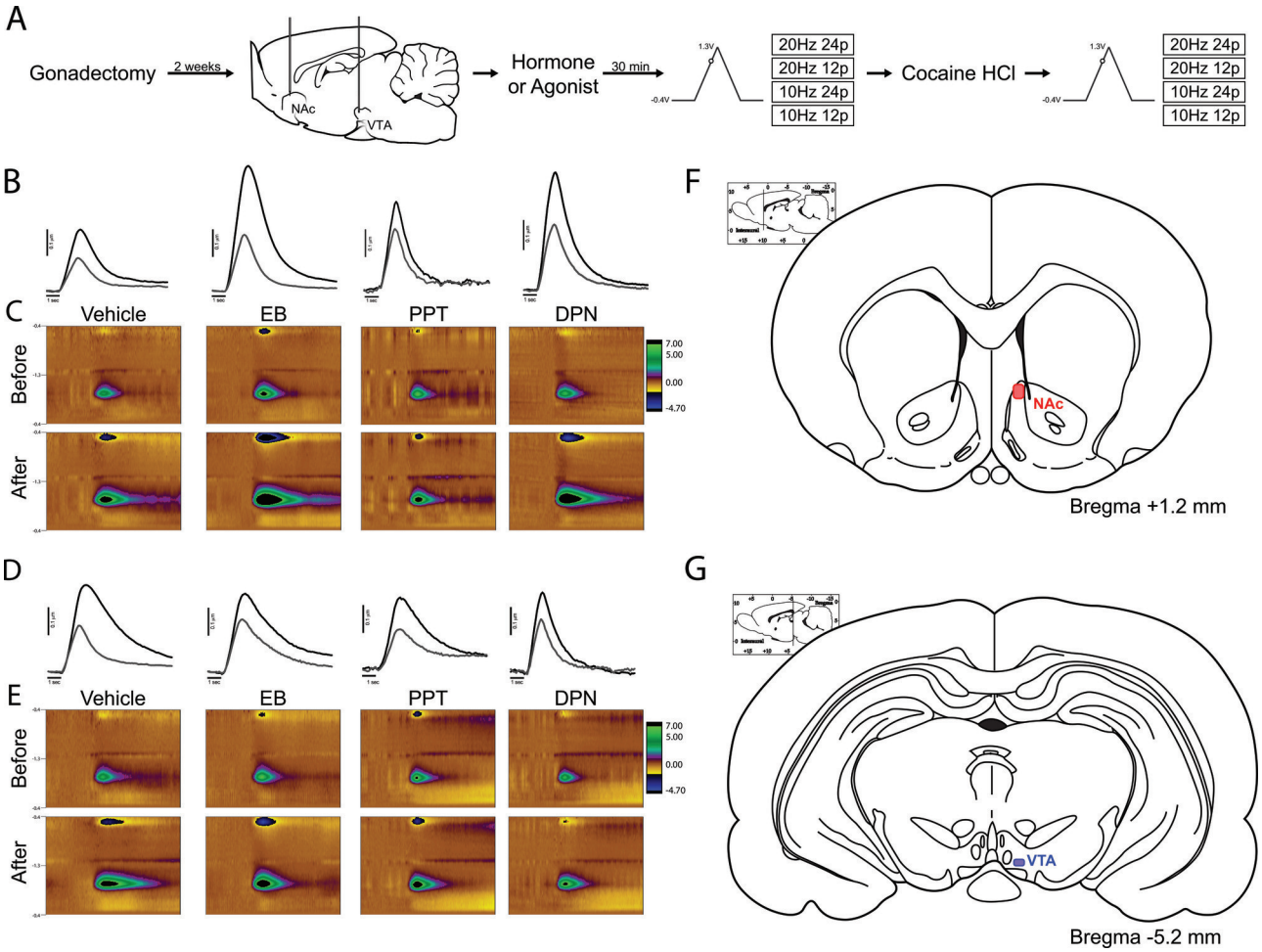
Figure 2. Estradiol enhances the effect of cocaine on NAc DA release in females but not in males. Estradiol treated females show significantly greater DA release after cocaine administration during (A) 20Hz 24p stimulation of the VTA but not (B) 20Hz 12p, (C) 10Hz 24p, or (D) 10Hz 12p stimulation. Values show mean % change in [DAc] after intraperitoneal administration of 10mg/kg Cocaine HCl. Error bars represent SEM. Scatter dots represent individual subject values. 20Hz 24p: n=6 for EB treated males; n=7 for vehicle treated females and males; n=8 for EB treated females. 20Hz 12p: n=6 for EB treated males; n=7 for vehicle treated females and males; n=8 for EB treated females. 10Hz 24p: n=6 for vehicle treated females, vehicle treated males, and EB treated males; n=8 for EB treated females. 10Hz 12p: n=5 for vehicle treated males, n=6 for vehicle treated females and EB treated males; n=8 for EB treated females. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect. EB, estradiol benzoate.

Figure 3. The effect of estradiol on DA release after cocaine is mediated by ERβ, DPN treated females show significantly greater DA release after cocaine administration during (A) 20Hz 24p stimulation of the VTA but not (B) 20Hz 12p, (C) 10Hz 24p, or (D) 10Hz 12p stimulation. Values show mean % change in [DAc] after intraperitoneal administration of 10mg/kg Cocaine HCl. Error bars represent SEM. Scatter dots represent individual subject values. 20Hz 24p: n=6 for DPN treated females; n=7 for vehicle treated females, vehicle treated males, and DPN treated males; n=8 for PPT treated females and PPT treated males. 20Hz 12p: n=6 for DPN treated females; n=7 for vehicle treated females, vehicle treated males, and DPN treated males; n=8 for PPT treated females and PPT treated males. 10Hz 24p: n=6 for vehicle treated females, DPN treated females, and vehicle treated males; n=7 for DPN treated males; n=8 for PPT treated females and PPT treated males. 10Hz 12p: n=4 for DPN treated females; n=5 for vehicle treated males, n=6 for vehicle treated females and DPN treated males; n=8 for PPT treated females and PPT treated males. * $p<0.05$ compared to within-sex vehicle, treatment effect. # $p<0.05$ compared by sex, sex effect. PPT, propyl pyrazole triol; DPN, diarylpropionitrile.

Figure 4. Estradiol attenuates the effect of cocaine on NAc DA reuptake in males but not in females. EB treated males show significantly less of an effect of cocaine on DA reuptake after (A) 20Hz 24p stimulation of the VTA. Reuptake is represented by the exponential decay constant Tau. Tau is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. There was no difference in the effect of cocaine on DA reuptake during (B) 20Hz 12p, (C) 10Hz 24p, or (D) 10Hz 12p stimulation of the VTA. Values show mean % change in Tau after intraperitoneal administration of 10mg/kg Cocaine HCl. Error bars represent SEM. Scatter dots represent individual subject values. Group sizes - 20Hz 24p: n=6 for EB treated males; n=7 for vehicle treated females and males; n=8 for EB treated females. 20Hz 12p: n=6 for EB treated males; n=7 for vehicle treated females and males; n=8 for EB treated females. 10Hz 24p: n=6 for vehicle treated females, vehicle treated males, and EB treated males; n=8 for EB treated females. 10Hz 12p: n=5 for vehicle treated males, n=6 for vehicle

treated females and EB treated males; n=8 for EB treated females. * $p<0.05$ compared to within-sex vehicle, treatment effect. EB, estradiol benzoate.

Figure 5. There is no effect of selective ER activation on DA reuptake in males or females. Reuptake is represented by the exponential decay constant Tau, which is inversely proportional to the decay rate, so that an increase in Tau corresponds to a decrease in reuptake. There is no difference in the effect of cocaine on DA reuptake during (A) 20Hz 24p, (B) 20Hz 12p, (C) 10Hz 24p, or (D) 10Hz 12p stimulation of the VTA. Values show mean % change in Tau after intraperitoneal administration of 10mg/kg Cocaine HCl. Error bars represent SEM. Scatter dots represent individual subject values. Group sizes - 20Hz 24p: n=6 for DPN treated females; n=7 for vehicle treated females, vehicle treated males, and DPN treated males; n=8 for PPT treated females and PPT treated males. 20Hz 12p: n=6 for DPN treated females; n=7 for vehicle treated females, vehicle treated males, and DPN treated males; n=8 for PPT treated females and PPT treated males. 10Hz 24p: n=6 for vehicle treated females, DPN treated females, and vehicle treated males; n=7 for DPN treated males; n=8 for PPT treated females and PPT treated males. 10Hz 12p: n=4 for DPN treated females; n=5 for vehicle treated males, n=6 for vehicle treated females and DPN treated males; n=8 for PPT treated females and PPT treated males. PPT, propyl pyrazole triol; DPN, diarylpropionitrile.



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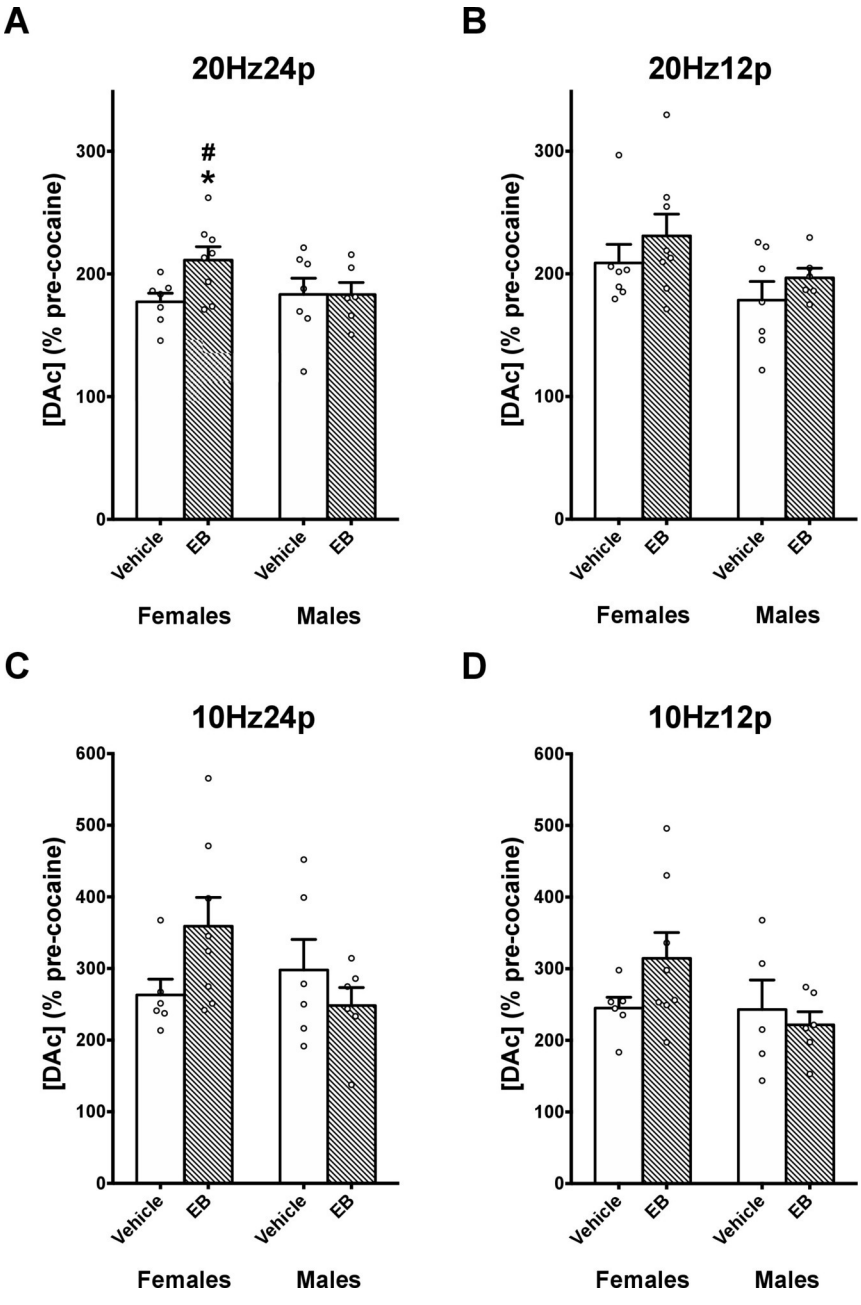

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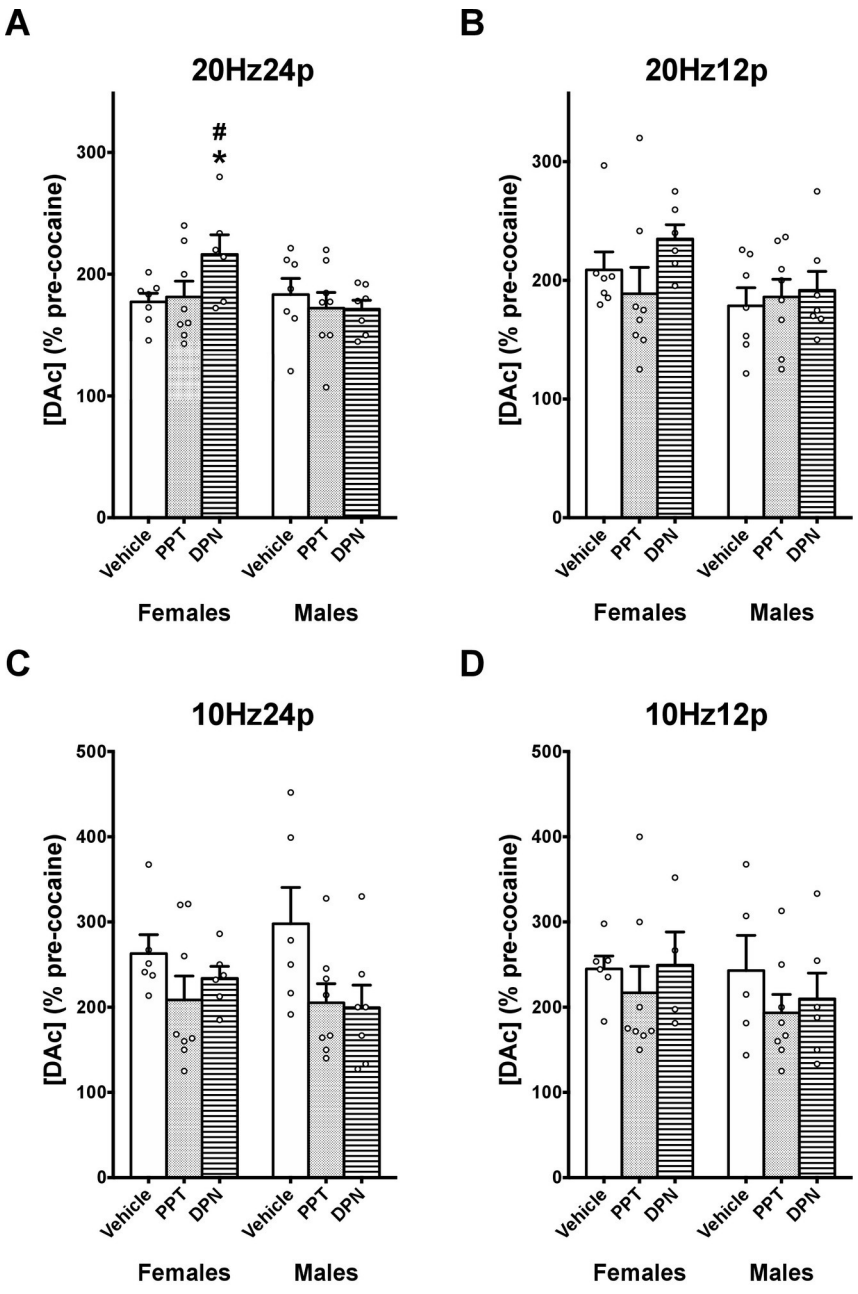

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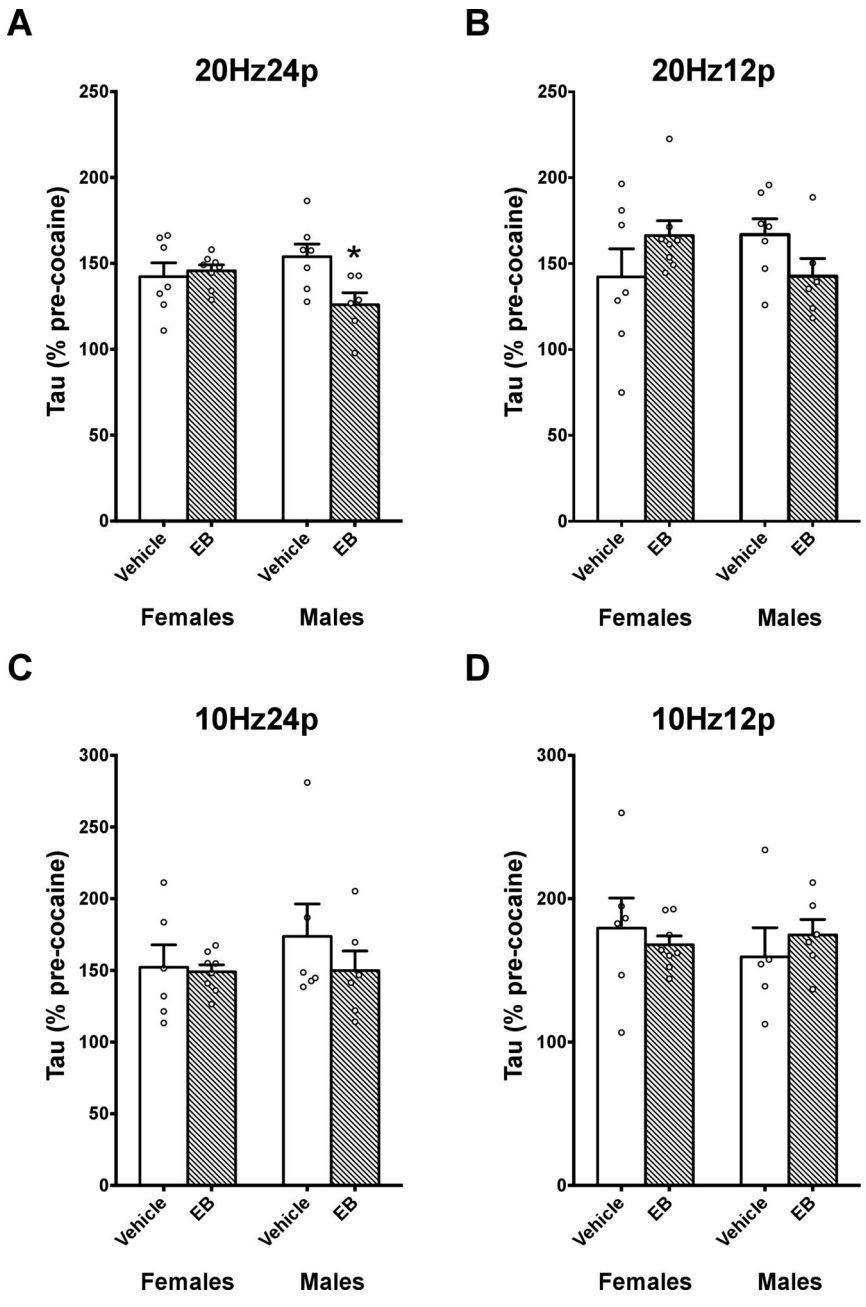

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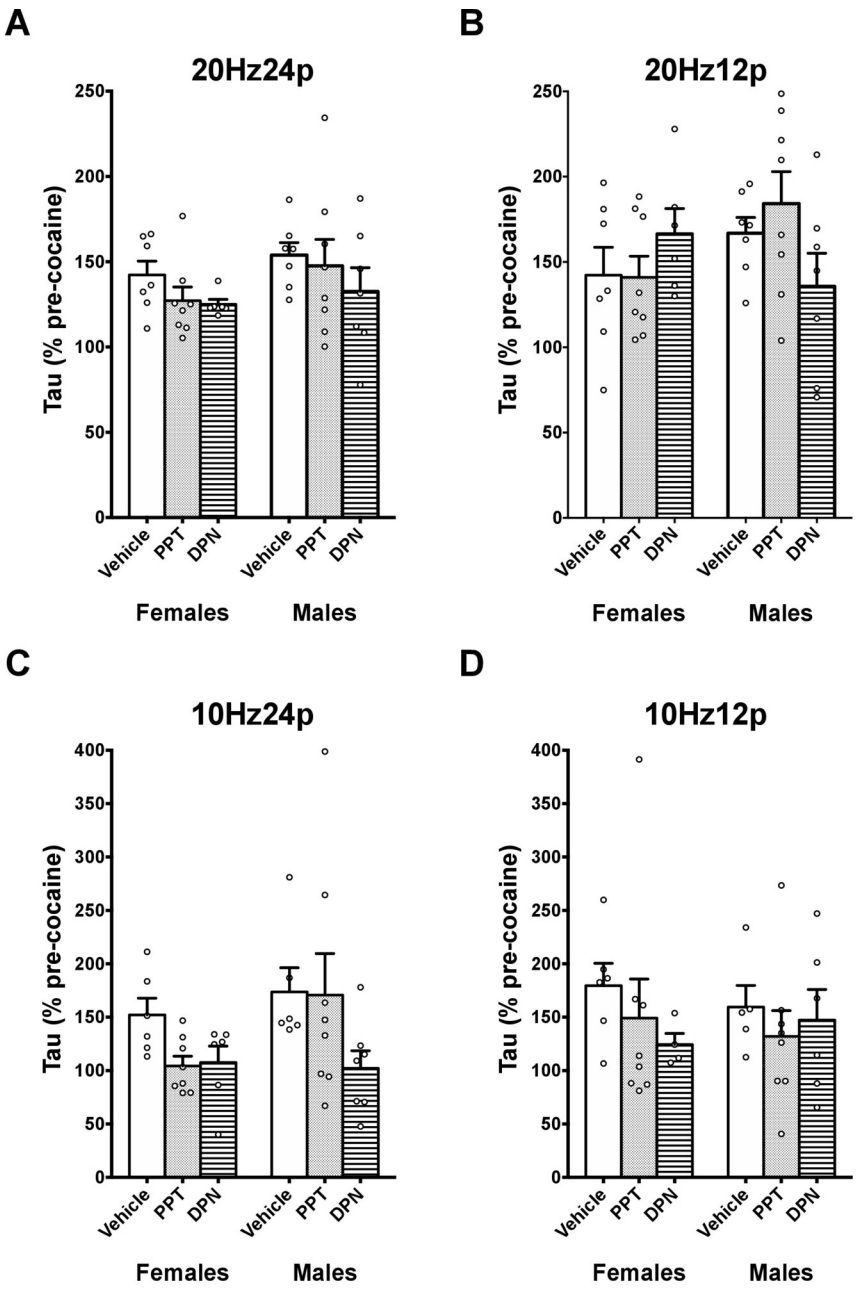

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	20Hz 24p			20Hz 12p			10Hz 24p			10Hz 12p		
	Baseline (μm)	Cocaine (μm)		Baseline (μm)	Cocaine (μm)		Baseline (μm)	Cocaine (μm)		Baseline (μm)	Cocaine (μm)	
Male												
Vehicle	0.52 ± 0.35	0.96 ± 0.66	n=7	0.14 ± 0.11	0.27 ± 0.24	n=7	0.18 ± 0.12	0.58 ± 0.45	n=6	0.09 ± 0.05	0.24 ± 0.19	n=5
EB	0.31 ± 0.19	0.58 ± 0.37	n=6	0.14 ± 0.08	0.28 ± 0.17	n=6	0.16 ± 0.10	0.41 ± 0.28	n=6	0.09 ± 0.03	0.21 ± 0.09	n=6
PPT	0.26 ± 0.22	0.40 ± 0.29	n=8	0.13 ± 0.12	0.22 ± 0.17	n=8	0.12 ± 0.10	0.21 ± 0.19	n=8	0.09 ± 0.10	0.14 ± 0.14	n=8
DPN	0.29 ± 0.13	0.49 ± 0.23	n=7	0.14 ± 0.23	0.24 ± 0.10	n=7	0.16 ± 0.10	0.30 ± 0.14	n=7	0.10 ± 0.07	0.18 ± 0.14	n=6
Female												
Vehicle	0.47 ± 0.38	0.82 ± 0.61	n=7	0.19 ± 0.13	0.37 ± 0.24	n=7	0.20 ± 0.19	0.55 ± 0.43	n=6	0.11 ± 0.08	0.30 ± 0.22	n=6
EB	0.30 ± 0.17	0.61 ± 0.33	n=8	0.13 ± 0.06	0.27 ± 0.14	n=8	0.12 ± 0.06	0.39 ± 0.17	n=8	0.07 ± 0.04	0.21 ± 0.11	n=8
PPT	0.25 ± 0.14	0.45 ± 0.25	n=8	0.10 ± 0.05	0.19 ± 0.10	n=8	0.14 ± 0.06	0.27 ± 0.12	n=8	0.08 ± 0.03	0.16 ± 0.06	n=8
DPN	0.33 ± 0.23	0.67 ± 0.52	n=6	0.20 ± 0.19	0.48 ± 0.46	n=6	0.20 ± 0.17	0.46 ± 0.35	n=6	0.16 ± 0.15	0.38 ±0.37	n=4

Table 1. Average stimulated DA release during baseline collections and after administration of cocaine. Values shown are mean ± SD after conversion to micromolar DA concentrations.